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Editor(s)

Dr. Triki Mohamed Amine

Department of Orthopaedic Surgery, Sahloul University Hospital, Sousse, Tunisia.
Email: mohamed-amine.triki@aphp.fr, Trikos@hotmail.fr, m.aminetriki@gmail.com;

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Preface

This book covers key areas of Medicine and Medical Research. The contributions by the authors include obesity, overweight, non-communicable diseases, obesity paradox, heart rate variability, chronobiology, stem cell, toxicology, bone marrow transplants, multipotent stem cells, adipose tissue, hydatid cyst, anaphylactic shock, laproscopic surgery, Parkinson disease experimental model, endocannabinoid system, oral rehydration, bone grafting, fixed appliance therapy, orthodontic tooth movement, urinary Schistosomiasis, NCIC FPC filing sequence, juvenile polyp, bleeding per rectum, colonoscopy, orbital cellulitis, endoscopic marsupialization, anterior orbitotomy, cascade stomach, reflux symptoms, dyspepsia, tooth preparations, chronic myeloproliferative neoplasms, chronic myeloid leukemia, primary myelofibrosis, polycythemia vera, elderly patients, chemotherapy, attorney health care. This book contains various materials suitable for students, researchers and academicians in the field of Medicine and Medical Research.

Chapter 1
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Health Impacts of Obesity: A Modern Era Pandemic

Shashi K. Agarwal [a#*]

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ABSTRACT

Obesity is a worldwide problem. It is usually diagnosed if the body mass index is >30, and/or a waist circumference >102 cm in men and >88 cm in women. Obesity is a chronic disease that increases the risk of heart disease, type 2 diabetes, obstructive sleep apnea, osteoarthritis, and many types of cancer. It reduces the health quality of life. It is a leading cause of global disability and mortality. It is, however, both preventable and treatable. Weight loss can be achieved by changing dietary patterns and exercising. Therapeutic interventions and surgical options are increasingly being instituted. Novel interventions like fecal transplantation appear promising. Even a loss of 5% to 10% of the total body weight is metabolically healthy. Prevention of obesity and a reduction in body weight in those with a BMI>30, will greatly improve the health of the world. It will also help bring health care costs down. This narrative review aims at highlighting the diagnostic criteria and the various factors that influence and cause obesity. It also summarizes its deleterious impact on humans, both in health and in disease. A look at the various modalities available for its mitigation is also discussed. This discussion should help health care providers to better manage their obese patients.

Keywords: Obesity; overweight; BMI; non-communicable diseases; obesity paradox.

1. INTRODUCTION [1-6]

The prevalence of obesity has substantially increased worldwide. Obesity is now seen in a third of the world's population. Its prevalence has nearly tripled between 1975 and 2016. In countries like the USA, obesity is now present in almost one-half of the population, with the Centers for Disease Control and Prevention (USA), estimating that it affected 42.4% of the population in 2017 – 2018. Overweight (including obesity) in the European Union is estimated to have affected 52.7% of the adult EU's population in 2019. In the Eastern Mediterranean Region, the prevalence of obesity has increased from 15.1% in 1980 to 20.7% in 2015. In Sweden, the obesity rate is 16.6%. The obesity burden is also affecting the less developed countries. In China, the combined prevalence of overweight and obesity rose by 27.5% for adults between 1980 and 2013, resulting in an overweight/obese population of 2.1 billion in 2013. In India, it is estimated that by 2030, the prevalence of overweight and obesity will be 27.8%, and 5.0%, respectively. Adult obesity has also been increasing at an alarming rate among African countries. Eastern Sudan has a prevalence of 26.8% for overweight and 32.2% for obesity. Uganda has a 17.8% prevalence of overweight while in Ethiopia, overweight/obesity increased significantly from 10.9% in 2000 to 21.4% in 2016. In South America, obesity is also common – for example in Mexico it is at 36.1%. In Brazil, a significant increase in the prevalence of obesity has occurred, from 11.8% in 2006 to 20.3% in 2019. According to the World Health Organization, in 2016, globally, 1.9 billion adults were overweight, and 650 million were obese. They also estimate that in 2030, 58% of the global adult population will be overweight and 20% will be obese. The recent pandemic with COVID-19 is expected to worsen this obesity epidemic. Excessive weight gain is not limited to adults and is also affecting children and adolescents. UNICEF estimates that in the pediatric and adolescent population, the prevalence rate of obesity is now >21.4%. Worldwide costs related to obesity care are extremely high - in the range of two to seven percent of the total healthcare costs. Obese patients often have several comorbid disorders. The cost of

[#] *Medical Director;*
[a] *Center for Contemporary and Complementary Cardiology, 2227 US Highway 1, Suite 309, North Brunswick, NJ 08902, USA.*
**Corresponding author: E-mail: usacardiologist@gmail.com;*

treatment of obese patients is, therefore, higher - it is estimated that they incur a 30% higher medical cost than those with a normal body mass index (BMI).

2. DISCUSSION

Obesity is not harmless. However, attempts to stem it has been essentially futile. Obesity increases the risk of a plethora of non-communicable diseases. It reduces the quality of life and increases premature mortality.

2.1 Predisposing Factors/Causes [7]

Normally, the body is in an equilibrium, where food intake (energy intake) equals physical activity (energy expenditure), and the person neither gains nor loses weight. When this energy balance is disturbed, and energy intake exceeds energy expenditure over an extended period, an increase in stored adipose tissue occurs, resulting in overweight and obesity. This is seen as hypertrophy of pre-existing adipocytes or recruitment (hyperplasia) of adipocyte precursors.

Obesity development is complex and multifactorial. Causes include a combination of biological, genetic, social, environmental, and behavioral factors. Obesity is more common with increasing age, in females, certain ethnic groups such as non-Hispanic blacks in the US, and in those with a family history of obesity. Other factors include living in rural areas and in areas where access to affordable and nutritious food is limited (called food deserts), and living in areas without walkability and high crime. Personal food choices (overconsumption of calories, consumption of energy-dense foods, fast foods, sodas, sugar-sweetened drinks, etc.), low education, poverty. lower social status, social ties with obese individuals, sedentary behavior (energy expenditure ≤1.5 metabolic equivalents (METs),) lack of planned exercise, poor sleep (both in duration and quality), exposure to air pollution, and environmental obesogens (chemicals such as flame retardants, pesticides, and insecticides, industrial materials such as bisphenol A, phthalates, polychlorinated biphenyls, and tributyltin) also play a role. It is also common in individuals with certain diseases (hypothyroidism, prolactinoma, polycystic ovarian syndrome, depression, and Cushing's syndrome) and with certain medications (psychotropic drugs, anti-diabetic medications, beta-blockers, corticosteroids, and anti-epileptic drugs). Certain viruses (greater Ad-36 viral loads) and gut microbiota also play a role. Genetics also have a say. Polygenic obesity (simultaneous presence of DNA variation in multiple genes) is the most common form, although monogenic (mutation or deficiency of a single gene) and syndromic obesity (Bardet–Biedl, Prader–Willi, Alstrom, and Smith–Magenis syndromes) is also seen. The epigenetic phenomenon may alter gene expression without changing the underlying DNA sequence and result in obesity.

2.2 Diagnosis of Obesity [8]

Adolphe Quetelet (1796-1874), a Belgian mathematician, astronomer, and statistician, described a practical index of relative bodyweight - an index that was the ratio of the weight in kilograms divided by the square of the height in meters (also called the Quetelet Index) in 1832. This was subsequently termed BMI in 1972 by Ancel Keys. BMI is nowadays, a widely used measure to define bodyweight. BMI can also be measured by this equation: BMI = [weight (lb)/height (in)] x 703). In adults (age over 18 years) BMI is categorized into several groups: < 19.9 kg/m^2 (underweight), 20.0–24.9 kg/m^2 (normal weight), and 25 to 29.9 kg/m^2, (overweight). Obesity is defined as a BMI exceeding 30 kg/m^2 and is subclassified into class 1 (30–34.9), class 2 (35–39.9), and class 3 or severe obesity (≥ 40). A BMI >50 Kg/m^2 is considered morbid obesity. The International Obesity Task Force recommends different BMI categories for Asians, due to their physical structure and increased percentage of abnormal fat accumulation. These are as follows: underweight (<18.5 kg/m^2), normal (between 18.5 and 23 kg/m^2), overweight (between 23 and 25 kg/m^2), obese (between 25 and 30 kg/m^2), severe obese (≥30 kg/m^2). BMI is not used for children and adolescents aged 2 to 18 years; instead, a percentile scale based on the child's sex and age is used.

BMI is only related to total body weight, and it does not indicate fat distribution or weight composition. Abnormal fat deposition, for example, extra-visceral or intra-visceral in the abdomen (central or visceral obesity) is more harmful than subcutaneous adipose tissue. Central obesity can be objectively ascertained by several anthropometric measurements. The commonest used is the waist circumference (WC). WC should ideally be <102 cm in males and <88 cm in females when measured to the nearest 0.1 cm at the umbilical level in a standing position. In the Asian populations, these numbers are <85 cm for males and, <80 cm for females. Higher values indicate central obesity. Many researchers have also used another anthropometric measurement, the waist to hip ratio. This is normally 0.85 or less for women and 0.9 or less for men. And higher values are abnormal. The waist-height ratio is calculated by dividing the WC by height. A ratio < 0.5 indicates no central obesity and ≥ 0.5 is consistent with central obesity.

2.3 Metabolically Healthy Obesity [9]

Metabolically healthy obesity (MHO) is present in individuals who have excessive body fat accumulation but do not have any cardiometabolic abnormalities (such as insulin resistance, impaired glucose tolerance, dyslipidemia). MHO is not uncommon, and many epidemiologic and clinical studies suggest that it is present in 10%–40% of obese individuals. However, MHO is not entirely benign. Most people with MHO are at a higher risk for metabolic diseases than people who are metabolically healthy and lean. Further, MHO is not a stable state, and it gradually shifts to become metabolically unhealthy obesity (MUO). Individuals with MHO therefore also benefit from weight-loss interventions

2.4 Thin Fat Obesity [10]

Normal weight obesity or the thin fat phenotype is defined as the presence of an increased body fat percentage in an individual with a normal body mass index.

2.5 Obesity and Health [11]

Obesity results in many harmful physiological and pathological changes in the human body, thereby promoting several acute and chronic diseases. The health complications from excess body fat increase as the BMI increases.

2.6 Obesity and Chronic Medical Disorders [12-14]

Obese individuals face an increased risk of a multitude of chronic health problems. The CDC defines chronic diseases as “conditions that last 1 year or more and require ongoing medical attention or limit activities of daily living or both”. Although already common in the West, most chronic diseases are gradually replacing infectious diseases as a major health concern in developing countries. These are discussed below.

Cardiovascular diseases (CVDs) are responsible for high morbidity and mortality rates worldwide. Many studies show that as the BMI increases, the risk for CVDs also increases. It is estimated that the risk of CVD increases 6% for each 1.1 kg/m^2 increase in BMI. A 10% body-weight reduction in the first-year results in a 21% lower risk of the primary CVD outcome and a 24% reduced risk of the secondary outcome compared with individuals who were weight stable. Obesity imparts an attributable risk of 13% to cardiovascular mortality. Chronic respiratory diseases were responsible for 3.8 million deaths, 9% of all non-communicable disease-related deaths, and 7% of all global deaths, in 2016. Increased BMI results in lower lung function, as demonstrated by a reduced forced expiratory volume in 1 second and a diminished forced vital capacity in these patients. Obesity is associated with an increased incidence of asthma and obstructive sleep apnea. Interestingly, reduced BMI is associated with high emphysema rates and COPD patients demonstrate an increased survival when compared with their underweight and/or normal-weight peers (obesity paradox discussed later in this manuscript). Mendelian randomization studies report that genetically predicted BMI, WHR, and insulin resistance increase the risk for lung cancer, especially for squamous cell and small cell lung cancer. Several studies have found associations between obesity and mental health problems. It is estimated

that obesity is associated with an approximately 25% increase in odds of mood and anxiety disorders. The prevalence of depression in obese individuals is twice as high as in those of normal weight. Obesity and non-alcoholic fatty liver disease (NAFLD) are closely related. NAFLD is a common condition and its prevalence among the obese population ranges from 30% to 37% - it rises to 84% to 96% in patients undergoing bariatric surgery. Obesity produces a 3.5-fold increased risk of developing NAFLD, with the relative risk increasing 1.20 for each 1 unit increase in BMI. Obesity increases adverse outcomes in patients with both alcoholic and viral hepatitis. Overweight/obesity also increases overall cancer risk. In 2015, obesity accounted for 15.1% of all cancer cases in Scotland and 6.3% of all cancer cases in England. In Germany, 7% of the cancers are attributable to excess body weight. In the USA, 7.8% of incident cancer cases in 2014 were attributed to excess body fatness. These included cancers of the esophagus, liver, gallbladder, pancreas, breast, stomach, uterus, ovary, kidney, colon/rectum, and the meninges. Several studies have shown that obesity causes a more rapid cancer progression, reduced response to certain chemotherapy drugs, increased cancer recurrence, and promotes the development of second primary cancers. Obesity is also associated with decreased survival in these patients. According to an American Cancer Society study, adult cancer subjects with a body mass index greater than 40 have higher death rates (52% higher in men and 62% higher in women) when compared to those with normal weight. Obesity is a major risk factor for type 2 diabetes (T2DM). Studies indicate that more than 85% of people with T2DM are overweight or obese. It is estimated that the risk of developing future T2DM is four times higher in obese children and obese adolescents. Reducing weight by a median of 5.5% for 2.8 years reduces the risk of prediabetes converting to diabetes by 58%. Bariatric surgery may not only result in a weight loss of 20% to 30% but may also induce remission of T2DM. Besides a reduced need for diabetic medications, these T2DM patients also reduce their hospitalizations and health care costs. Weight reduction is also associated with an improvement in the high cardiovascular morbidity and mortality in these patients. Obesity is a major cause of chronic kidney disease (CKD). It is also associated with a more rapid progression of the disease. and results in worse outcomes, both pre and post renal transplantation surgery. In obesity-related glomerulopathy, a weight loss of 12% results in a decrease in proteinuria by >80%. Weight loss with bariatric surgery also results in resolution, improvement, or stabilization of kidney function in CKD patients. An association between obesity and nephrolithiasis has also been described, particularly calculi made up of uric acid and calcium oxalate minerals. Obesity is also associated with cancers of the kidneys. In a meta-analysis, it was estimated that the increased risk for kidney cancer was 1.82 (pooled RR) for men and 2.64 for women. Obesity during mid-life may lead to a much higher incidence and prevalence of Alzheimer's disease in later life. It has been seen that individuals who are obese during midlife developed dementia at an increased rate with a mean odds ratio of 3.88. An Australian study estimated that in 2050, dementia in old age can be reduced by 10% by decreasing midlife obesity by 20%. Obesity is often present in individuals with knee and hip osteoarthritis. Obese patients undergoing total knee arthroplasty experience increased revision rates, lower functional scores, and increased complications when compared to non-obese patients. Although knee and hip arthritis in obese individuals is commonly ascribed to obesity-related mechanical issues, obese individuals also suffer from increased osteoarthritis in non-weight-bearing joints. The actions of pro-inflammatory adipokines and cytokines are implicated in this. Obesity is also significantly associated with rheumatoid arthritis (RA) with a relative risk of 1.31. It accelerates the progression of RA. Gouty arthritis is also causally connected with obesity. In an evaluation of 10 prospective studies, involving 27,944 cases with a median follow-up of 10.5 years, it was found that the relative risks for gouty arthritis were 2.67, 3.62, and 4.64 for persons with a BMI of 30, 35, and 40 kg/m2, respectively, compared with persons with a BMI of 20 kg/m2. Many other obesity-related conditions exist. Obesity is detrimental for more than 90% of chronic ailments affecting society today.

2.6 Obesity and Acute Medical Disorders [15]

Obesity increases the risk for fractures of the ankle, leg, and humerus. It however appears to have a protective effect with regards to the wrist, hip, and pelvic fractures. Obesity-related osteoporosis has also been implicated in increasing the risk of low-impact, fragility fractures. Rotator cuff tendonitis and shoulder impingement syndrome are more common in obese individuals and are associated with poorer surgical outcomes. Carpal tunnel syndrome is also more common with obesity. Lower limb disorders common in the obese population include plantar fasciitis, Achilles tendonitis, and posterior tibial tendon dysfunction. Meniscal tears are not only more common in patients with high BMI, but also

require surgical intervention more often when compared to those with a normal BMI. Obese patients involved in traumatic injuries have longer stays in the intensive care unit, higher rates of complications, and mortality, compared to non-obese patients despite equivalent injury severity. Besides these orthopedic problems, obese adults and children are at an increased risk of nonallergic rhinitis, with an adjusted odds ratio (OR) of 1.43 for adults and 0.88 for children. There is an increased risk of infections in the obese population, including those at the surgical-site, nosocomial, urinary tract, and skin infections. Obese women do not respond well to hepatitis-B vaccination. Obesity is associated not only more frequently in patients with periodontitis but also accelerates its progression. Several cohort studies have shown that obesity is associated with more tooth loss. Periodontal conditions in obese individuals are significantly worse 2 and 6 months after dental treatment compared with those in non-obese individuals. Obesity has also been recognized as an independent risk factor for COVID-19 infections.

2.7 Pregnancy and Obesity [16]

Maternal obesity is common - up to 30% of women of reproductive age are obese. Obesity may increase infertility. Preconception obesity is also associated with early pregnancy loss. Obese pregnant women face several health problems, including an increased risk of gestational diabetes mellitus, pre-eclampsia, eclampsia, preterm birth, a higher need for cesarean section, infections, and post-partum hemorrhage. The fetus has a higher risk of being still-born or suffering from fetal distress syndrome. Post-partum, obese mothers are more prone to depression, deep vein thrombosis, hypertension, and dyslipidemia. In the long term, they are more susceptible to developing T2DM and obstructive sleep apnea.

2.8 Obesity and Health Problems in Children [17]

Pediatric obesity is associated with an earlier onset of chronic disorders such as T2DM, dyslipidemia, NAFLD, obstructive sleep apnea, and polycystic ovary syndrome. Females may also experience early puberty and menstruation irregularities. Obese children are also at risk for slipped capital femoral epiphysis. Further, obesity in children portends future obesity in adults. It is estimated that almost three-fourth of children with overweight/obesity will be overweight or obese in adulthood.

2.9 Psycho-social effects of Obesity [18]

In adults, obesity is associated with feelings of rejection, shame, low self-esteem, and guilt. Obese individuals may also be the subject of weight bias and stigma from others, including health care providers. In addition, obesity frequently leads to disqualification from the military. Children and adolescents with obesity also experience an increased risk of psychological problems such as school absenteeism. They are often a target of bullying and rumors/lies, name-calling, teasing, physical abuse, isolation, and experience lower hourly wages in the future.

2.10 Obesity and QOL, DALYs and Mortality [19]

Obesity reduces the health quality of life. According to recent data, obesity was related to 148 million DALYs and 4.72 million deaths worldwide. It remains a major cause of preventable death. The Global Burden of Disease project estimated that mortality has increased by 45% for grade I obesity (BMI 30.0 to <35.0 kg/m2), by 94% for grade 2 obesity (BMI, 35.0 to <40.0 kg/m2), and 176% for grade-3 obesity (BMI, 40.0 to <60.0 kg/m2). It is estimated that obese individuals (BMI of 30–35 kg/m²) lose about 2-4 years of life while those with severe obesity (BMI >40 kg/m², lose about 8-10 years of life. Losing weight is therefore beneficial. The Swedish Obese Subjects Study showed a 29% reduction in overall mortality in obese patients (after 10.9 years) who underwent bariatric surgery. Obesity represents only 10% of the global BMI >25 but accounts for >60% of the disease and mortality burden.

2.11 Mechanisms of Obesity-induced Health Damage [20]

White adipose tissue and its macrophages produce pro-inflammatory cytokines - tumor necrosis factor-alpha and interleukin-6. The cytokines induce increased inflammation and higher insulin

resistance, resulting in endothelial dysfunction and atherosclerosis. There is also increased pro-atherogenic dyslipidemia and a prothrombotic effect. Obesity induces insulin resistance, hyperinsulinemia, and an abnormally increased blood level of insulin-like growth factor (IGF). Visceral fat, which is rich in white adipose tissue, is also closely related to tumorigenesis and the progression of tumors. Many other harmful factors come into play in obese individuals.

2.12 Obesity Paradox [21]

In the general population, mortality is higher in both the underweight and the overweight, when compared with those with normal weight – indicating a 'U-shaped' relationship. Some diseases, however, demonstrate improved survival in overweight/obese individuals, when compared with normal-weight individuals, and still demonstrate increased mortality in those underweight. This reverse "J-shaped" curve is referred to as the "obesity paradox" and has been described in several chronic diseases such as heart failure, coronary artery disease, chronic obstructive pulmonary disease, and end-stage kidney disease. It has also been noted in some acute conditions such as pneumonia, sepsis, and acute respiratory distress syndrome. Several hypotheses have been advanced to explain this phenomenon. Studies have found that patients demonstrating the obesity paradox have increased lean mass, despite the obesity, and better cardiorespiratory fitness. Obesity paradox has also been noted in some cancers.

2.13 Treatment [22]

Practically, weight loss should be about 5–15% over 6 months, except in those with BMI over 35, where a higher and faster weight loss may occur with surgical intervention. A slow and gradual weight loss of 1 to 2 pounds/week (approximately 0.5 to 1 kg/week) prevents drastic changes in metabolism and is associated with better long-term improvement in BMI, WC, and body composition. Obesity management also helps co-morbidities.

2.14 Nutrition [23]

Weight loss and weight-loss maintenance require a long-term reduction in caloric intake, without interfering with the nutritional needs of the body. Recommended daily calorie intakes in the US are around 2,500 for men and 2,000 for women. Since one pound of weight is equal to 3500 Kcal, a deficit of 500 to 1000Kcal/day should help reduce about 1-2 lbs. per week. Several different diet plans have become popular for losing weight:

2.15 Calorie Based Diets [24]

Very-low-calorie diet: This limits the calorie intake to 600–900 per day This should be switched to a low-calorie diet after a period of 2 weeks to 3 months.

Low-calorie diet: This diet generates a deficit of 500-750 calories per day by restricting caloric intake to 1,000–1,500 calories per day.

Most people underestimate the calories they consume.

2.16 Macronutrient Based Diets [25]

Low-fat diet: Diets consisting of low consumption of fat (< 15%–20% of daily calories and < 7%–10% saturated fats) are considered low-fat diets. There is no convincing data to support that these diets work any better than other dietary interventions to achieve long-term weight loss.

Low-carbohydrate diet: Low carbohydrate diets entail the consumption of carbohydrates as < 45% of daily calories or < 130 mg/day. However, the long-term results are not any better than those seen with a low-fat diet this diet may however help in the control of type 2 diabetes, often seen in obese individuals.

Ketogenic diet: A marked decrease in carbohydrate intake (<10% of daily calories or <20–50 g/day), results in ketosis. In this diet, there is an associated increase in the proportions of protein and fat consumed. To preserve lean body mass, daily protein intake is usually 0.8–1.5 g/kg of ideal body weight. Ketogenic diets help decrease appetite and increase lipolysis, but long-term safety is unknown.

High-protein diet: These diets increase the intake of protein to >30% of total daily caloric intake (as high as 43 %) or >1–1.2 g/kg of the ideal body weight. Proteins from vegetable sources are safer. They increase satiety and decrease fat mass, resulting in weight loss. High-protein diets should be used with caution in individuals with or at risk of chronic kidney disease.

2.17 Mediterranean Diet [26]

The Mediterranean diet consists of high consumption of fruits and vegetables, poultry, fish, dairy products, and monounsaturated fats, (high intake of extra virgin olive oil) with little to no consumption of red meat. A glass of red wine is often consumed with meals. Mediterranean diet shows the strongest evidence for weight loss (compared to other diets) and improvements in cardiometabolic parameters.

Intermittent fasting [27]: Intermittent fasting is practiced in many ways - alternate-day fasting, 5:2 intermittent fasting (fasting or consuming 900–1,000 calories for 2 days each week), and daily time-restricted feeding (fasting for 16–18 hours a day). Fasting not only reduces calorie intake but also helps in reducing insulin resistance and strengthening the immune system. Diabetics on hypoglycemic agents may want to avoid this technique.

Other Dietary Interventions [28]: The timing of the diet also appears to matter. Eating at the wrong time disrupts the circadian rhythm, leading to several metabolic changes that can lead to obesity. Overweight and obesity are increased if breakfast is skipped. Similarly, late-night eating is associated with an increased risk of obesity. Ideally, breakfast should be high in calories and late-night eating should be avoided, resulting in an overnight fast.

Food should be balanced, even if the caloric intake is reduced. Recommendations include intake of 32 g/day of dietary fiber, 400 g/day of fruit and non-starchy vegetables, 0 g/week of processed meat, <500 g/week of red meat, 0 gm/week of trans fats, total intake of fats less than 30%, and saturated fats less than 10% of total energy intake. Saturated fats should be replaced with unsaturated fats - preferably with polyunsaturated fats. Salt intake should be limited so that sodium intake is less than 2,300 mg a day and sugar intake should be restricted to less than 10% of total caloric intake.

Nutritional diets help weight reduction initially (over 6 months) but most improvements in weight and cardiometabolic factors disappear after 12 months. Concomitant exercise helps in more weight loss and further cardiometabolic improvements. Exercise also helps reduce the diet-induced loss of muscle from almost 27% to 13% (of the weight lost).

Exercise [29]: Physical activity (PA) is defined as any bodily movement produced by the contraction of skeletal muscles that results in a substantial increase in resting energy expenditure. Exercise, which is a subcategory of physical activity, is defined as 'any sport or activity that works large groups of muscles, is continually maintained and performed rhythmically'. Aerobic activity is an ideal exercise as it involves the repeated and continuous movement of large muscle groups. Activities such as walking, cycling, jogging, and swimming are aerobic exercises. Several professional organizations recommend that adult men and women accumulate at least 150 min of moderate-intensity physical exercise per week (spread out throughout the week). This can be achieved by exercising 30 minutes a day, five days a week. Muscle-strengthening activity should be included twice a week. Children and young people aged 5–17 years should accumulate at least 60 min of physical exercise of moderate to vigorous intensity daily. Adults aged 65 and older should do at least 150 minutes a week of moderate-intensity activity (such as brisk walking), resistance exercises at least two days a week (two sets of 10 repetitions, each resistance exercise involving different major muscle groups), and improve balance (such as standing on one foot). Pregnant or postpartum women are also recommended to do at least

150 minutes of moderate-intensity aerobic physical activity per week (such as brisk walking), with their doctor's approval. Exercise at this level helps improve cardiorespiratory fitness and exerts a mitigating effect on several co-morbid conditions seen in those obese. Exercise has also been beneficial in individuals with drug addiction. Older individuals with excess body weight notice improved mobility. There are also beneficial psychological effects. Regular exercise improves the quality of life, even in healthy individuals. Higher levels of total physical activity, at any intensity, and less time spent sedentary, are associated with a substantially reduced risk for premature mortality. Moderate- to high-intensity exercise has been shown to increase life expectancy by 1.3 to 3.7 years.

However, although physical exercise is widely thought to prevent and treat obesity, it is not an effective method of weight reduction, especially at the recommended levels for general fitness. It appears that exercise closer to 300 min/week is needed for weight loss, as there is approximately 1000-kcal/week compensatory response that accompanies exercise. This stems from exercise-induced biological changes in appetite and postprandial satiety. Exercise may also lead to a decrease in daily physical activity. The US National Research Council recommends an exercise regimen of 60 minutes of moderate-intensity physical activity per day, or a total of 300 minutes per week, to reduce weight and to prevent weight gain. Besides the reduction in BMI, exercise also reduces intra-abdominal fat and increases lean (muscle and bone) mass. Sedentary behavior is an independent contributor to obesity and abnormal metabolic profile, despite any exercise activity being performed. Avoiding sedentary behavior helps improve cardiovascular fitness, reduces morbidity, and reduces all-cause mortality independent of weight loss. It improves mobility in older persons. It also helps improve their emotional and social state.

Increasing exercise for weight loss and maintenance of weight loss takes significant effort and time. Most patients find this difficult. It is estimated that adherence to the high-intensity exercise program is <50% at 6 months and <40% at 12 months. Successful maintenance of weight loss is seen in only 25% after 3 years, mainly due to poor adherence in the rest. Adherence is enhanced by exercising in shorter bouts – each session of exercise being around 10 minutes. This may even result in more weight loss than from long bouts of exercise.

A combination of physical activity combined with nutritional restriction is more effective in weight loss and weight loss maintenance. One review reported a 20% greater weight loss with the combination of physical activity and dietary modification than with dietary modification alone. In a study of 1079 patients in the Diabetes Prevention Program, given dietary and physical activity directions, a follow-up was performed at 1 and 3-year. Mean PA levels were 224 and 247 minutes, and weight loss goals were reached in 49% and 37% of patients at 1 and 3 years respectively. However, overall adherence to dietary restriction and combined exercise remains poor. A cross-sectional study assessing 109,000 people in the USA using Behavioral Risk Factor Surveillance found that amongst those people attempting to lose weight, only 20% can reduce their energy intake and combine it with 150 minutes of exercise per week. Further, maintenance of weight loss is extremely difficult. One study reported in 2005 found that 80% of 4000 patients in the National Weight Control Registry failed to maintain weight loss after 1 year. It is estimated that even if there is a significant weight reduction, approximately one-third, more than half, and almost the total population with obesity return to their original weight within a year, 2 years, and 5 years, respectively. Behavior therapy is therefore important.

Psychological and Behavioral Interventions [30]: Behavior therapy is a useful adjunct for weight loss and weight maintenance. Lifestyle changes, such as healthy nutrition and increased physical activity need adherence that can be sustained over time. Cognitive-behavioral therapy helps compliance with these changes. Despite the psychological intervention, the rate of long-term adherence to lifestyle modifications, however, remains low, and most patients with obesity lose only modest weight. If further weight loss is needed to improve health and well-being beyond what can be achieved with nutrition, exercise, and behavioral modification, more intensive pharmacologic, mechanical assist devices, and surgical therapeutic options must be considered.

Pharmacotherapy [31]: Pharmacological treatment is given as an adjunct to calorie restriction, increased physical activity, and behavior modification. The U.S. National Institutes of Health recommends anti-obesity drugs for individuals with BMI ≥30 or ≥27 kg/m^2 with comorbidities. The

Asia-Pacific obesity treatment guidelines recommend that anti-obesity drugs should be considered for those with BMI ≥25 or ≥23 kg/m2 who have at least one weight-related comorbidity. Treatment should be discontinued in non-responders, and in those with safety or tolerability issues – alternate drugs or other modes of treatment may then be considered in these patients. This manuscript discusses drugs currently approved in the USA for weight reduction.

Orlistat [32]: Orlistat is available over the counter in a dose of 60mg, to be used by adults and adolescents ≥12 years of age for weight loss. It is taken before each meal. It inhibits gastric and pancreatic lipases, preventing the breakdown of triglycerides into fatty acids. It is estimated that there is approximately a 30% decrease in the absorption of intestinal triglycerides, thereby reducing caloric intake and resulting in weight loss. Common side effects of orlistat include fatty/oily stools, fecal urgency, incontinence, and flatus. It may also cause small decreases in the absorption of fat-soluble (A, D, E, and K) vitamins. Supplementation with fiber-containing supplements such as psyllium, and fat-soluble vitamins usually reduce the side effects and prevent deficiencies.

Phentermine/Topiramate [33]: Phentermine/topiramate extended release is the first combination agent for the long-term management of obesity. Phentermine enhances the release of norepinephrine, dopamine, and serotonin thereby suppressing appetite. Topiramate is a gamma-aminobutyric acid agonist, glutamate antagonist, and carbonic anhydrase inhibitor, that also causes taste abnormalities. Adverse effects include dry mouth, insomnia, paresthesia, headaches, dizziness, dysgeusia (distortion of the sense of taste), and constipation. It may also cause congenital disabilities, especially when used in the first trimester of pregnancy.

Naltrexone/Bupropion [34]: Naltrexone antagonizes an opioid-dependent feedback loop that limits the effects of bupropion on the POMC neurons – increasing the activity of the POMC neurons. Bupropion is a non-selective inhibitor of the dopamine and norepinephrine transporters – this leads to a reduction in appetite and an increase in energy expenditure by increasing the activity of POMC neurons. These drugs, therefore, work synergistically. The dose is once daily initially and is gradually increased weekly – the final dose is two tablets taken twice daily by week four. Side effects include nausea, headache, dizziness, insomnia, and vomiting. Bupropion/ Naltrexone carries a black box warning regarding increased suicidal risk and ideation in young adults and hence requires careful monitoring.

Liraglutide [35]: Liraglutideis, a glucagon-like peptide 1 (GLP-1), is given in a dose of 3.0 mg subcutaneous injection daily. It acts on the hypothalamus, limbic/reward system, and cortex to increase satiety Side effects may include nausea, vomiting, diarrhea, constipation, and dyspepsia. The side effects and the need for a daily injection, leading to a high discontinuation rate – this is seen in 13% of patients.

Semaglutide [36]: Semaglutide is a GLP-1 receptor agonist that has shown a 15%-18% weight loss over 68 weeks during clinical trials. It is injected in a 2.4-mg/week subcutaneous dose and is used in conjunction with diet and exercise interventions. Indications include an initial BMI of 30 kg/m^2 or greater or a BMI of 27 kg/m^2 or greater (overweight) in the presence of at least one weight-related comorbid condition.

Setmelanotide [37]: Setmelanotide is a synthetic melanocortin 4 receptor agonist which acts in the hypothalamus to suppress appetite. It is meant for patients aged 6 years or older for weight control in obesity caused by rare genetic conditions such as proopiomelanocortin (POMC), proprotein convertase subtilisin/kexin type 1 (PCSK1), and leptin receptor (LEPR) deficiencies. Clinical trials showed that it caused a weight loss of at least 10% in 80% of patients with obesity caused by POMC or PCSK1 deficiency. It also caused weight loss in 45.5% of patients with obesity due to LEPR deficiency.

Children [38]: Besides the use of setmelanotide in syndromic obesity, Orlistat has been approved for use in children between the ages of 12-18 years. Metformin may have some impact on weight loss in children with insulin resistance but is not FDA approved. Both medicines have GI-related side effects.

Emerging Anti-obesity Drugs [39]: Tesofensine inhibits the synaptic reuptake of serotonin, noradrenaline, and dopamine. It decreases the desire for food and helps reduce weight. Besides this, several other treatment options against obesity are also being studied in clinical trials, including cannabinoid type 1 receptor blockers, amylin mimetics, peptide YY, neuropeptide Y inhibitors, fibroblast growth factor 21 analogs, and vaccines.

Lorcaserin was removed from the market in the United States in 2020 due to an increased risk of cancer.

Cellulose and citric acid (Plenity^{R}) [40]: Although available and ingested in the form of a capsule, Plenity is considered a medical device rather than a drug. This is due to its 'mechanical' mechanism of action. Non-aggregating particles created by the cellulose and citric acid in Plenity increase the volume of contents and elasticity of the stomach and small intestine - this gives a feeling of fullness. The weight loss is approximately 10% and individuals may see several inches reduced from their WC. The cellulose/citric acid hydrogel matrix is effective, safe, and well-tolerated. The particles are broken down in the large intestine and expelled into the feces.

Bariatric Surgery [41]: Bariatric surgery is an option for obese patients with a BMI > 40 or BMI > 35 with comorbidities, and who are unable to lose weight by non-surgical interventions. Bariatric surgery is effective in ensuring long-term weight loss. There are improvements noted in the co-morbidities, including a potential remission of T2DM, hypertension, and hyperlipidemia. It is also effective in improving NAFLD and nonalcoholic steatohepatitis. Other conditions helped by bariatric surgery include obstructive sleep apnea, heart failure, asthma, esophagitis, pseudotumor cerebri, osteoarthritis, thromboembolism, and urinary incontinence. These patients also notice a reduction in the medications used and they experience a better quality of life. Although the surgical mortality may be as high as 2%, the long-term survival improves. It is estimated that around 685,000 weight-loss operations are performed every year worldwide. Several techniques exist, such as laparoscopic sleeve gastrectomy (LSG), Roux-en-Y gastric bypass (RYGB), adjustable gastric banding, gastroplasty, and bilio-pancreatic diversion and duodenal switch (BPD/DS). RYBG and LSG, are the most performed – accounting for 76% of all bariatric surgeries. The adjustable gastric band is the third most common bariatric surgery.

LSG consists of a vertical resection involving the greater curvature and the fundus of the stomach. The gastric resection involves approximately 80% of the stomach and the remnant gastric area is left with a capacity of about 100 ml. The pylorus remains intact. The gastric reduction does not require a gastrointestinal anastomosis or bypass which makes it easier than RYGB or BPD/DS. LSG is associated with a significantly lower rate of major complications in the first month after surgery and has a shorter operative time. However, this type of surgery cannot be reversed because part of the stomach is permanently removed.

RYGB consists of the creation of a small gastric pouch and connecting it to the lower cut end of the middle jejunum, causing nutrients to bypass the remnant stomach and duodenum. The stomach acids and digestive enzymes from the bypassed stomach, bile from the gall bladder, and pancreatic juices drain into the duodenum and jejunum normally and are mixed with food as the biliopancreatic limb – the upper end of the cut jejunum is anastomosed to the small intestine distally. RYGB induces a significant weight loss (mean 51 kg in 18 months) and this tends to be maintained over 36 months in a follow-up study. Studies indicate that with RYGB, obese individuals lose two-thirds of their excess body weight over 2 years, and more than 50% of the excess body-weight loss is maintained at 8-9 years of follow-up. RYGB results in more weight loss than do other procedures but has more complications and is therefore performed less often when compared to LSG.

Besides the weight loss, there is also an improved metabolic profile following bariatric surgery – the mechanisms behind this are not fully understood. Both RYGB and LSG procedures are associated with an increase in the postprandial secretion of anorexigenic hormones including glucagon-like peptide-1, peptide YY, and oxyntomodulin. There is also downregulation of ghrelin with a subsequent decrease in food intake. The reduction of adipose tissue also reduces the release of several hormones and substances such as leptin, adiponectin, resistin, visfatin, and retinol-binding protein-4.

In addition, patients with RYGB surgery have increased plasma bile acid concentration due to a more rapid passage of bile acids to distal parts of the small intestine. This increase results in improved insulin sensitivity. Bariatric surgery is also accompanied by a reduction in chronic inflammation as evidenced by reduced serum C-reactive protein, tumor necrosis factor α, and interleukin 6 levels. Bariatric surgery also alters the gut microbiota. The overall gut microbial richness increases after bariatric surgery. There are also alterations of the gut microbiome bacterial population, and this is characterized by increased Gammaproteobacteria and Akkermansia muciniphila and a decreased ratio of Firmicutes to Bacteroidetes. And finally, RYGB may be involved in the downregulation of genes involved in metabolic pathways and inflammatory responses. Bariatric surgery has also become a therapeutic option in adolescents The number of these operations is increasing in Europe and the United States and in other countries. According to the pediatric metabolic and bariatric surgery guidelines, vertical sleeve gastrectomy is the most recommended surgical procedure in adolescents with severe obesity, due to its technical ease, good results, and fewer complications.

Complications of RYGB surgery include stricture at the gastrojejunal anastomosis site (3%-7%), and ulceration on the jejunal mucosa (1%-16%). Gastro-gastric fistula between the surgically created pouch and excluded remnant stomach occurs in 1%-2%. Cholelithiasis and choledocholithidasis is also seen, the former being more common and may occur in 32% to 42% of patients. Small bowel obstruction may occur in between 1.5% to 5% while early dumping syndrome is diagnosed in about 13% of patients, usually young females. Late dumping syndrome is also reported. Most of these complications are seen with RYGB. It is estimated that about 15% of the patients may require an additional operative procedure for complications following the initial surgery. Nutritional deficiencies are also common. B12 is low in 30% to 35%, iron in 30% to 50%, and folate in 6% to 35% of patients. Thiamine deficiency has also been reported. Low calcium and vitamin D levels are also common following surgery. Low levels of zinc, copper, selenium and vitamin C have also been reported. Nutritional deficiencies can be prevented or treated with supplementation. A weight regain of about 14% of the lost weight may occur at 5 years post-surgery. However, this weight gain is small compared with the initial weight loss. Because of frequent complications associated with bariatric surgery, an extensive preoperative evaluation of surgical risks is required and is usually routinely done. Relative contraindications include non-understanding and non-compliant patients (surgery involves significant post-surgery lifestyle changes, office visits, and exercise programs, etc.), Crohn's disease, psychosocial disorders, and drug or alcohol use disorders. Bariatric surgery is also not performed during pregnancy. Other contraindications include end-stage renal disease, unstable coronary artery disease, severe heart failure, cirrhosis, portal hypertension, and active cancer.

Other surgical procedures [42]: Experiments are showing that gastric pacing may result in weight loss. In one study, with a pacing device laparoscopically implanted, obese patients had a mean weight loss of about 25% during a 3-year follow-up. Other experimental surgical procedures include visceral fat removal, omentectomy, subcutaneous fat panniculectomy, and large-volume subcutaneous fat liposuction. Poor results have been noted with procedures such as jaw wiring, vagotomy, and insertion of a gastric balloon or a gastric wrap and these should not be performed.

Fecal Microbiota Transplantation (FMT) [43]: Gut microbiota plays a role in several biological pathways that control food intake and regulate energy balance. FMT has shown encouraging results in weight loss and its maintenance. When fecal material from human twins discordant for obesity was transplanted into germ-free mice, it was noted that the mice with obese individuals' microbiota developed obesity, while those with healthy individuals' microbiota remained lean. FMT causes a shift towards a leaner phenotype by increasing butyrate-producing bacteria and Bacteroidetes in the gut. Further randomized placebo-controlled trials are needed to confirm these benefits and to address safety concerns.

Probiotics [44]: Probiotics alter the gut microbiota and may therefore help in combatting obesity. Probiotics can restore "lean gut microbiota. Studies have shown that ingestion of strains of Lactobacillus and Bifidobacterium, alone or in combination, result in the reduction of body weight, BMI, WC, and body fat. However, the reductions are small. A meta-analysis of 14 trials in 881 adults, showed that probiotics promote a mean loss of 0.54 kg in adults. No reductions were seen in children or infants. In a systematic review and meta-analysis of randomized controlled trials, it has been confirmed that the reductions are small. Probiotics result in a reduction in body weight by 0.6 kg, body

mass index by 0.27 kg/m2, and fat percentage by 0.6%, when compared to placebo. A more recent systematic review and meta-analysis, which included 19 randomized trials in 1,412 participants, reported no effect on body weight or BMI, and only a small reduction in waist circumference (0.82 cm). Although the common probiotic species are safe and the data on their effectiveness in preventing or treating obesity appears promising, human trials show only limited reductions. Obviously, more data is needed.

Fat Substitutes [45]: Olestra is a fat substitute that is not digested or absorbed in the human body. It has no caloric value. It works by interfering with the absorption of other lipophilic substances and thereby helps the individual to lose weight. It is used in foods like potato chips. Adverse effects reported include bloating, flatulence, and loose stools. Because of the danger of decreased absorption of fat-soluble vitamins, vitamin supplements should be taken along with olestra. Although certain kinds of margarine (containing stanols and sterols) can block cholesterol absorption in the intestine, they only work to reduce LDL cholesterol and have no effect on body weight.

Water Intake [46]: Drinking water before a meal helps aid weight loss in overweight and obese middle-aged and in older adults on a hypocaloric diet. The additional weight loss is reported to be 44% more in adults aged 55-75 years and with a BMI of 25-40 kg/m^2 when compared to individuals on a hypocaloric diet without pre-meal water consumption. Drinking cold water also helps overweight children to lose weight. Water increases the resting energy expenditure. It is estimated that a loss of 1.2 kg per year can be induced by drinking 10ml/kg of cold water daily.

Goals [47]: Most individuals should aim at reducing 1-2 lb. per week, according to guidelines released in 2013 by the American College of Cardiology, the American Heart Association, and The Obesity Society. Even a weight loss of 1%-2% improves the metabolic profile and provides substantial health benefits with obesity-related comorbidities. The practical goal should be to lose 5% or more of the bodyweight in 12 weeks. Failure to achieve this should result in a re-evaluation of the treatment strategies.

Follow-Up/Compliance [48]: Obesity is a chronic disease, and besides not becoming overweight/obese, and losing excess weight, the long-term aim is also to avoid regaining lost weight. Diet, exercise habits and behavioral patterns initiated for the treatment of obesity must be maintained. A follow-up and continued supervision are therefore usually necessary. This will also help in monitoring and treating co-morbidities.

3. CONCLUSION

Overweight and obesity, defined as excess body weight for height, have genetic, behavioral, socioeconomic, and environmental origins. The global prevalence of obesity has nearly tripled since 1975 and continues to grow at an exponential rate. It is growing in all ages, populations, and ethnic groups, irrespective of the socioeconomic status of the individuals. Obesity has become the number one lifestyle-related risk factor for premature death. A 5% to 10% weight loss can significantly improve health, quality of life, and longevity. Data from the Nurses' Health Study and the Health Professionals Follow-up Study estimates that maintaining normal body weight and adherence to the other four healthy lifestyles increases the lifespan at age 50 by 14 years in females and 12.2 years in males when compared with those with zero low-risk factors. The increase in obesity all over the world, the difficulty in losing and maintaining an ideal weight, the huge impact on major diseases, and its major effect on increasing premature death, make obesity a dangerous health adversary.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Biography of author(s)

Shashi K. Agarwal, MD
Center for Contemporary and Complementary Cardiology, 2227 US Highway 1, Suite 309, North Brunswick, NJ 08902, USA.

Research and Academic Experience: Published several hundred scientific articles both nationally and internationally.

Research Area:
Cardiovascular Diseases
Integrative Medicine

Number of Published papers: >100

Special Award: Presented several abstracts, posters and talks on medical subjects both nationally and internationally.

Any other remarkable point(s): Reviewer for several respectable scientific journals

Chapter 2
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Study on Heart rate Variability and Heart Rate under General Anesthesia in Rats of Both Sexes

P. Svorc [a,b*], D. Petrasova [c] and P. Svorc Jr. [b]

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ABSTRACT

Objectives: It is known that general anesthesia weakens autonomic function and baroreflex control. Intravenous anesthetics may have different qualitative and quantitative effects on the peripheral autonomic nervous system (ANS) and, can thus, alter the activity of sympathetic or parasympathetic divisions of the ANS. Presently, there are relatively little data regarding sex differences in ANS activity or sex differences in ANS activities during anesthesia.
Aims: The primary objective of this study was to determine the impact of spectral powers of heart rate variability (HRV) on changes in heart rate (HR), total spectral power of HRV, and low-frequency (LF)/high-frequncy (HF) ratio in healthy, sexually mature rats of both sexes spontaneously breathing under zoletil anesthesia in the light (inactive) and the dark (active) period of their regimen day.
Materials and Methods: Experiments were performed using male and female zoletil-anesthetized (30 mg/kg [intraperitoneal]) Wistar rats after a four-week adaptation to a light-dark (LD) cycle (12h:12h). The animals were divided into four experimental groups (n=20 each) according to sex and light period. HR, spectral powers of HRV (very low frequency, LF, and HF), as well as LF/HF ratio were evaluated 20 min after administration of anesthesia.
Results and Conclusions: Zoletil exerted a tachycardic effect in both sexes and in both light periods of the regimen day. In females, the autonomic nervous system was involved in HR changes in both light periods, while in males, HR exhibited no dependence on autonomic nervous system activity; as such, the authors speculate that it was predominantly determined by other factors. In females, HRV was determined by sympathetic and baroreflex activity in both light periods, while in males, HRV was determined by parasympathetic activity. LF significantly influenced LF/HF ratio in females, but not in males, while the effect of HF on the LF/HF ratio was negligible in both sexes and in both light periods.

Keywords: Zoletil anesthesia; heart rate variability; chronobiology; sex differences; rats.

1. INTRODUCTION

General anesthesia is known to potentiate parasympathetic activity and weaken sympathetic and baroreflex activity [1]. These effects should be avoided as much as possible because it limits a subject's ability to respond to physiological perturbations during surgery [2]. Therefore, the choice of anesthetic is very important and, no less important, is to know to what extent the tone of the autonomic nervous system (ANS) is affected under general anesthesia and the potential impact on the cardiovascular system, which is primarily under ANS control.

Heart rate (HR) is the result of mutual interactions between vagal and sympathetic activity. In this regard, the use of the HR variability (HRV) method can be an effective tool in evaluating autonomic heart control, in which changes in HRV are a useful indicator of tendencies of heart rhythm disorders. The study by Yamamoto et al. [3] points to changes in heart rate variability during exercise in men. Also the study of Tsuji et al. [4] emphasizes that reduced HRV can be an important indicator of risk

[a] *Department of Physiology, Medical Faculty Safarik's University, Kosice, Slovak Republic.*
[b] *Department of Physiology and Pathophysiology, Medical Faculty Ostrava University, Ostrava, Czech Republic.*
[c] *Laboratory of Research Bio-models, Medical Faculty Safarik's University, Kosice, Slovak Republic.*
**Corresponding author: E-mail: pavol.svorc@upjs.sk;*

for new cardiac events in a large community-based population. In cardiology, prior studies have documented adverse prognostic implications of reduced heart rate variability in patients after myocardial infarction and with other pathological conditions [5] an association between reduced heart rate variability and risk for all-cause mortality in elderly original participants in the Framingham Heart Study. The relation of altered heart rate variability to risk for cardiac events has not been studied in the general population [6].

However, there are currently relatively little data describing changes in ANS activity during general anesthesia as well as possible sex differences. A concept that sex-based variations should also be taken into account has been proposed because different cardiovascular regulation has been described in females [7]. Koresh et al. [8] referred to this phenomenon, in which male rats exhibit significantly higher it does not affect vital functions. However, the unanswered question is whether there are sex differences depending on the time of day general anesthesia is administered. The primary objective of this study was to determine the impact of spectral powers of HRV on changes in HR, on total spectral power of HRV and on low-frequency (LF)/high-frequency (HF) ratio in healthy, sexually mature rats of both sexes, spontaneously breathing under zoletil anesthesia in the light (inactive) and the dark (active) period of their regimen day.

2. MATERIALS AND METHODS

The present study conformed to the Guide for the Care and Use of heart rhythm and lower HRV parameters than females in the both active as well as non-active periods of the rat regimen day. However, there is no supportive evidence of changes in HRV in rats over a 24 h period.

Zoletil anesthesia is used by veterinarians mainly in domestic animals and those living in the wild, but is not commonly used in experiments. It is reported that this type of anesthesia is suitable because Laboratory Animals published by the United States National Institutes of Health (publication number 85-23, revised 1996). The study protocol was approved by the Ethics Committee of the Medical Faculty of Safarik University (Kosice, Slovak Republic; permission number 2/05 and permission number ŠVPS SR: Ro-4234/15-221).

The experiments were performed using zoletil-anesthetized (Zoletil, 30 mg/kg, Virbac, France) Wistar rats (mean weight, 340 ± 40 g, 3-4 months of age) and after adaptation to a light-dark (LD) cycle (12h light:12h dark) for four weeks. The rats were fed a standard pellet diet, with ad libitum access to food and water. The depth of anesthesia was assessed according to whether a painful stimulus caused noticeable motor movements (minimal limb movement, muscle tension change) or cardiovascular responses, such as change in HR or onset of heart rhythm disorders. Animal handling was performed by professional staff at the menagerie. Animals were randomly divided into four experimental groups (n = 20 each) according to sex and light conditions.

HRV was analyzed using the ID Instruments computer system for biopotential recording from an average of 220 heart cycles, 20 min after administration of anesthesia. The focus was on the assessment of the impact of the spectral powers of HRV on changes in HR, total spectral power of HRV, and LF/HF ratio.

Correlations were calculated as a correlation coefficients, in which the coefficient was statistically significant in the range of -0.4 to -1, and from +0.4 to +1. The experiments were conducted throughout the year and the results were averaged independent of season and, in females, independent of estral cycle.

3. RESULTS AND DISCUSSION

3.1 HR

In the present study, we focused on sex differences in HR during the active and nonactive periods of the rat regimen day (what may be considered parallel to circadian rhythm). In females, LD differences in HR (light 441 ± 61 versus [vs.] dark 446 ± 29 beats/min) were eliminated, unlike in males (light 415

± 24 vs. dark 482 ± 19 beats/ min), in which they differed significantly ($p < 0.001$). Significant sex differences ($p < 0.001$) were observed only in the dark period with higher values in males.

Because HR in non-anesthetized rats exhibits circadian variation; in the dark period, it varies from 347 to 363 beats/min and, in the light period from 309 to 321 beats/min. Molcan et al. [9] reported that recorded HR was increased in both sexes and in both light periods. In females, LD differences were eliminated (possibly modified) and, in males, LD differences were preserved. Based on this result, we speculate that males probably respond diffferently to general anesthesia than females with regard to maintenance of circadian rhythms in HR.

Based on our results, however, the question emerged that if tachycardia in both sexes and both light periods of the regimen day was detected during a period dominated by parasympathetic activity, what is the impact of spectral powers and the total spectral power of HRV on changes in HR? To answer this question, we used correlation coefficients calculated for the spectral forces of VLF, LF, HF, and HR (Table 1), which revealed sex differences. In females, changes in HR were significantly dependent on ANS activity in both light periods, in which parasympathetic activity was more prominent. A negative correlation could, in part, explain the increase in HR. Similar results were also obtained for total spectral power of HRV and HR (Table 1). In males, changes in HR demonstrated no dependence on ANS activity in both light periods; therefore, we assume that it was predominantly determined by other factors.

Table 1. Correlation coefficients for spectral powers of HRV, total spectral power of HRV and HR

HR (beats/min – ms2)	Female, light	Female, dark	Male, light	Male, dark
HF - HR	r=- 0.76	r=- 0.54	r=- 0.23	r=- 0.06
LF - HR	r=- 0.72	r=- 0.51	r=- 0.27	r= 0.12
VLF - HR	r=- 0.59	r=- 0.55	r=- 0.34	r= 0.13
Total power of HRV - HR	r=- 0.83	r=- 0.54	r=- 0.29	r=- 0.06

Bolded values indicate a statistically significant correlation. HR: Heart rate; VLF: Very-low frequency; LF: Low-frequency; HF: High-frequency; HRV: Heart rate variability

Table 2. Correlation coefficients for spectral powers of HRV and total power of HRV

ms2	Female, light	Female, dark	Male, light	Male, dark
HF, total power of HRV	r=0.73	r=0.90	r=0.96	r=0.97
LF, total power of HRV	r=0.97	r=0.99	r=0,59	r=0.95
VLF, total power of HRV	r=0.92	r=0.98	r=0.58	r=0.95

Bolded values indicate a statistically significant correlation. VLF: Very-low frequency; LF: Low-frequency; HF: High-frequency; HRV: Heart rate variability

Thus, a paradox emerges in which, on one hand, there is clear evidence of increased parasympathetic activity and, on the other, increased HR. This paradox has also been described in dogs under inhalation anesthesia, in which it is assumed that different anesthetics have a different impact on vagolytic activity [10]. Such an explanation is described in the review by Yuan and Silberstein [11] regarding the vagal nerve and its stimulation, which is currently undergoing many trials to investigate their potential for various clinical disorders. Although this is an example of bronchodilation after stimulation of the vagal nerve, the authors allude to experimental work in guinea pigs, in which low- voltage stimulation of the vagal nerve caused a moderate increase in plasma levels of adrenaline and noradrenaline.

3.2 Total Power of HRV

Total spectral power of HRV reflects overall autonomic activity. The total spectral power of HRV demonstrated sex differences in both light periods. However, what is the proportion of VLF, LF and HF in the total power of HRV? The changes in HRV in both sexes depended on all spectral powers of

HRV in both light periods. It appears that in females, the proportion of VLF and LF are more pronounced than HF, while in males, HF predominates in both light periods (Table 2).

Table 3. Correlation coefficients for LF and HF, and LF/HF ratio

	Female		Male	
	Light	**Dark**	**Light**	**Dark**
LF (ms2), LF/HF	r= 0.90	r= 0.99	r= 0.39	r= 0.19
HF (ms2), LF/HF	r= 0.34	r= 0.29	r=- 0.08	r=- 0.11

Bolded values indicate a statistically significant correlation. LF: Low frequency; HF: High frequency; LF/HF: Ratio between LF and HF.

Table 4. Summarized results and conclusions

	Females	**Males**
Heart rate	Tachycardia, loss of LD differences	Tachycardia, maintenance of LD differences
Effect of HRV on HR		
Light	Yes	No
Dark	Yes	No
Impact of spectral powers on		
HRV	LF and VLF prevail	HF prevails
Light	LF and VLF prevail	HF prevails
Dark		
Impact of LF, HF on the ratio		
LF/HF	LF prevails, HF without effect	LF and HF without effect
Light	LF prevails, HF without effect	LF and HF without effect
Dark		

HRV: Heart rate variability; LD: Light-dark; LF: Low frequency; HF: High frequency; VLF: Very low frequency; LF/HF: Ratio between LF and HF.

3.3 LF/HF Ratio

The LF/HF ratio was originally based on 24 h recording, in which the aim was to estimate the ratio between sympathetic (LF) and parasympathetic (HF) activity; however, there is a disagreement with the LF component. Some studies have suggested that LF is a quantitative indicator of sympathetic modulations; in other studies, LF appears to reflect both sympathetic and parasympathetic activity. Thus, any changes in HR, such as tachycardia, may be result of the preservation of sympathetic and decreased parasympathetic activity, or increased sympathetic and preservation of parasympathetic activity or, simultaneously, increased sympathetic and decreased parasympathetic activity.

The question is to what extent is the LF/HF ratio influenced by the spectral powers LF and HF in our model, although parasympathetic activity is higher in both sexes and in both light periods? The results of our study show that dependence of the LF/HF ratio on LF and HF also depends on sex and on the light periods. LF significantly affected the LF/HF ratio in females but not in males, while the effect of HF on the LF/HF ratio was negligible in both sexes and in both light periods (Table 3). However, these conclusions, in the light of these doubts, should be interpreted with caution.

4. CONCLUSIONS

Zoletil has a tachycardic effect in both sexes and in both light periods of the rat regimen day. In females, ANS is involved in HR changes in both light periods, while in males, HR exhibited no dependence on ANS activity. Therefore, we speculate that it was predominantly determined by other factors. In females, HRV was determined by sympathetic and baroreflex activity in both light periods, while in males, HRV was determined by parasympathetic activity. LF significantly influenced the LF/HF ratio in females, but not in males, while the effect of HF on the LF/HF ratio was negligible in both sexes and in both light periods. Our conclusions are summarized in the table below (Table 4).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

Prof. P. Svorc PhD.
Department of Physiology, Medical Faculty Safarik's University, Kosice, Slovak Republic and Department of Physiology and Pathophysiology, Medical Faculty Ostrava University, Ostrava, Czech Republic.

He was born in 1958. He graduated from the Faculty of Science, General Biology in Kosice. Currently he works at the Faculty of Medicine of the P. J. Safarik's University, Department of Physiology in Kosice, Slovak Republic as a professor in the field of Normal and Pathological Physiology as well as he is head of the Department of Physiology and Pathophysiology, Medical Faculty, University of Ostrava, Czech Republic. He is member of International Society for Chronobiology, Slovak Physiological Society as committee member and Slovak Medical Society. His professional orientation is chronobiology of the cardiovascular and autonomic nervous system and currently he analyzes the effect of general anesthesia on these systems depending on sex and light-dark cycle. He also studies the effects of apneic episodes and reoxygenation on electrophysiological parameters and activities of the autonomic nervous system. He was the coordinator as well as coordinator of 9 scientific projects and has a rich publishing activity. He is the author of two monographs and the author of 253 scientific and professional works in domestic as well as foreign journals. He is cited 263 times, of this 190 citations in foreign publications registered in citation indexes of the Web of Science and the SCOPUS database.

Dr. P. Svorc Jr. PhD.
Department of Physiology and Pathophysiology, Medical Faculty Ostrava University, Ostrava, Czech Republic.

He was born in 1981. He graduated from the Faculty of Science, specialization in special ecology and physiology of individuals and populations. He works at the Faculty of Medicine of the Ostrava University, Department of Physiology and Pathophysiology in Ostrava, Czech Republic as a assistant professor in the field of Normal and Pathological Physiology and currently holds the position of Deputy Head of this department. He is a member of Slovak Physiological Society and Slovak Medical Society. He specializes in two directions. In vivo experiments in rats in cooperation with the P.J.Safarik University in Košice, dealing in chronobiology of the cardiovascular and autonomic nervous system research, and currently he analyzes the effect of general anesthesia on these systems depending on sex and light-dark cycle. The second field of study is in sensory deprivation with a focus on the cardiovascular, autonomic, ocular and endocrine system using the REST method using long exposure of darkness to the human subject. He was the coordinator of 4 scientific projects focusing on Dark therapy.

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A Brief Overview on Stem Cell and Toxicology

Doaa Abdel Wahab El Morsi [a*#]

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ABSTRACT

Undifferentiated cells were initially utilized as a part of bone marrow transplants in 1956. Since the late 1990's when embryonic undifferentiated cells were found in people, the advances in undeveloped cell science have been rapid. However, a significant part of the work in the field of stem cell science is centered around comprehension ordinary advancement and disarranges of the human body and for toxicology screening (testing new medications) [1].
Multipotent stem cells could possibly help in the treatment of tissue that can't recover itself. This would be of advantage for spinal cord injuries and injuries to tactile organs, which also have likewise constrained repair abilities. Once more, research on these particular cell types can aid scientists in discovering treatment choices for different issue, not necessarily just using stem cells themselves to treat the disorder [2]. This chapter aims to introduce stem cell in toxicology, a new era of in vitro toxicology that provides effective and efficient alternatives to classic toxicology tests.

Keywords: Stem cell; toxicology; bone marrow transplants; multipotent stem cells; adipose tissue

1. BACKGROUND

Stem cells are undifferentiated natural cells that can differentiate into particular cells and can divide (through mitosis) to deliver more stem cells. They are found in multicellular living beings. In warm blooded animals, there are two types of stem cells: embryonic cells, which are disengaged from the internal mass of blastocysts, and adult cells, which are found in different tissues [3].

Stem cells in adult act as a repair framework for the body, renewing tissues. In embryo, stem cells can separate into all specific cells-ectoderm, endoderm and mesoderm. Additionally, keep up the typical turnover of regenerative organs, for example, blood, skin, or intestinal tissues [4].

There are four known wellsprings of grown-up stem cells in people [5]:

i. Bone marrow (commonly the femur or iliac crest).
ii. Adipose tissue (lipid cells), which requires extraction by liposuction.
iii. Blood, where blood is drawn and went through a machine that concentrates the stem cells and returns different parts of the blood to the donor.
iv. Stem cells can likewise be taken from umbilical cord blood soon after birth.

Occasionally, stem cells are used as a part of therapeutic treatment (e.g., bone marrow transplantation). They can now be isolated into particular cell sorts with characteristics dependable with cells of various tissues, for example, muscles or nerves. Embryonic cell lines and autologous embryonic cells created have additionally been proposed as promising possibility for future therapies [6].

[#] Professor, Member of Medical Education Department;
[a] Departments of Forensic Medicine and Clinical Toxicology and Medical Education, Mansoura Faculty of Medicine, Mansoura University, Egypt.
**Corresponding author: E-mail: dr.doaa2014@outlook.com;*

The classical properties of stem cells:

i. Self- reestablishment: the capacity to experience various cycles of cell division while keeping up the undifferentiated state.
ii. Potency: the ability to separate into specialized cell types. This requires stem cells to be either totipotent or pluripotent - to have the capacity to offer ascent to any full-grown cell, although multipotent or unipotent progenitor cells are sometimes referred to as stem cells. Aside from this, it is said that stem cells are managed in a feedback system.

2. HUMAN STEM CELLS AS A NEW TOOL IN TOXICOLOGY

Even though the stem cells could be used to assess the potential of any toxin/toxicant; it may be insightful examine some theoretical and practical matters related to our current state of understanding basic stem cell biology and their roles in normal development and any stem cell–related disease [7].

In spite the concept of stem cells has long been for quite some time a center of scholarly examination in the fields of embryology, developmental biology, plant biology, and cancer research. The detachment of human embryonic immature stem cells has brought stem cells into investigative/therapeutic groups. The hypothetical plausibility of applying human immature microorganisms for enhancing numerous human illnesses appeared to be conceivable before achieving any crucial comprehension of the perplexing variables which control an undeveloped cell's behavior [8].

However, right now of attempting to distinguish the reasonable possibilities about the stem cells, endeavors are being made to apply immature cells for wide various applications, for example, for testing toxicity. To date, stem cells have been thought to be utilized for (1) essential comprehension of how stem cells manage the genome and its cell behavior (cell multiplication in a symmetrical or asymmetrical form, separation, apoptosis, and so forth.), (2) regenerative medication or stem cell treatment, (3) drug discovery, (4) study poisoning of pharmaceuticals and stem cell treatment, (5) genetic treatment, and the role in stem cell-derived sicknesses and in the aging procedure. It is difficult to look at the possibilities and a limitation of all these potential applications [9].

Similarly essential, our comprehension of the pathogenesis of numerous human illnesses is not known. Thus, attempting to foresee the pathogenesis of a human disease (or how to counteract or cure the infection) by utilizing information, got from deficient toxicity testing, just depicts the insufficiencies of our present condition of knowledge [10].

2.1 Stem Cell Biology

At the time of fertilization, the fertilized egg (''totipotent'' stem cell), which contains genome of individual, should now confront the complex interactions of the external environment's effect on the pregnant female, of her genetic background, her nutritional status and diet, lifestyle behavior, mental stress level, and of any medical treatments. From this dynamic association, the implantation of the totipotent stem cell begins a link of occasions to confer this cell to shape the embryonic or pluripotent stem cell [11,12].

As these cells show up in the blastocyst, they, change the microenvironment leading to a further restriction of these pluripotent stem cells to become, multipotent cells that are committed to specific organ types. At last, progenitor stem cells, such as those seen in the lymphohematopoietic system appear. Transit-amplifying cells, or those derived from a stem cell are, somehow, committed to a life span. The terminally separated cells, such as a red blood cells or a lens cell, is limited from proliferating [13,14].

Operationally, a stem cell is characterized as a cell that can isolate either symmetrically or asymmetrically after being given an appropriate external signal. Despite: the mechanism for controlling whether a stem cell divides, symmetrically or asymmetrically it is still unknown; some growth conditions do influence this cellular decision, including specific growth factors or O2 levels and antioxidants [15,16].

2.2 Nature of Normal Development, and Health

In utilizing human stem cells for potential poisoning; one must remember the dynamic nature of stem cell biology *in vivo* so that any *in vitro* result will not be misinterpreted to potential toxicities *in vivo*. The developmental process of the cells, involving an undifferentiated totipotent stem cell, with the total genetic information to produce an individual, includes numerous interactions and organization of molecules, biochemical reactions, organelle formation within cells, that regulate tissues and organisms, which form organ systems to create human being. All this occurs via unique environmental (physical, chemical, biological) interactions with specific genes [3].

2.3 Toxicity End Points at the Cell Level

At the cell level, exposure of cells to physical, chemical, biological agents, and even psychological stress will be translated into some response: The cancer problem is not a cell problem; rather it is a problem of cell interaction. But we must also remember that the integration of normal cells with the welfare of the whole organism is brought about by molecular messages acting on molecular receptors [9].

It appears that three outcomes to this exposure will be (1) mutation, caused by either an error in DNA repair or by an error of DNA replication (genotoxicity), (2) cell death by necrosis or apoptosis (cytotoxicity), and (3) altered gene expression at the transcriptional, translational, or posttranslational levels (epigenetic toxicity). For genotoxicity and cytotoxicity assessments; various molecular and *in vitro* assays have been used and till now no consensus has been achieved [6].

The consequences of these assays were (1) not reliable, (2) did not assess what they were assumed to measure, (3) extracted from cell types not relevant to the human system, (4) derived from the wrong conditions for measuring toxicities that occur *in vivo* at different conditions, and (5) in many cases, were misinterpreted. *Invitro* assays carry many limitations to measure ‘‘epigenetic toxicants’’ or, as some refer to them as ‘‘non genotoxicants’’. The endogenous - and exogenous induced intracellular signaling mechanisms were evolutionarily designed to regulate gene expression [17].

Genetic or environmentally induced abnormal expression of genes is the basis of epigenetic toxicity. However, the recent microarray technologies, still leave much to be desired. Clearly, patterns of altered gene expression from exposed or diseased tissues can be reproduced and even have useful applications. However, extracting molecular expressed genes from normal, treated, or diseased tissue is extracting messages from a heterogeneous mixture of cell types [18].

Moreover, some cells are stressed; others are dying of apoptosis, or mutated, and others are invading cells. So, the total message examined is the result of expression of all these types of cells. This point has been studied as they titled as: ‘‘Traditional microarrays’’; which can neither distinguish between variations in gene expression resulting from an actual physiological change versus differences in cell-type frequency nor identify the contributions of different cell types to the total measured gene expression [19].

In addition, ionizing and ultraviolet light radiation can produce chromosome and gene mutation, respectively, they can, induce oxidative stress, which, can altered gene expression. In effect, they can have epigenetic effects. It should also be stated that, at toxic doses, any agent (radiation, chemicals, biological) can be an indirect epigenetic toxicant, so the released substances from cell killing can act to stimulate the surviving cells to wound healing [20].

In vivo toxicity involves the tissue’s response to the exogenous chemical or therapeutic stem cell, as well as to the consequences of the chemical or stem cell products on the immune system. As any substance (chemical or stem cell product) enters the body, it will directly or indirectly interact with (1) the three types of cells (adult stem cells, transit amplifying cells, and the terminally differentiated cells) in the tissue and (2) with cells of the immune system. In both cases, intracellular signaling occurs. In the case of the cells of the immune system, various bioactive factors are secreted that can interact with the epithelial/endothelial cells that might have also been ‘‘primed’’ by the same toxicant [11].

Study of how chemicals or stem cell products, could induce specific intracellular signaling to trigger epigenetic changes *in vivo*, will be a challenge. One of the characteristic properties of this class of agents is also to inhibit apoptosis, in that tumor promoting chemicals [9].

In transplantation studies, it is noticed alteration in the apoptotic rate of cells. On contrary, if the agent causes upregulation of either cells of the immune system or cells of the affected tissues, cells could differentiate or go to apoptosis. So; the stem cell itself will be subject to *in vivo* endogenous factors that could lead to symmetrical division (proliferation), induced asymmetrical division (differentiation, apoptosis), or senescence [7]. These cells also can affect the immune response by secreted factors that could illicit a cellular response in the targeted tissue. This type of unexpected response has been reported [19,20].

2.4 The Limitations of Current *In vitro* Assays

To use *in vitro* mammalian cells, most assays have used either immortalized or cancer cells. Moreover, without an immunological or dynamic physiological environment, these abnormal could not predict how any of the three classes of normal human cells (the adult stem cells, the progenitor or terminally differentiated cells) would react *In vivo* [5].

The different cell types (the few stem cells, the many progenitors and differentiated cells) is physiologically different. So, there will be a differential response despite the toxic potential of any agent. In fact, there is proof that these stem cells express drug transporter genes that pump out various classes of toxic chemicals. This might be one potential limitation for the use of pure stem cell populations to test for genotoxic chemicals [21,22].

3. CONCLUSION

It could be concluded that the stem cells could be used to assess the potential of any toxin/toxicant. It may be instructive to examine some theoretical and practical matters related to understanding basic stem cell biology and their roles in normal development and any stem cell–related disease using stem cells.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Biography of author(s)

Professor Doaa Abdel Wahab El Morsi, MSc, MD.
Departments of Forensic Medicine and Clinical Toxicology and Medical Education, Mansoura Faculty of Medicine, Mansoura University, Egypt.

Research and Academic Experience: She has More than 20 years' experience in teaching forensic medicine and clinical toxicology for under and postgraduates in Egypt and Libya.
Also teaching ethics for Mansoura – Manchester students and coordinator of elective course "First Aid in Medicine" in Mansoura – Manchester Program.
In addition to managing acute intoxicated patients with different poisoning, now I'm the supervisor of Toxicology Unit – at Mansoura Emergency Hospital.
She organized the 1st Scientific Day of Mansoura Toxicology Unit.

Research Area: Her Research Area includes Environmental toxicology, Nanotoxicology, Drug of Abuse, Genetics.

Number of Published papers: She has 30 Research articles.

Special Award: Best Doctorate Thesis all over the Mansoura Faculty of Medicine – Mansoura University - 20/5/2007.

- Contribution and Participation in the training course "Junior Doctor: The Right Start". Dakahlia Medical Syndicate. 20 – 21 February 2020 (10 CME).
- Scientific Publication Prize from Mansoura University, 2019.

Any other remarkable point(s): She is a Instructor in European and Egyptian Resuscitation Council. She got other qualifications such as Master of Health Profession Education, Total Quality management Diploma and Healthcare and Hospital Management Diploma.

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Chapter 4
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Laparoscopic Management of Anaphylactic Shock Following Abdominal Trauma Revealing Intravascular Rupture of Cystic Echinococcosis (A Case Report and Brief Literature Review)

Haif Assia [a,b*]

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ABSTRACT

Accidental traumatic intraperitoneal rupture resulting by anaphylactic shock is the well-known complication, unlike to the anaphylactic shock caused by the intravascular spread of cyst contents which is rare. One of the common complications of hydatid disease is cyst rupture after trauma or spontaneous rupture. This article reports an unusual case description about 13 years old boy, following a trauma admitted to the public hospital with few signs. Ultrasonography demonstrated a hydatid cyst in the liver with the drainage of the cyst contents into the right hepatic vein without free peritoneal fluid. A shortly afterwards anaphylactic shock developed and the patient transferred to our emergency. An abdominal laparoscopic examination, after stabilization of patient confirmed rupture of the cyst, which was treated laparoscopically. Additionally, the patient received albendazole. The aim is to evaluate the feasibility and safety of laparoscopy in the management of complicated Hepatic echinococcosis, duration of surgery and hospital stay. We think that laparoscopic surgery can be recommended in this kind of clinical presentation of Echinococcosis.

Keywords: Hydatid cyst; rupture; anaphylactic shock; vessels; laparoscopic.

1. INTRODUCTION

Echinococcosis is common term currently used according to an International consensus on terminology, it designates the disease (s) related to infection with parasites of the Echinococcus (E) genus [1]. The disease continues to be a major public health issue in many regions of the world especially in the Maghreb countries mostly Algeria which are highly endemic [2].

Hydatid cyst may develop in any body organ, it may remain clinically silent for many years and are often an incidental finding on ultrasonography performed for unrelated reasons [3]. One of the common complications of hydatid disease is cyst rupture after trauma or spontaneous rupture [4]. It may rupture into the peritoneal cavity, rupture to the gastrointestinal tract, rupture of a cyst directly into a blood vessel, caused most probably by constant pressure of the cyst's wall on the blood vessel [5].

Accidental traumatic intraperitoneal rupture resulting by anaphylactic shock is the well-known complication, unlike to the anaphylactic shock caused by the intravascular spread of cyst contents which is a rare and a review of the literature shows few case reports.

[a] *Department of Pediatric Surgery, University Hospital Center of SETIF, Algeria.*
[b] *Laboratory of Applied Pediatric Surgery, Ferhat Abbas Setif University 1, Algeria.*
**Corresponding author: E-mail: haif_chu.setif@yahoo.fr, a.haif@univ-setif.dz;*

2. CASE PRESENTATION

A 13-year-old boy assaulted by kick in the abdomen by his young brother and admitted to the public hospital with less abdominal pain. Ultrasonography demonstrated a cyst in the liver without free peritoneal fluid.

After a few hours, the general condition of the patient progressively worsened with vomiting, tachycardia, oliguria, hypotension, and respiration was rapid and superficial leading to dyspnea.

The patient was oriented in our emergency: Clinical examination showed generalized tenderness without any palpable mass and increasingly severe abdominal pain.

Laboratory examination revealed severe leukocytosis. A second ultrasonography showed the drainage of the cyst contents into the right hepatic vein (Fig. 1), but there was no free fluid in the abdomen.

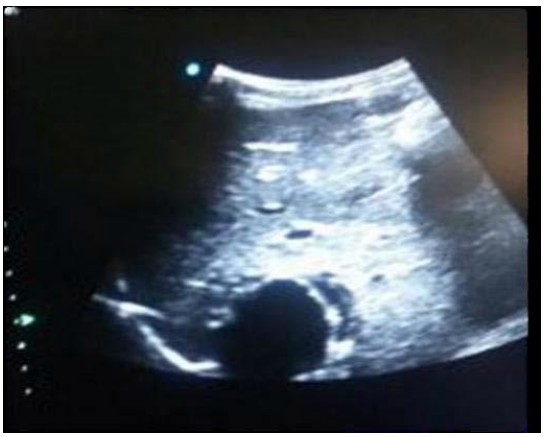

Fig. 1. Extracapsular free fluid

After resuscitation measures without resorting to intubation, the patient became stable. Albendazole was immediately started. After stabilization of the patient (48 h), abdominal laparoscopic examinations are realized and confirmed the absence of free intraabdominal fluid.

On the seven-liver segment, there was an intact wall cyst 5 cm to 6 cm.

The approach consisted to the puncture which confirmed the rupture to the blood vessel by containing bloody fluid (Fig. 2), aspiration, injection of hypertonic saline solution into the cyst. After 15 min to 20 min respiration of all the fluid.

Fig. 2. Hematic aspect of fluid

The cyst was totally evacuated; the residual cavity was explored carefully by optics for the presence of cysto-biliary communications. A partial cystectomy is done completed by drainage.

A parasitological study found that the liquid contained protoscolices.

Postoperatively there were no complications and the boy was discharged 5 days later. Albendazole was continued for two months.

The final cosmetic aspect is showed into (Fig. 3).

There was no recurrence after 36 months of follow-up either in liver or in other organ.

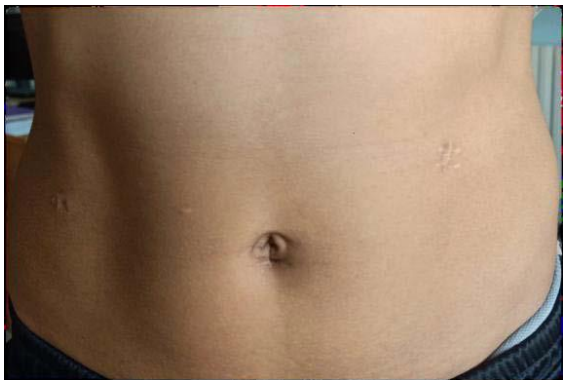

Fig. 3. Final cosmetic aspect

3. DISCUSSION

Rupture of hepatic hydatid cyst is very rare complication (1% to 8%) and can occur spontaneously owing to increasing pressure of cystic fluid or iatrogenically [6], following serious injuries or even minor trauma as in our case.

Young age is a risk factor of rupture because of the greater frequency of traumatic events and higher prevalence of hydatid disease in children and adolescents than in adults [7].

Two similar cases were described as rupture into the right hepatic vein and bile ducts, in which one of them was caused by trauma requiring conventionnel surgery [5,8]. The spillage of hydatid fluid as a result of cyst perforation has been shown to trigger serious anaphylactic reaction, the bloody contents of the cysts, most probably due to a partial drainage of its contents into the systemic circulation through the injured blood vessel.

One case report of a 13-year-old girl with an intact hydatid cyst was described as sudden anaphylactic shock after a blunt abdominal trauma. The cause of leakage of cystic fluid into the bloodstream was confirmed by absence of peritoneal fluid and existence of protoscolices in the hepatic venules ruptured what led to the death [3]. Elmali et al. [9] in his case report of a 15-year-old boy showed that emergency surgery in this case should be minimized, and he recommend initial albendazole treatment and delayed surgical intervention.

Another revue of literature reports a rare case of anaphylaxis to cystic hydatid disease initiated by leakage into the vasculature following trauma was reported and treated with laparotomy by Marriott [10].

A rare case is reported about rupture of a retroperitoneal hydatid cyst into the infrarenal abdominal aorta, without prodromal symptoms [11].

A recent study showed that sudden death due to hydatid cyst was more frequent among males. This predominance can be related by the occurrence of minimal trauma (fall) as a factor in cyst rupture, which is most common in males. In the same study among 20 sudden death, autopsy finds that cyst content was discharged into vessels in five cases, which caused fatal anaphylaxis [12].

According to the Lewell's classification, our patient cannot be included via the communicating rupture and we join Buyuk who suggests that the classification should be extended to include rupture in the vessels [13,14].

Radiologists can play a primary role in reaching diagnosis by their imaging techniques. The presence of a cyst in the liver of a patient who developed anaphylactic reaction, with detection of membrane like structures inside the cyst and discontinuity of the wall are considered diagnostic for ruptured hydatid cyst [9].

Management of rupture hydatid disease of the liver involves both medical and surgical treatment. Surgery is the gold standard for complicated cysts, already realized by laparotomy, the role of laparoscopic surgery in the treatment of intraperitoneal rupture of a hydatid cyst has not been proven but only Feleppa et al. [6] contended that, if the general condition of the patient is not severe, laparoscopy can be very useful and even more effective than traditional surgery.

Controversy in the time of surgery between different team over the world about anaphylaxis shock following rupture cyst [9,15-17]. Increasing experience of laparoscopic therapy in combination with albendazole therapy has been shown to be safe and efficient and can offer many of the advantages of minimal access surgery, including low morbidity, short hospital stay, resumption to school and the good cosmetic result, and also reduce recurrence [18].

There was no case reported in the management of intravascular rupture of hydatid cyst trigger to anaphylaxis following trauma using laparoscopy, which can be a challenge in our experience.

4. CONCLUSION

The traumatic rupture of cyst in the blood vessels is very rare entity, but should be kept in mind in a context of anaphylaxis shock especially in endemic areas.

Laparoscopic surgery can be recommended in this kind of clinical presentation of echinococcosis which is safe, feasible and effective combined to anti-parasitic treatment.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Biography of author(s)

Prof. Haif Assia
Department of Pediatric Surgery, University Hospital Center of SETIF, Algeria and Laboratory of Applied Pediatric Surgery, Ferhat Abbas Setif University 1, Algeria.

She is the head of Pediatric Visceral Surgery Unit, University Hospital Center of SETIF, Algeria. She is a Teacher-researcher at the Faculty of Medicine, FERHAT ABBAS SETIF 1 UNIVERSITY Algeria, Team leader in the research Laboratory of Applied Pediatric Surgery, FERHAT ABBAS SETIF 1 UNIVERSITY Algeria. She has been serving as a Member of the Algerian Society of Echinococcosis (SAEH), Member of the Algerian Society of Pediatric Surgery (SACP), Member of the World Federation of Association of Pediatric Surgeons (WOFAPS), and Member of the World Association of Echinococcosis (WAE).

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Chapter 5
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L-DOPA/Capsazepine or L-DOPA/Rimonabant Co-Administration in an Experimental Parkinson Disease Model: Behavioral and Cellular Consequences

José Luis Ordoñez-Librado [a], Ana Luisa Gutierrez-Valdez [a], Jesus Espinosa-Villanueva [a], Enrique Montiel-Flores [a], Patricia Aley-Medina [a], Javier Sánchez-Betancourt [a], Claudia Dorado-Martínez [a], Leonardo Reynoso-Erazo [b], Rocío Tron-Alvarez [b], Vianey Rodríguez-Lara [c] and Maria Rosa Avila-Costa [a*]

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ABSTRACT

Objective: using the 6-OHDA lesion in rats, we evaluated the ability of rimonabant or capsazepine with the addition of L-DOPA in: (1) the severity of LIDs, the dyskinetic effects were assessed using measures of abnormal involuntary movements (AIMs); (2) the protection of dopaminergic cell loss; and (3) in the cytological differences between treatments through analyzing the number of dendritic spines of the striatal medium-sized spiny neurons and the neuropile preservation. Oral co-administration of each antagonist with L-Dopa significantly decreased LIDs. Our data demonstrate that co-administration of L-DOPA with CB1 or TVPR1 receptor antagonists result in a very efficient treatment to reduce AIMs by conserving some functional dopaminergic cells, which implies the well-preserved synaptology of less denervated striatum. Thus, consistent with other reports, cannabinoid antagonist-based therapy would not only be aimed at alleviating specific motor symptoms but also at delaying/arresting the degeneration of striatal and substantia nigra compacta cells.

Keywords: L-DOPA; dyskinesia; Parkinson disease experimental model; endocannabinoid system antagonists; CB1, TRPV1; ultrastructure; cytology.

1. INTRODUCTION

Parkinson disease (PD) is a chronic and progressive neurodegenerative disorder of essentially unknown etiology, first described by James Parkinson more than 180 years ago and now affecting tens of millions of people worldwide, with an associated high socioeconomic burden. The clinical features of the disease are represented by poverty of voluntary movements (akinesia), slowness and impaired scaling of voluntary movement (bradykinesia), muscle rigidity, and limbs tremor at rest. These symptoms seem to represent the downstream effect of a pathological cascade resulting in the degeneration of midbrain dopaminergic (DAergic) neurons of the substantia nigra pars compacta (SNc) projecting to the striatum the main input station of the basal ganglia neural circuit [1,2].

The discovery of dopamine (DA) deficiency in PD and the subsequent introduction of replacement therapy with the DA precursor L-3,4-dihydroxyphenylalanine (L-DOPA) initially revolutionized the

[a] Neuromorphology Lab, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.
[b] Health Education Project, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.
[c] Department of Cell and Tissue Biology, Faculty of Medicine, UNAM, Mexico City, Mexico.
**Corresponding author: E-mail: nigraizo@unam.mx;*

management of the disease [3]. Unfortunately, motor fluctuations and dyskinesias complicate L-DOPA treatment in most patients (>90%) within 5–10 years of treatment initiation. L-DOPA-induced dyskinesias (LIDs) are characterized by choreiform and dystonic movements and are classified according to their temporal profile as **"**peak-dose**"** (occurring at peak L-DOPA concentration in the brain), **"**diphasic**"** (at the beginning and end of dosing), and **"**off**"** dyskinesias (when L-DOPA concentration is low) [4,5].

In rats, LID can be modeled via intracerebral injection of the neurotoxin 6-hydroxydopamine (6-OHDA), which damages the nigrostriatal pathway followed by chronic administration of low doses of L-DOPA, which causes characteristic abnormal involuntary movements (AIMs) and dyskinesias [6].

Hence, since L-DOPA treatment induces motor disturbances after 5-10 years, other therapies targeting non-DAergic systems have been proposed to manage PD [7]. Thus, while PD is predominantly associated with the loss of DA, it is becoming widely accepted that this disease is also associated with disruptions in non-DAergic mechanisms [8]. A signaling system, which is becoming increasingly implicated in PD is the endocannabinoid system (ECB) [9,10].

The major components of the ECB system are two endogenous lipids -N-arachidonoylethanolamine or anandamide (AEA) and 2-arachidonoyl-glycerol (2-AG), which are specific ligands of G-protein-coupled receptors named CB1 and CB2 receptors, respectively [11-14]. Specifically, the CB1 receptor and its main endogenous ligands, AEA [15,11] and 2-AG [16], are known to be present in high concentrations in the basal ganglia [17]. CB1 receptors are expressed on the presynaptic terminals of the striatonigral and striatopallidal neurons, as well as on the presynaptic terminals of corticostriatal neurons. They are supposed to exert a tonic inhibitory effect on these neurons via retrograde signaling from postsynaptic neurons [18,19,20]. CB1 receptors are also expressed on striatal interneuron subtypes containing parvalbumin, nitric oxide synthase, and Choline acetyltransferase [21]. Therefore, normal motor function is mediated by a highly balanced signaling loop consisting of GABAergic striatonigral and striatopallidal projections, glutamatergic corticostriatal projections, and striatal interneurons under the tonic regulatory influence of nigrostriatal DA as well as ECBs [22] to modulate basal ganglia neural network dynamics and long-term forms of synaptic plasticity [23]. A growing body of evidence suggests that the ECB system is altered in both parkinsonian patients [24,25,26] and experimental models of PD [27,28,29,30,31].

On the other hand, the transient receptor potential (TRP) receptors are ligand-gated ion channels that generate a cation inward flow upon activation [32]. Experimental evidence indicates that cannabinoids and ECBs directly interact with at least five distinct TRP channels, among which the vanilloid receptor VR1 (TRPV1) [33] emerges as the best-characterized "ionotropic" cannabinoid receptor [34]. This receptor is activated by vanilloids, such as capsaicin [35,36], endogenous ligands, including AEA [37,36], and N-arachidonoyl-dopamine [38].

TRPV1 receptors are present in the striatum, globus pallidus, and substantia nigra [39,40,41]. In the latter region, they affect the excitability of DAergic neurons by modulating excitatory inputs from glutamatergic terminals [42,43], suggesting that TRPV1 might be involved in the control of movement [44,42]. In support, treatment with TRPV1 agonist capsaicin decreases DA release in the striatum [45,46] and decreases motor activity [37,47]. Furthermore, pharmacological modulation of these receptors has been reported to influence striatum-dependent functions [48] and modulate motor symptoms originating from striatal dysfunction [47,49]. In the striatum, TRPV1 receptors co-localize with CB1 receptors [39,50], suggesting a close functional interaction between these receptors.

It has been mentioned that early presymptomatic phases in PD are associated with down-regulation/desensitization of CB1 receptors [51,52,9] since the activation of CB1 receptors inhibits glutamate release. Thus, down-regulation/desensitization of these receptors observed in PD may be associated with enhanced glutamate levels, and excitotoxicity plays an essential role in disease progression [9]. At later stages, a significant up-regulation of CB1 receptors was found in PD, which is caused by adaptive responses and is also compatible with the akinetic profile of these patients [44,9]. It has also been reported that GABAergic medium-sized spiny neurons contain many CB1 cannabinoid receptors [53]. Activation of these receptors causes presynaptic inhibition of GABA

release *in vitro* [54,23,44,55,102] and profoundly affects motor behaviors *in vivo* [27,57,58,59]. Moreover, the interdependency between CB1 and DA receptors and their high abundance in the striatal system has led to the hypothesis that targeting the CB1 receptor could be of value to improve motor deficits in neurodegenerative motor diseases such as PD [60]. Thus, abnormalities in DA signaling, as reported in PD and related animal models, may disrupt this feedback mechanism [61] and lead to a functional state characterized by motor disturbances [62].

Experimental evidence suggests that the ECB transmission is increased in the basal ganglia of humans affected by PD [26] and in experimental models of this disease [27,28,63]; thus, the blockade of cannabinoid CB1 or TRPV1 receptors might be beneficial to alleviate PD symptoms. Evidence from nonhuman primates and rodents has shown that the administration of the antagonist SR141716 (Rimonabant) improves motor symptoms in models of PD [64,65,66,67,68], and direct activation of CB1 receptors significantly attenuated L-DOPA-induced AIMs and the elevation of brain AEA via pharmacological blockade of its catabolism produced an antidyskinetic effect in the presence of TRPV1 antagonist capsazepine [69]. Altogether, the above data support the concept of targeting the CB1 and TRPV1 systems in the treatment of movement disorders, such as LID.

Dyskinesia is a highly disabling clinical manifestation of PD induced by the long-term use of L-DOPA therapy; it has been proposed the antagonism of CB1 or TRPV1 as an alternative for the regulation of the up-regulated cannabinoid system. Therefore, using the 6-OHDA-medial forebrain bundle (mfb) lesion, we evaluated the ability of rimonabant (RIM) or capsazepine (CZP) with the addition of L-DOPA (LD) in: (1) the presence of LD-induced dyskinesia, the severity of dyskinetic effects were assessed using measures of abnormal involuntary movements (AIMs); (2) the protection of SNc DAergic cell loss; and (3) in the structural and ultrastructural differences between treatments through analyzing the number of dendritic spines of the striatal medium-sized spiny neurons and the neuropile preservation.

2. MATERIALS AND METHODS

The experiments were carried out in 30 male Wistar rats weighing 180–200 g at the beginning of the study. The rats were individually housed in hanging plastic cages under controlled light conditions (12 h light/h dark regime) and fed with Purina Rat Chow and water *ad libitum*. Bodyweight was recorded daily. The experimental protocol was conducted following the Animal Act of 1986 for Scientific Procedures. All efforts were made to minimize the number of animals used and their suffering.

2.1 Stereotactic Surgery and Treatments

The rats were anesthetized with sodium pentobarbitone (35 mg/Kg i.p.) and placed in a stereotaxic apparatus. The rats were injected with 4 µl of a saline solution containing 8 µg of 6-OHDA (Sigma Chemical, USA) and 0.2 mg of ascorbic acid into the right mfb (n = 24), and a sham lesion was made with vehicle (n = 6 (control group)). The injections were given over a 4-min period with a Hamilton syringe attached to a glass micropipette with a tip diameter of 20–50 µm. The stereotaxic coordinates were as follows: AP = –4 mm anterior of the ear bar; L = 1.4 mm lateral of bregma; V = –7.7 mm vertical of the dura (according to [70]). After recovery from the anesthesia, the animals were returned to their home cages. Apomorphine (Sigma Chemical, USA; 0.25 mg/Kg i.p.) induced rotational behavior was tested one day after lesioning. Only those animals exhibiting more than 200 complete turns in a 30 min period were used [71]. One day after the test, 6 experimental animals were treated with 15-mg/kg LD (Sinemet® (Carbidopa-L-DOPA 25/250) 1:10 ratio), 6 were treated with 1-mg/kg rimonabant co-administrated with 15-mg/kg LD (LD/RIM), 6 with 1mg/kg capsazepine co-administrated with 15-mg/kg LD (LD/CZP) (Cayman Chemicals, USA). Sinemet tablets were crushed, and the powder was dissolved in 10 ml tap water. The corresponding antagonists' doses were added to Sinemet solution and given orally with an insulin syringe for two months. The other six lesioned rats without treatment, as well as control animals, were kept for the same time. It must be considered that there is evidence that administration of DAergic agents to 6-OHDA-lesioned rats can affect the course of DAergic lesion, and agonists such as apomorphine can induce a priming effect enhancing the emergence of DAergic hypersensitivity [72], so we decided not to use the apomorphine-induced circling behavior to evaluate motor recovery.

2.2 AIMs Rating

AIMs were scored every 14 days (4 evaluations). LD-induced AIMs were scored according to a rat dyskinesia scale [73]. Rats were placed individually in transparent plastic cages and observed every 20th min, from 20 min before taking the dose to 180 min after the treatment (10 monitoring periods of 1 min each). Four subtypes of AIMs were classified according to their topographic distribution as locomotive, axial, forelimb, or orolingual. The rating did not include enhanced manifestations of otherwise normal behaviors, such as rearing, sniffing, grooming, and gnawing. AIM severity was assessed using the published method of Cenci and Lundblad [73], which assigns a score from 0 to 4 to each of the four AIM subtypes listed above according to the proportion of time/monitoring period during which the AIM is present. Borderline scores, such as 0.5, 1.5, 2.5, and 3.5, were allowed to increase the sensitivity of the evaluation.

2.3 Video Recording

Performance during AIM analysis was video recorded using a Panasonic camcorder (SDR- H80 model). The video editing software Final Cut Pro captured representative still frames from digital video recordings. Pictures were cropped and adjusted for color and brightness contrast in Adobe Photoshop but were not altered in any other way.

2.4 Tissue Preparation

All rats were perfused via aorta under sodium pentobarbital anesthesia immediately after the two-month treatments, with a saline solution followed by a fixative containing 0.2% glutaraldehyde and 4% paraformaldehyde in 0.1M-phosphate buffer (PB). The brains were removed and placed in a fixative solution for 1 hour.

2.5 TH Immunocytochemistry

Coronal sections (50 μm) were obtained on a vibrating microtome through the mesencephalon for immunocytochemistry. Tyrosine hydroxylase (Chemicon International, Inc. CA, USA, 1: 1000) immunostaining with the ABC detection method (Vector Lab MI, USA) was performed for light microscope analysis. The analysis was conducted with a computer-assisted system (Image-Pro Plus, Media Cybernetics, L.P. Del Mar, CA, USA) connected by a CCD camera to Optiphot 2 microscope (Nikon, Japan). The number of TH-positive neurons was counted in 1500 μm^2 from 7 SNc sections of each animal [74].

2.6 Golgi Method

Blocks from the striatum were cut into 90-μm- thick sections and processed for the rapid Golgi method [75]. The histological analysis consisted of counting the number of dendritic spines in a 10-μm-long section from 5 secondary dendrites from 10 medium-size spiny neurons [74].

2.7 Electron Microscopy

Fragments from contralateral and ipsilateral striata were carefully taken. After washing in PB, the fragments were treated for 60 minutes with 1% osmium tetroxide in PB, soaked for 30 minutes in PB, dehydrated with graded ethanol, and flat-embedded in Araldite. Ultrathin sections were collected, counterstained with uranyl acetate and lead citrate, and examined in a ZEISS SIGMA Gemini 300 electron microscope.

2.8 Ultrastructural Analysis

Synapses were defined by the presence of an apparent postsynaptic density facing at least three presynaptic vesicles. Ultrastructural analysis was performed in 50 randomly selected synaptic endings per striatum. In each synaptic bouton, we observed all its membrane and organelle features. We

measured the diameter of the presynaptic bouton using two axes, which were perpendicular to each other and intersected at the center of the synaptic terminal (Fig. 1); the diameter was measured directly from the electron microscope screen with a grid placed inside the eyepiece [76].

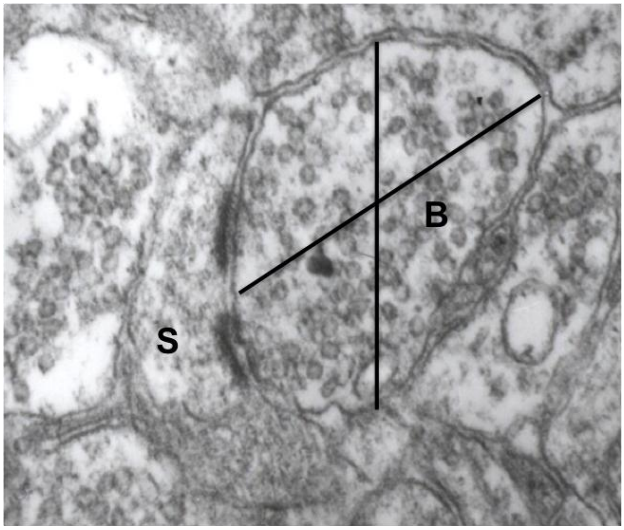

Fig. 1. Synaptic ending (B) shows the two axes measured, establishing a synaptic contact with a dendritic spine (S)

2.9 Statistical Analysis

Dyskinesias were expressed as the median of total AIMs score and analyzed using the Kruskal–Wallis test followed by Dunnett's multiple comparison test. The threshold for statistical significance was set at $p < 0.05$. One-way ANOVA was used to analyze the number of TH-immunoreactive cells, the number of dendritic spines, and synaptic ending diameter and expressed as mean ± SEM. Group differences were considered statistically significant at $P < 0.05$. When appropriate, *posthoc* comparisons were made with the Tukey test. Analyses were performed by GraphPad Prism version 5.0 for Mac (Graph Pad Software, San Diego, CA, USA).

3. RESULTS

After two months, neither clinical nor significant weight changes were detected in the experimental animals compared with controls.

3.1 Abnormal Involuntary Movements (AIMs)

3.1.1 Time course and overall incidence AIMs

To get an overview of the development of dyskinesia in the different groups, we carried out the summation of all subtypes of AIMs (axial + locomotive + limb + orolingual). Fig. 2A depicts the total sum of the severity of all AIM's induced by LD in each experimental condition at different treatment times. LD/RIM-treated rats presented less severe AIMs than the group treated only with LD. A similar effect showed LD/CZP-treated group.

Nevertheless, the attenuation of AIMs induced by CZP persisted to 56 days. As shown in Fig. 2B (treatment day 42), the temporal manifestation of AIMs after a single dose of LD alone or LD co-administrated with antagonists was similar in all groups, resembling the time course of peak-dose dyskinesia in PD [4,5]. Thus, AIM severity gradually increased during the first 20 min post-treatment, remained elevated for an additional 60 min, and then slowly returned to baseline between 80 and 140 min post-treatment. The AIMs severity is much more evident in the LD-only treated group since the co-administration with RIM or CZP significantly reduces the AIMs severity.

3.1.2 Representation of AIM subtypes

According to Cenci and Lundblad [73], the animals were evaluated on four different topographic subtypes of AIMs. Different AIM subtypes were mainly characterized in the LD-only treated group and with less severity in the antagonist-treated groups (Fig. 3). The development of orolingual (3A), axial (3B), and forelimb (3C) AIMs during chronic drug treatment differed significantly among groups. Indeed, chronic therapy with LD alone produced increasingly severe AIMs affecting the trunk, limb, and orolingual muscles. However, from the first to the last testing session, the co-administration with LD/RIM and LD/CZP attenuate the development of AIMs, being more effective LD/CZP co-administration.

Moreover, LD/RIM- and LD/CZP- treated groups, demonstrate less impaired motor behavior. It is essential to note that LD/CZP-treated rats showed ipsilateral axial AIMs, sometimes more frequently than contralateral ones (data not shown). Finally, locomotive AIM was barely observed in all experimental groups; both LD-alone and antagonist-treated groups manifest turning behavior involving two members (axial AIM). Only locomotive movements concerning all four limbs are rated under this AIM category.

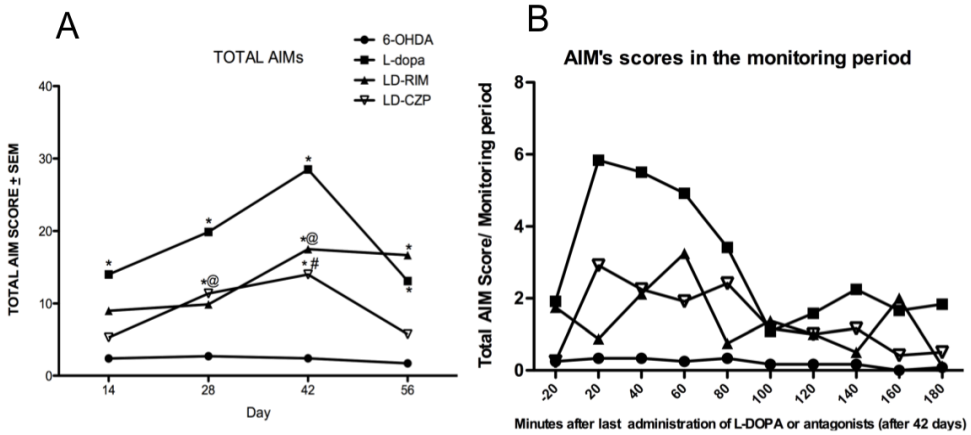

Fig. 2. Time Course and Overall Incidence AIMs. The three treated groups, 6-OHDA+LD, 6-OHDA+LD/RIM, and 6-OHDA+LD/CZP, confer certain susceptibility to dyskinesia during the experiment, but the overall AIM severity is most pronounced in rats with 6-OHDA+LD treatment. (A) Time course of AIM development during the chronic LD and LD/antagonist treatments. Values give total (locomotive + axial + orolingual + limb AIMs) integrated AIM scores per testing session as group median scores. (=P < 0.001 LD and antagonist-treated groups versus 6-OHDA-lesion; @ =P < 0.005 LD/RIM versus LD group; #=P < 0.005 LD/CZP versus LD treated group, Kruskal–Wallis followed by Dunn's multiple comparison test). (B) Time course of total AIM scores/monitoring period after a single treatment of LD or antagonists (treatment day 42)

3.2 TH Immunocytochemistry

The number of TH-positive neurons in the control group, both contra and ipsilateral SNc, remained unaffected (94 ± 1.9 and 93 ± 1.7 neurons, respectively) (Figs. 4 and 5). In contrast, we found a significant loss of TH-positive neurons in the SNc of 6-OHDA lesioned animals in both contralateral (73 ± 1.9 neurons) and ipsilateral (5 ± 1.6 neurons) SNc compared to controls as shown in figures 4 and 5; likewise, LD-treated rats (59 ± 1.0 and 6 ± 2.0 neurons contralateral and ipsilateral, respectively), in contrast, LD/RIM (78 ± 2.2 neurons contralateral and 20.3 ± 1.2 ipsilateral SNc) and LD/CZP- (81 ± 1.8 contralateral and 18 ± 0.9 ipsilateral SNc) treated rats show significant bilateral loss of TH-positive cells comparing to control group, however, when compared to LD-only treated group we found significant differences in both sides. As shown in Fig. 5, both antagonists revealed a protective effect on DAergic cell survival, being more effective LD/RIM co-administration.

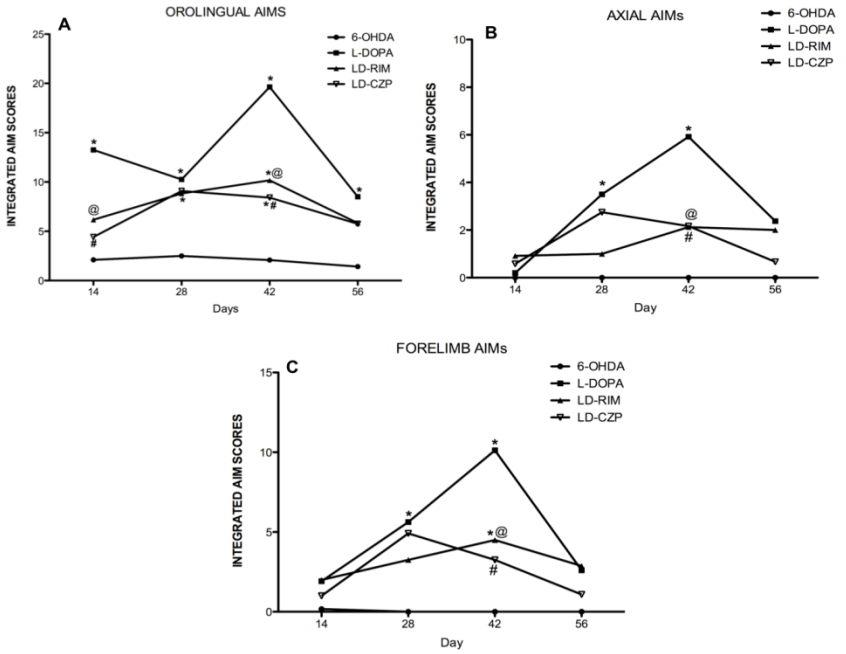

Fig. 3. Representation of AIM Subtypes. Integrated AIM scores were generated separately for orolingual (A), axial (B), and forelimb (C) AIMs. (*=P < 0.005 treatments versus 6-OHDA-lesion; @ =P < 0.005 LD/RIM versus LD group; #=P < 0.005 LD/CZP versus LD treated group, Kruskal–Wallis followed by Dunn's multiple comparison test)

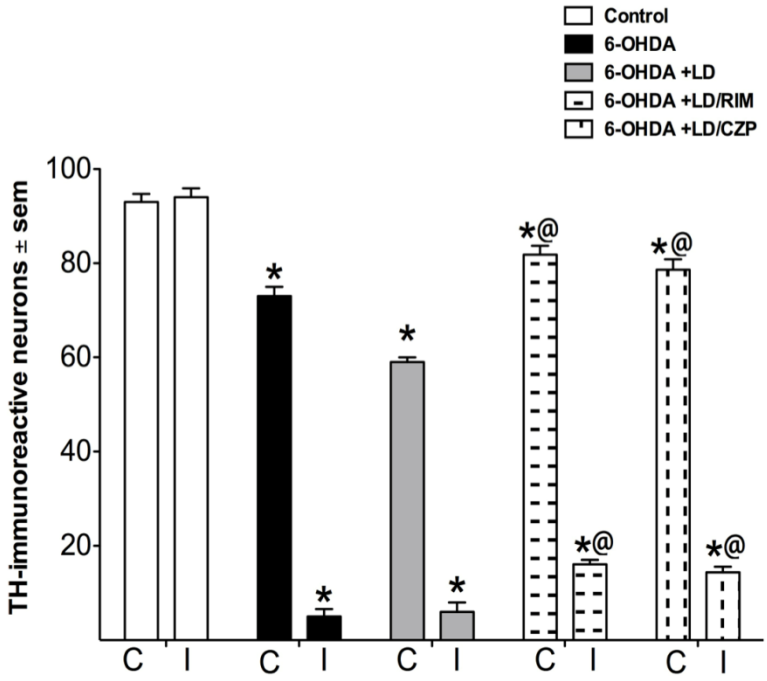

Fig. 4. TH-immunoreactive cell counts from the SNc. The data are presented as the mean ± SEM. A statistically significant decrease in TH-immunoreactive cells was detected in both contralateral (C) and ipsilateral (I) SNc in the four experimental groups; however, LD/RIM and LD/CZP treatments showed evident protection (* = P < 0.05 versus control group; @ = P < 0.05 versus LD-treated group; ANOVA test)

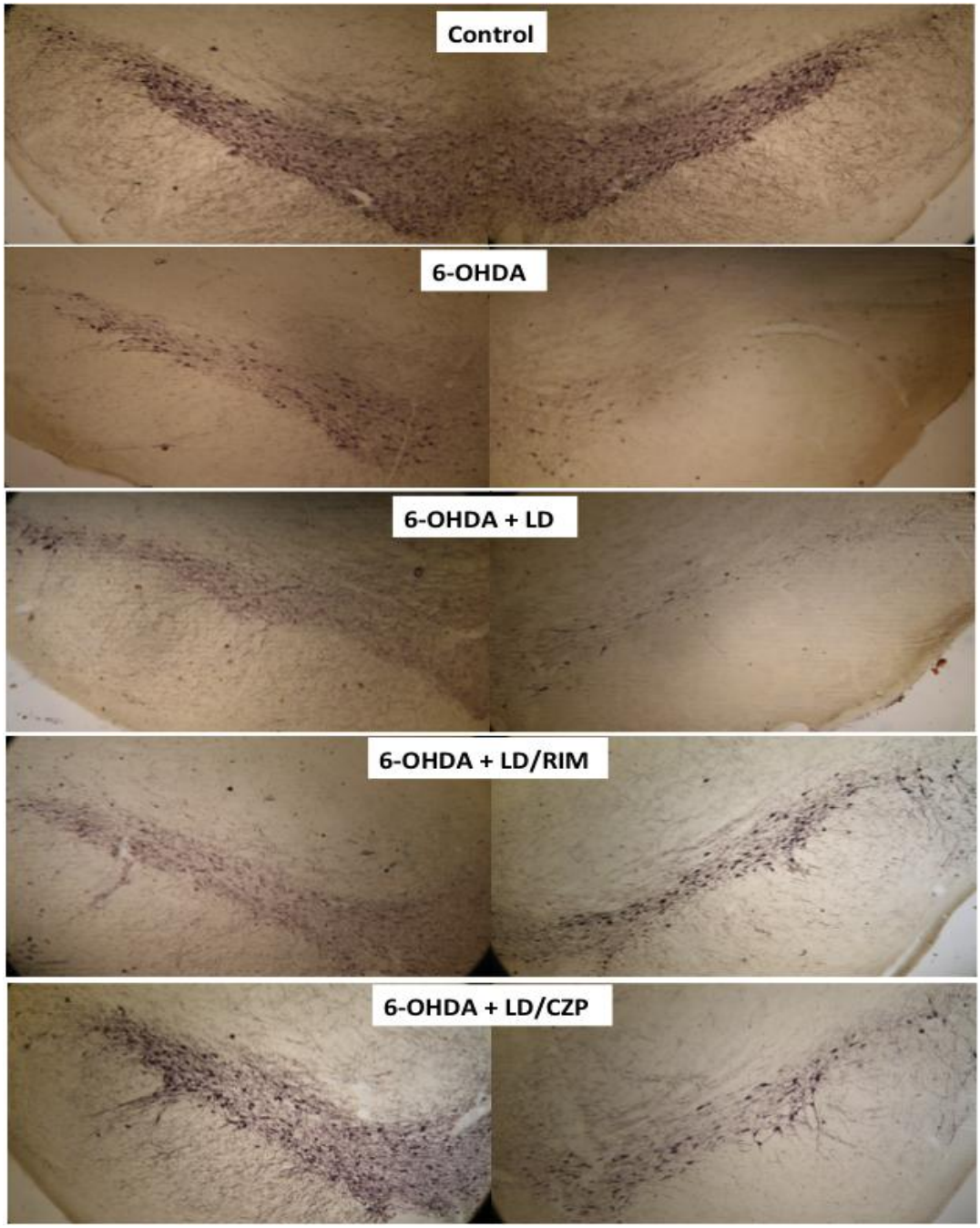

Fig. 5. Representative TH-immunostained from coronal sections containing the SNc of control, 6-OHDA, 6-OHDA+LD, 6-OHDA+LD/RIM, and 6-OHDA+LD/CZP treated rats. Note the profound cell loss in the ipsilateral SNc in the four experimental groups, being more evident in 6-OHDA and LD-only treated ones; in contrast, it is striking the cellular protection provided by the antagonists-treated groups (Magnification 10x)

3.3 Histological Analysis

Figs. 6 and 7 show the effects of all the treatments on dendritic spine density. The spine densities in the control group, both contra, and ipsilateral striatum, remained unchanged (15.23 ± 0.36 and 14.7 ± 0.30 spines, respectively (Figs. 6 and 7A)). In contrast, 6-OHDA lesion and LD treatment induced a substantial loss of dendritic spines in both striata (Figs. 6 and 7B and C), likewise, LD/CZP (Figs. 6

and 7D) and LD/RIM groups (Figs. 6 and 7E) show significant differences in the ipsilateral striatum compared to control group but in less proportion. When comparing the three treated groups, we found that the contralateral striatum of both antagonist-treated groups shows similar values to those found in the control group striatum, *Post hoc* analysis showed a significant reduction in spine density in the LD-treated group compared to LD/CZP, and LD/RIM treated groups.

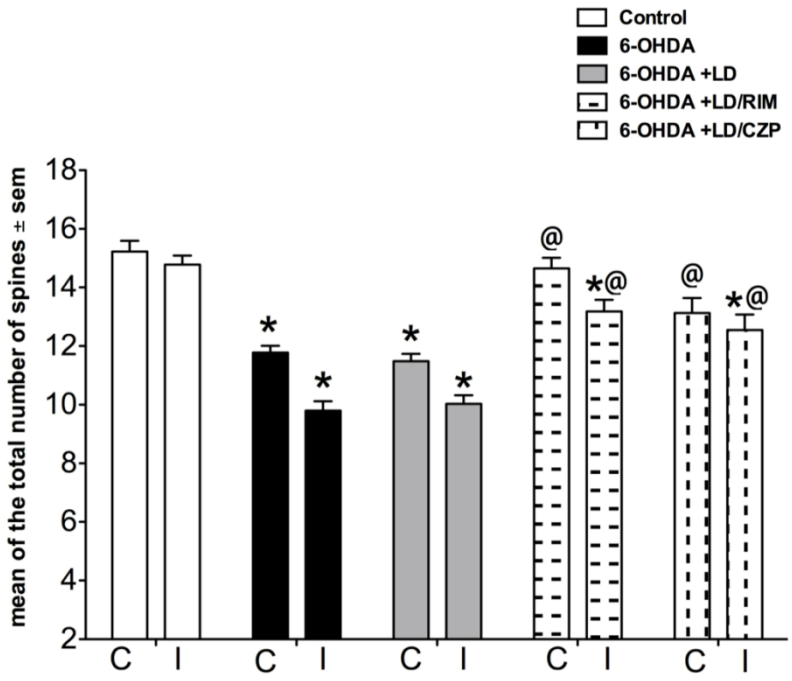

Fig. 6. Golgi stain analysis. Dendritic spines density of medium-size spiny neurons of the contralateral (C) and ipsilateral (I) striata after two months. (* = P < 0.05 versus control group; @ = P < 0.05 versus LD-treated group; ANOVA test)

3.4 Electron Microscopy

Control rats did not show any differences between both striata synaptic endings diameter and neuropile alterations after sham surgery (Figs. 8 A, B, and 9A). As shown in Figure 8, the synaptic endings of the control group, the major axis presented an average of 696.8 ± 9.4 nm on the contralateral striatum and 700 ± 9.6 nm on the ipsilateral one; the minor axis mean 474.9 ± 9.6 nm on the contralateral and 477.0 + 9.6 nm on the ipsilateral striatum. The 6-OHDA-lesioned group showed an evident increase in the size of synaptic boutons (1379.7 ± 18 and 980.3 ± 16.13 nm major and minor axis, respectively on the ipsilateral striatum), the same pattern was observed in the 6-OHDA + LD-treated group (1340.0 ± 13.20 and 966.0 ± 12.10 nm major axis and minor axis of the ipsilateral side, respectively) there were statistically significant differences in both groups comparing to control group (Figs. 8 A, B, and 9 B, C). 6-OHDA + LD/RIM group showed fewer presynaptic buttons with edema (987 ± 12.00 nm and 765 ± 13 nm major and minor axis of the ipsilateral side, respectively); likewise, LD-/CZP rats displayed fewer swollen synaptic endings (1078 ± 12 nm and 878 ± 8.98 nm major and minor axis of the ipsilateral striatum, respectively), showing significant differences comparing to 6-OHDA untreated and LD-only treated groups. The contralateral (non-lesioned side) of all experimental groups showed no significant differences compared to the control group (Fig. 8 A, B). The neuropile of 6-OHDA and LD-only treated groups was severely altered (Figs. 9 B, C); in contrast, the neuropile of LD/RIM and LD/CZP was well preserved with no evident alterations (Figs. 9 D, E).

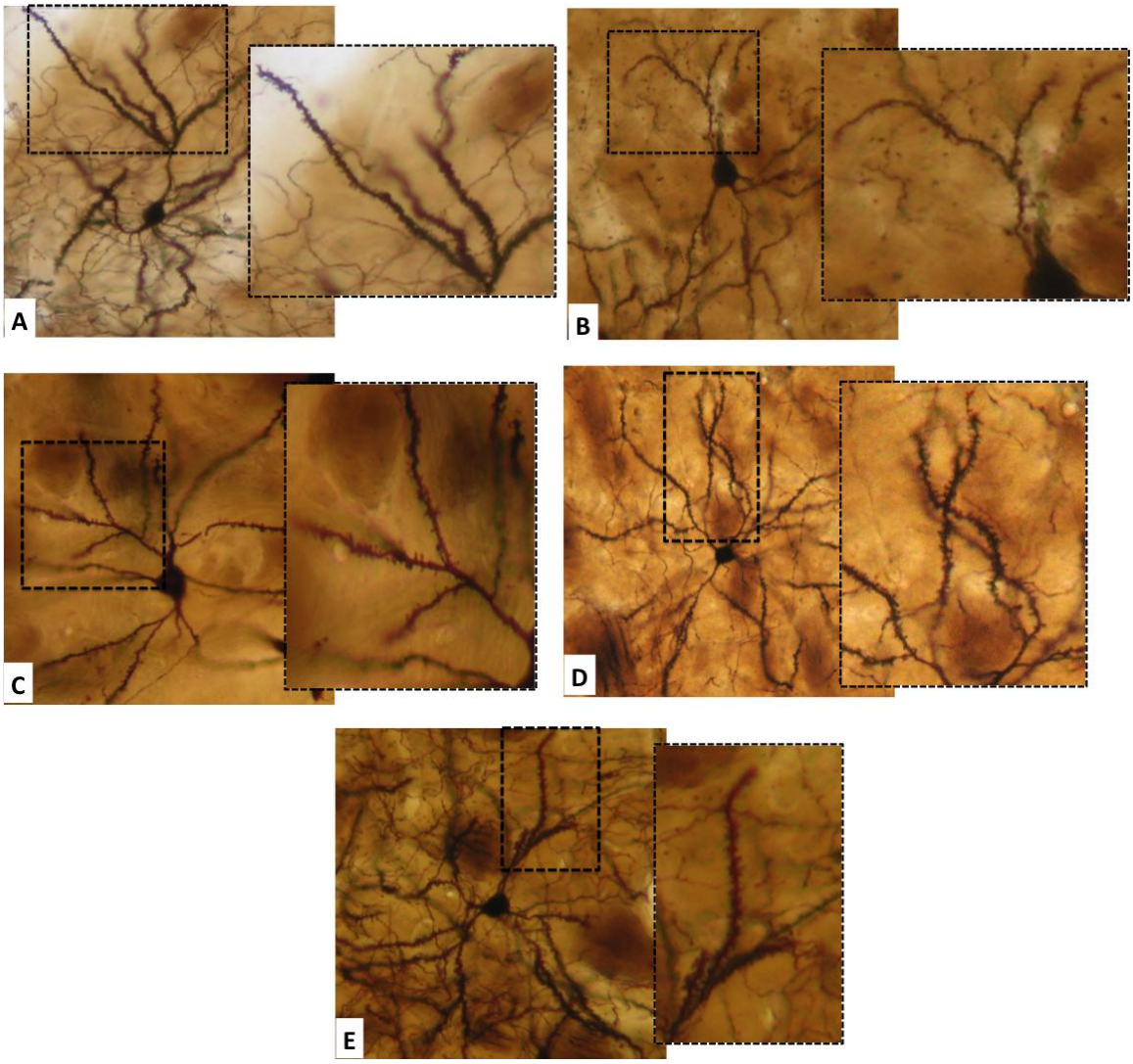

Fig. 7. Dendritic spine density. Photomicrographs of representative Golgi-stained medium-sized spiny neurons of the ipsilateral striatum with a representative box of dendritic spine densities from the control group (A), 6-OHDA-lesion (B), 6-OHDA + LD-treatment (C), 6-OHDA + LD/RIM-treatment (D), and 6-OHDA + LD/CZP-treatment (E). Both 6-OHDA lesion and LD-treatment significantly decreased the total number of spines, mainly in the ipsilateral striatum. In contrast, antagonist-treated groups show well-preserved dendritic spines density (Magnification 40X and 100X)

4. DISCUSSION

The data of this study demonstrate that co-administration of L-DOPA with CB1 or TVPR1 receptors antagonists result in a very efficient treatment to reduce AIMs through the conservation of some functional SNc DAergic cells, which in turn imply the well-preserved synaptology of less denervated striatum. Thus, cannabinoid antagonist-based therapy would not only be aimed at alleviating specific motor symptoms but also at delaying/arresting the degeneration of striatal and SNc cells.

4.1 AIMs

The evidence of an increase in the ECB transmission in the basal ganglia in PD patients and animal models of this disease [63,26] supports the potential of Rimonabant or other CB1 receptor antagonists to alleviate PD symptoms. The data found in this work confirm this hypothesis since we showed that

blockade of CB1 receptors significantly attenuated 6-OHDA-induced hypokinesia. Moreover, the finding that the combination of 1 mg/kg Rimonabant and 15 mg/kg L-DOPA reduces LID severity appears to be an important demonstration that a cannabinoid antagonist can be adjunctively therapeutic in an animal model of PD. This finding is consistent with the intimate linkage between the DA and ECB systems within the basal ganglia [55,58,25] and suggests that adjunctive use of a cannabinoid antagonist might enable a reduction of the dose and, therefore, the side effects of L-DOPA needed to treat PD.

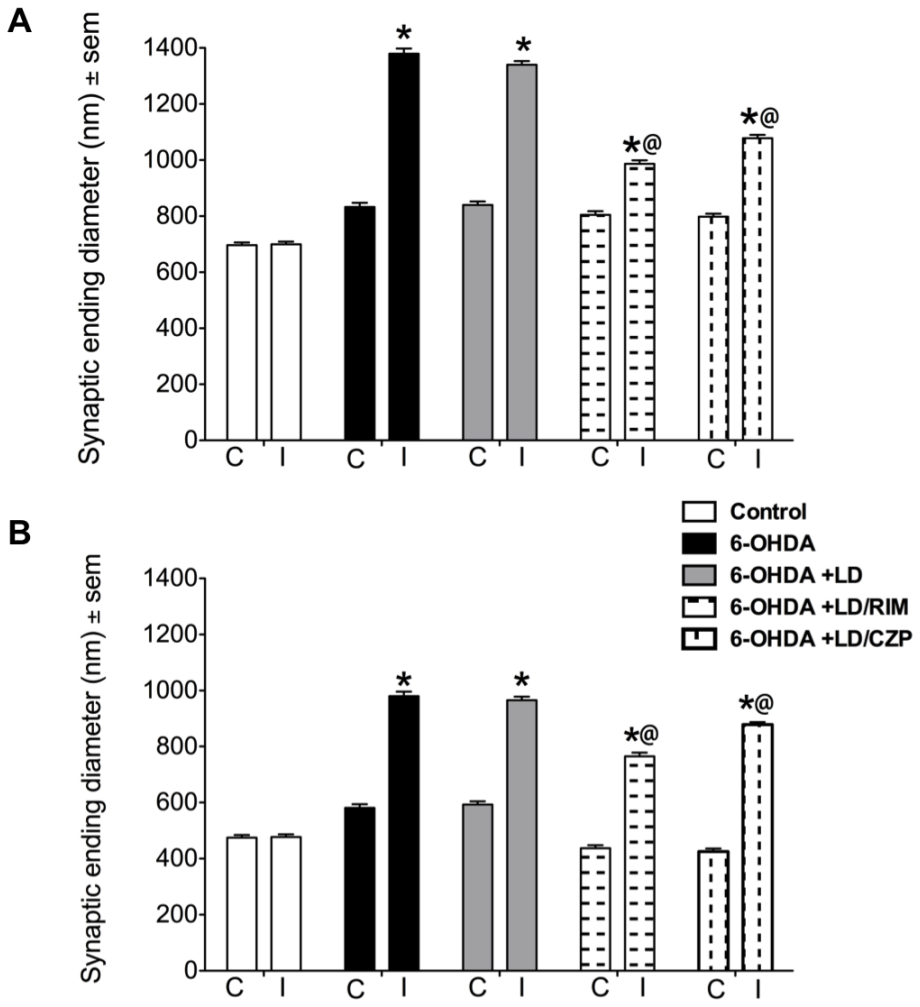

Fig. 8. Ultrastructural analysis. Synaptic ending diameter in ipsilateral (I) and contralateral (C) striata after stereotactic surgery and treatments, major (A) and minor (B) axes. (* = P < 0.05 versus control group; @= P < 0.05 versus LD-treated group; ANOVA test)

On the other hand, the pharmacological blockade of TRPV1 unmasked the antidyskinetic effect of the fatty acid amide hydrolase (FAAH) inhibitor URB597 in a rat PD model [69]. The existence of a crosstalk between CB1 and TRPV1 is evidenced by studies showing that CB1 stimulation can alter the functional state of TRPV1 [44,77,78,36,46,69,83,79,79]. Thus we conclude that after blocking the CB1 receptor with Rimonabant, the improvement in LID could be due to the changes in the TRPV1 receptor, which, as CB1 is localized in the striatum.

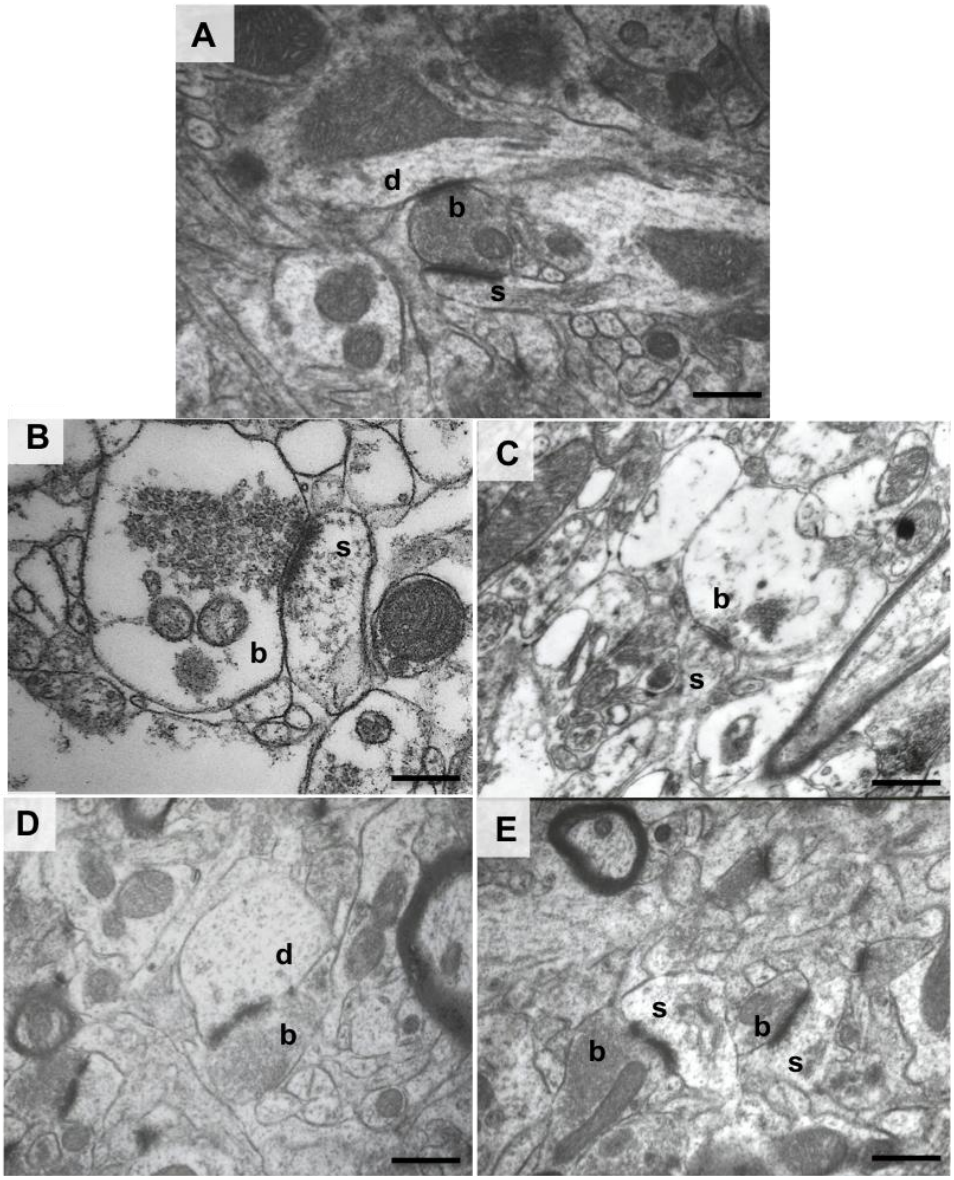

Fig. 9. Striatal ultrastructure. Electron micrographs from ipsilateral striatum neuropile. (A) In the control group, the mean size of the synaptic buttons (b) was 700 X 696 nm; it can be observed that the neuropile is well preserved, (s) dendritic spine, (d) dendrite. (B) This image shows a swollen synaptic button (b) of a 6-OHDA rat establishing a synaptic contact with a dendritic spine (s); note the altered mitochondria and some vacuoles within the neuropile. (C) This image demonstrates swollen presynaptic ending (b) of a LD-treated rat establishing a synaptic contact with a dendritic spine (s); this rat also showed severe neuropile alterations. (D) LD/RIM and (E) LD/CZP, both synaptic buttons and neuropile of antagonist-treated groups were well preserved similar to control group, (b) synaptic bouton, (s) dendritic spine, (d) dendrite. Bar 0.2μm

These and other data led to conclude that the blockade of CB-1 and TRPV1 by ECBs has opposite effects on LID. Hence, the data reported here are somehow in agreement with the results of these authors since CB1 and different paths blocked TRPV1 receptors; however, further analysis is needed to understand why the co-administration with L-DOPA reduced the severity of AIMs. On the other hand, the fact that Rimonabant in our study demonstrates a reduction in the severity of AIMs is in agreement with the data reported by Fernández-Espejo et al. [65], who found that the systemic administration of cannabinoid CB1 Rimonabant antagonist exerted antiparkinsonian effects in animals

with very severe SNc degeneration. In their group of rats, nigral TH+ cell loss was higher than 95% (analog of the last stage of human PD), and turning, akinesia, sensorimotor neglect, and right forepaw use were significantly ameliorated. The mfb 6-OHDA lesion produces a severe SNc degeneration, corroborated by apomorphine-induced circling behavior [71] and TH+ cell count (See Figs. 4 and 5). According to Fernández-Espejo et al. [65], previous contradictory results after CB1 antagonists in parkinsonian rats and monkeys could be attributable to the differential efficacy of these ligands depending on the level of nigral degeneration. In support of this hypothesis, the reserpine rat model of PD is known to induce a substantial DA depletion with a rapid development of striatal DAergic supersensitivity (within 12–24 h after reserpine) [81]. Cannabinoid CB1 antagonists are effective in ameliorating immobility in this model [50]. However, cannabinoid CB1 antagonists have been revealed not to be effective in long-term MPTP-treated parkinsonian monkeys [82]. Other authors have shown that most severely injured parkinsonian animals subjected to chronic MPTP regimens present 70–80% nigral TH+ cell loss and >95% striatal DA depletion [83]. According to the present study, the percentage surviving population of nigral TH+ neurons is a critical factor regarding the antiparkinsonian nature of cannabinoid CB1 antagonist, and its oral administration appears to exert functional effects when nigral loss of DAergic neurons is more than 95%. Therefore, since this antagonist only improved motor inhibition in 6-OHDA-lesioned rats with considerably DAergic degeneration (>95%), it was proposed that low to moderate doses of Rimonabant might only be effective at the advanced phases of PD characterized by severe denervation of the striatum. This observation might serve as a basis to develop anti-parkinsonism agents in a stage of the disease when classic DAergic therapy generally fails [84].

On the other hand, Maccarrone et al. [30] demonstrate that in a rat model of PD induced by unilateral nigral lesion with 6-OHDA, the striatal levels of the ECB AEA were increased, while the activity of its membrane transporter (AMT) and FAAH were decreased, these effects were completely reversed by chronic L-DOPA treatment; for that reason, treatment with L-DOPA may be effective at the beginning of the disease. It has been reported that corticostriatal glutamatergic transmission is enhanced following DA denervation [85,86,63]. This effect also reflects the loss of the D2 receptor-mediated control of corticostriatal transmission [87]. Interestingly, D2 and CB1 receptors share the same signal transduction pathway and cooperate closely in the negative regulation of striatal excitatory transmission [82]. Thus, the finding that endogenous levels of AEA are higher in parkinsonian rats may reflect a compensatory mechanism trying to control the cortical glutamatergic drive to the striatum. However, this mechanism seems insufficient in chronic L-DOPA-treated rats since spontaneous excitatory activity, manifested as AIMs, is still higher in these animals. In keeping with this hypothesis, it has been shown that chronic L-DOPA increase CB1 mRNA levels in the striatum of rats unilaterally lesioned with 6-OHDA [88].

Moreover, Ferrer et al. [61] demonstrate that L-DOPA selectively elevates AEA in different areas of the basal ganglia of normal rats via activation of dopamine D1/D2 receptors. This elevation was prevented by pharmacological blockade of either D1- or D2-like receptors in striatum and Globus Pallidus and by D1 antagonists only in SNc. The authors hypothesize that this increase represents negative feedback to enhanced DAergic transmission following L-DOPA administration. Interestingly, in our experiment, the pharmacological blockade of the CB1 or TRPV1 receptors in combination with L-DOPA notably attenuates AIM severity.

Regarding capsazepine protection, it has been shown that the administration of the endocannabinoid AEA to rats produces hypokinesia in parallel to a decrease in the activity of nigrostriatal DAergic neurons [89]; this effect is also able to activate TRPV1 receptors, and that these receptors are located on nigrostriatal DAergic neurons, suggest that the activation of vanilloid-like receptors rather than CB1 receptors might be responsible for AEA-induced hypokinesia and decreased nigrostriatal dopaminergic activity. These effects were completely reversed by the TRPV1 receptor antagonist capsazepine, thus indicating a role of these receptors in mediating the hypokinetic effects of AEA. *In vitro* studies using perfused striatal fragments support this vanilloid-like receptor-mediated direct action, which would not be available for classic cannabinoid agonists. These observations reinforce the notion that the blockade of TRPV1 receptors in the basal ganglia circuitry plays an essential role in controlling movement and, complementarily to CB1 receptors, represents another target susceptible of analysis for a potential application in motor disorders.

4.2 Cytological Alterations

Dendritic spine counting showed that 6-OHDA and L-DOPA only-treated groups exhibit significant dendritic spine loss in both striata analyzed, and severe TH-positive cell loss in both ipsilateral and contralateral SNc; it seems that the contralateral side is damaged after DA depletion but in less proportion than the ipsilateral one [90,76]. Yang et al. [91] argue that changes in the ipsilateral SN affect the contralateral side because their electrophysiological data have shown that the SNc from the contralateral brain side influences nigrostriatal DA cell activity. Moreover, Fass and Butcher [92] and Emsley et al. [93] have reported that nigrostriatal projection is primarily ipsilateral but also comprises a small contralateral component. Our results also show that both antagonists co-administrated with L-DOPA prevented 6-OHDA- induced spine loss. Since L-DOPA- treatment alone did not avoid this loss, preventing dendritic spine loss may be crucial to the antidyskinetic effects [94] of Rimonabant or capsazepine treatments. Profound plastic changes have been demonstrated to affect the striatal medium-sized spiny neurons during the progressive loss of DA input [95,90,96,97]. Hence, we hypothesized that the loss of spines and the alterations in the synaptic connectivity observed after 6-OHDA lesion or L-DOPA treatment might participate in the development of adverse motor events related to L-DOPA therapy [98] because it would alter information flow through the striatum and rest of the basal ganglia nuclei, and since both antagonists prevent the striatal synaptic alterations, it is possible that CB1 or TRPV1 blockade actively participate in the preservation of the striatal synaptic connectivity. Indeed, the excitability of DA receptors is elevated after DA depletion [99,100]. This adaptation of excitability in parallel with the loss of connectivity could be intimately linked to the development of dyskinesias and might be related to the ECB system since El-Banoua et al. [101] demonstrate that CB1 antagonism reduced motor asymmetry in parkinsonian rats after injections into the striatum, globus pallidus, and to a lesser extent, subthalamic nucleus. At the level of the dorsal striatum, Rimonabant effects were mediated through opposite modulation of D1 and D2 DA receptor function. Likewise, Martín et al. [58] found that the ECB system is a relevant negative modulator of dopamine D1 and D2 receptor-mediated behaviors through its actions on striatal neurons co-expressing DA and cannabinoid receptors. Moreover, the antidyskinetic effect of capsazepine may be related to the fact that TRPV1 receptors co-localize with CB1 receptors [39,50], suggesting a close functional interaction between these receptors.

On the other hand, we found a less severe but significant loss of TH-positive cells in both contralateral and ipsilateral SNc in the groups co-treated with L-DOPA and antagonists; however, this loss was not enough to alter the dendritic spine density in the striatal medium-sized spiny neurons; the possible explanation is that capsazepine seems to promote DA liberation in striatal terminals [89] and Rimonabant modulates glutamate release over medium-size spiny neurons [82], both events protect and/or enhance dendritic spine density.

In this way, it is possible that TH-positive cell death prevention was caused by the antagonism of TRPV1 receptors, which impedes the increased calcium influx [43] effect, which might account for the increase of striatal glutamate release by the agonist capsaicin.

Some reports suggest that cannabinoid agonists may offer neuroprotection in PD [102]. This has been studied in rats with hemiparkinsonism generated by unilateral injection of the neurotoxin 6-OHDA [103,104]. D9-THC and cannabidiol were the first cannabinoids shown to be capable of attenuating the damage to nigrostriatal DAergic neurons caused by this neurotoxin [104]. Neuroprotective effects of cannabinoids blocked by CB1 receptor antagonists/inverse agonists such as Rimonabant have also been found in other *in vivo* models of neuronal injury [105], such as trauma [106] and multiple sclerosis [107]. Moreover, Rimonabant has been used in many studies to assess whether neuroprotective effects of agonists or indirectly acting compounds are mediated via this receptor [105] in models of cerebral ischemia [108,109,110], trauma [111], PD [68], and neuronal damage induced by NMDA [112]. Thus, we assume that AEA is elevated after DA denervation [30,113] and PD patients [26]. That increase stimulates the overproduction of glutamate, leading to cell death [87,63]; the antagonistic effect by Rimonabant impedes AEA over activation preventing in part DAergic cell death. Besides, Rinaldi-Carmona et al. [114] show that Rimonabant is a functional antagonist of the brain CB1 receptor with excellent oral bioavailability and a long duration of action.

The fact that both antagonists have been effective, although it has been proposed to have opposite effects, lies mainly in the CB1 or TRPV1 receptors status and also in the severity of DA depletion. As we mentioned above, activation of DA receptors is accompanied by the release of AEA throughout the basal ganglia and is disrupted after lesioning the nigrostriatal pathway; thus, the blockade of TRPV1 receptors by capsazepine promotes the release of DA and, in consequence, improves neuronal survival preventing motor alterations. Regarding Rimonabant, we have mentioned earlier that ECB transmission is augmented after DA depletion, suggesting that the blockade of cannabinoid CB1 receptors might help reduce motor disturbances. In this regard, it has been demonstrated that Rimonabant improved motor inhibition in 6-OHDA-lesioned rats with extremely high degeneration of DAergic neurons (as in our case and the case of patients with advanced disease). Moreover, it has been proposed that local decreases and increases of ECB tone occur in the striatum of animals and humans with impaired nigrostriatal DA signaling causing different effects on glutamatergic inputs from subthalamic nucleus GABAergic outputs on the Globus Pallidus outer segment and substantia nigra reticulata. Decreases (possibly due to decreased D2 signaling) might occur in glutamatergic neurons impinging on the indirect pathway, whereas increases (possibly due to decreased D1 signaling) might occur in glutamatergic neurons affecting the direct pathway. This would result in stimulation or reduction of GABAergic outputs in the two pathways, respectively, thereby causing enhanced GABAergic activity from both the internal layer of the globus pallidus and the substantia nigra reticulata onto thalamic (and hence cortical) outputs, with subsequent locomotor impairments [115]. Therefore, CB1 receptor blockade by Rimonabant could regulate GABAergic transmission to the thalamus, enhancing glutamatergic transmission to the cerebral cortex, preventing motor alterations.

5. CONCLUSION

In summary, our results demonstrate that oral co-administration with L-DOPA/Rimonabant or L-DOPA/capsazepine results in an evident improvement of the AIMs severity, which correlates with enhanced performance, DAergic cell survival and, in consequence, to a well-preserved striatum sinaptology.

Advances in defining the role of ECBs in both normal basal ganglia function and the pathophysiology of PD and LID provide the basis for ECB-targeted therapy for these and other movement disorders. However, experimental evidence indicates that the effects of CB1 or TRPV1 receptor agonists or antagonists in the basal ganglia circuits are site-specific and probably dose-dependent. Recent studies in PD models also suggest that the impact of these drugs depends on the severity of DA denervation. Given these multiple variables, the results of clinical trials assessing the efficacy of ECB receptor agonists or antagonists in PD and LID still need to be interpreted with caution. However, the potential therapeutic value of these drugs, not only as adjunctive but also as potential neuroprotective therapy, warrant further studies better to define their use in PD and other movement disorders.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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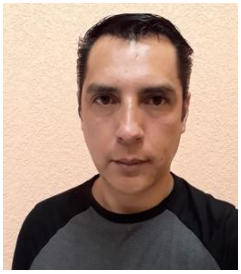
Biography of author(s)



José Luis Ordoñez-Librado
Neuromorphology Lab, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.

Research and Academic Experience: He Graduated from a Biology degree, a master's degree in Experimental Biology and PhD in Neurobiology. He has 10 years of teaching experience in medicine. He has directed and participated in the evaluation of multiple undergraduate theses; He has participated in various national and international conferences.
He has carried out biomedical research contributing to the characterization of the model for the study of Parkinson's disease by manganese inhalation.

Research Area: His Research Area includes Neurobiology of Nuerodegenerative Diseases.

Number of Published Papers: He has 17 research articles in the national and international journals.

Any Other Remarkable Points: He has 15 Chapters in international Books.

Ana Luisa Gutierrez-Valdez
Neuromorphology Lab, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.

Research and Academic Experience: She has Experience in basic biomedical research on physiological and behavioral aspects in neurodegenerative processes, as well as the impact of drugs on the adaptive plasticity of the nervous system.
She has Presentations at national and international conferences, Support and advice for pre graduate students.

Research Area: Neurodegeneration.

Number of Published Papers: She has 22 research articles in the national and international journals.

Any Other Remarkable Point: She has Published chapters (15) and Books (2).

Jesus Espinosa-Villanueva
Neuromorphology Lab, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.

Research and Academic Experience: He has 36 years of Experience as a Histology Technician.

Research Area: Neuromorphology.

Number of Published Papers: He has 35 research articles in the national and international journals.

Enrique Montiel-Flores
Neuromorphology Lab, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.

Research and Academic Experience: He has 27 years of Research and Academic Experience.

Research Area: His Research Area includes Neurosciences, neuromorphology, animal models of Parkinson's and Alzheimer's disease.

Number of Published Papers: He has 14 research articles in the national and international journals.

Patricia Aley-Medina
Neuromorphology Lab, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.

Research and Academic Experience: She has 33 years of Experience as a Electron Microscopy Technician.

Research Area: Neuromorphology.

Number of Published Papers: She has 12 research articles in the national and international journals.

Javier Sánchez-Betancourt
Neuromorphology Lab, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.

Research and Academic Experience: He graduated from Universidad Nacional Autonoma de Mexico (UNAM) with a degree in psychology and obtained a doctorate in behavioral neuroscience from UNAM. He has participated in lines of research in which the inhalation of manganese in rodents has been proposed as a reliable model for the study of Parkinson's disease. One of the articles with the greatest impact is entitled "Manganese mixture inhalation is a reliable Parkinson disease model in rats". He has been a contributor to research projects on the use of treatments including melatonin and other drugs to improve

treatments used in Parkinson's disease. He currently works at the Universidad Autonoma de Baja California (UABC) as a professor-researcher developing research that associates lifestyle (quality of sleep, diet and physical activity) with brain functions. He has published work evaluating the effect of melatonin on cognitive functions and mood. He runs a social service program in which neuropsychological evaluations are given to older adults. In 2008 he received the award of "To the best of psychology in Mexico" for his contributions to the knowledge of the pharmacokinetics of antidepressants. Additionally, he has taught courses on neuroanatomy, psychophysiology and statistics at institutions such as UNAM and UABC. One of his main goals is to remain a member of the National System of Researchers (SNI) and to consolidate an academic body.

Research Area: His Research Area includes Parkinson Disease, Lifestyle and brain.

Number of Published Papers: He has 11 research articles in the national and international journals.

Special Award: "A lo mejor de la investigación en psicología 2008" by AMERSE.

Claudia Dorado-Martínez
Neuromorphology Lab, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.

Research and Academic Experience: She has B.Sc. in Pharmacy, complemented by a five-year long Medicine program and a two-year long Neuroscience program.
She has Three years of working Experience as part of a neurodegenerative disease research group. She has 15 years of Experience as a university professor of pharmacology and development biology at a medicine school.

Research Area: Neurodegenerative diseases, medical education.

Number of Published Papers: She has 18 research articles in the national and international journals.

Special Award: Travel Award from the International Behavioral Neuroscience Society (IBNS).
Honorable Mention in the professional exam of the master's in education.

Leonardo Reynoso-Erazo
Health Education Project, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.

Research and Academic Experience: He is the head of Behavioral Medicine Residency program.

Research Area: Stress and chronic diseases.

Number of Published Papers: He has 54 research articles in the national and international journals.

Rocío Tron-Alvarez
Health Education Project, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.

Research and Academic Experience: She has Experience on Methodology and Statistic.

Research Area: Neuroscience.

Number of Published Papers: She has 22 research articles in the national and international journals.

Vianey Rodríguez-Lara
Department of Cell and Tissue Biology, Faculty of Medicine, UNAM, Mexico City, Mexico.

Research and Academic Experience: She is a Professor at undergraduate and postgraduate level in Medical School and researcher in cell and cancer biology areas.

Research Area: Her Research Area includes CELL BIOLOBY, HORMONES, and CANCER.

Number of Published Papers: She has 32 research articles in the national and international journals.

Special Award: SNI I (national), PRIDE C (institutional).

Maria Rosa Avila-Costa
Neuromorphology Lab, Facultad de Estudios Superiores Iztacala, UNAM, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Edo. Mex. 54090, Mexico.

Research and Academic Experience: She has PhD degree,
She is a Full time Titular Professor,

Research Area: Neurobiology of Neurodegenerative diseases.

Number of Published Papers: She has published 5 articles in national journals and 52 in high impact international Journals. She has published one national book and four international ones; she is also the author of 20 chapters in national and international books.

Special Award:

- Sor Juana Inés de la Cruz medal in 2007
- State Prize for Science and Technology in the area of Health Sciences in 2011.

Any other remarkable point(s): She studied a degree in Psychology, a master's degree in Neurosciences and a PhD in Biological Sciences at UNAM. She was awarded by the UNAM, with the Sor Juana Inés de la Cruz medal in 2007 and with thecState Prize for Science and Technology in the area of Health Sciences in 2011. She belongs to the National System of Researchers Level II. Her field of study focuses on the Neurobiology of Neurodegenerative diseases, mainly Parkinson's disease. She is responsible for the Neuromorphology Laboratory where she has trained undergraduate, master and doctoral students. Her publications have been cited more than 550 times. Recently, She proposed a novel experimental model for the study of Parkinson disease, which has had a great impact at the international level. She has participated in the review and update of academic programs, has made 90 reviews on scientific articles for specialized international journals, has evaluated several SEP-CONACyT projects, and provides specialized advice to research projects. She currently collaborates with the Epilepsy Genetics / Genomics Laboratories and Comprehensive Epilepsy Program group, UCLA School of Medicine and VA GLAHS West Los Angeles Medical Center.

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Chapter 6
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Novel Pyruvate-Enriched Medical Solutions and Beverages

Zhou, Fang-Qiang [a*]

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ABSTRACT

The objective of this review is to introduce novel uses of pyruvate-enriched fluids. Pyruvate has been extensively and intensively studied since a half century ago. An abundance of experimental research in both animals and humans, *in vitro* and *in vivo*, demonstrate that pyruvate is a unique anion, which is more beneficial in protection of multiple cell/organ metabolism and function than anions in commercial medical fluids and health beverages. The robust advantages of pyruvate action are mainly enhancement of anoxia/hypoxia tolerance, correction of hypoxic lactic acidosis and improvement of glucometabolic disorders in addition to anti-oxidative stress/inflammation and protection of mitochondria, leading to reversal of the Warburg effect in various pathogenic attacks, including severe hypoxia/ischemia, hypo/hyperglycemia, trauma/burn and sepsis. Many investigations in animals and humans, *in vivo*, reveal pyruvate protections with absence of clinical adverse effects. Innovative pyruvate-enriched fluids, both crystalloids and colloids, would be more favorable than current fluids in clinical resuscitation due to therapeutic effects additionally as a volume expander. Pyruvate-enriched oral rehydration salt (Pyr-ORS, equimolar pyruvate replacement of alkalizers in WHO-ORS) also would be more beneficial than WHO-ORS in oral rehydration, peri-operative fluid management and prehospital rescue. Alternatively, oral Pyr-ORS-based beverages may be favorable in plateau tourism, diabetes care and anti-aging. This review cited most important animal experiments and human tests with applicated pyruvate dosages, demonstrating the clinical effectiveness and safety and revealing innovative Pyr-ORS-based beverages as both medical care fluids when short of medical supply and functional drinks in endurance exercises. Pyruvate, as a novel nutritional componence, applications in clinical scenarios would be another most important medical advance in this century.

Keywords: Beverage; endurance; pyruvate; oral rehydration salt; resuscitation.

1. INTRODUCTION

Pyruvate is a key metabolite in glucose metabolism, which holds several beneficial biomedical and pharmacological properties superior to current medical anions in cell/organ metabolism and function. Pyruvate-enriched fluids have been demonstrated to be advantageous over commercial fluids in resuscitation from various severe injuries in many animal models [1,2]. Pyruvate-enriched oral rehydration salt/solution is a novel ORS that cannot be only a therapeutic solution, but also a health functional beverage [3,4]. However, sodium pyruvate is non-FDA approved for medical uses to date. This review mainly focuses on the possibility and feasibility of pyruvate-enriched ORS as a novel functional beverage.

2. PYRUVATE-ENRICHED ORS PROTECTION AGAINST VARIOUS INJURIES IN ANIMALS

In 2012, sodium pyruvate (SP, pyruvate)-enriched oral rehydration salt/solution (Pyr-ORS) was first innovated by equimolar pyruvate replacement of alkalizers in World Health Organization-guided ORS

[a] Fresenius Medical Care, Dialysis Centers in Chicago, IL, USA.
**Corresponding author: E-mail: fqzh60130@yahoo.com;*

that contains salts and glucose for treatment of children diarrhea. WHO-ORS saved a couple of million young lives worldwide per year. It was discovered that enteral Pyr-ORS was advantageous over traditional bicarbonate-based WHO-ORS (I) in intestinal absorption of sodium and water and protection of barrier structure and function in rats subjected to severe shock [3]. Then, Pyr-ORS was further demonstrated with more protection of intestinal energy metabolism, additionally via hypoxia-inducible factor-1α (HIF-1α) activation, inhibition of oxidative stress/ inflammation, increase of systemic and visceral blood flow and preservation of multiorgan (brain, heart, liver, kidney and intestine) function, resulting in severe acidemia correction and significant survival improvement than WHO-ORS (I; II and III: citrate-based) in severe shock-injured models with hemorrhage, burns or cardiac arrest [4-8]. Notably, even the buffer capacity is equal (30 bicarbonate mmol/L) in both Pyr-ORS and WHO-ORS, neither bicarbonate-, nor citrate-based WHO-ORS corrected hypoxic lactic acidosis (one of lethal complications in critical care patients), but did Pyr-ORS, robustly prolonging survival in animal studies [4,5,7]. That is because only can pyruvate consume hydrogen ($[H^+]$) in three metabolic pathways as a prospective alkalizer: 1) the pyruvate reduction coupled with the nicotinamide adenine dinucleotide reduced form (NADH) oxidative reaction by stereo-specific lactate dehydrogenase (LDH) to raise the oxidized form NAD^+/NADH ratio throughout the body, 2) the reactivation of pyruvate dehydrogenase (PDH) activity to promote oxidative phosphorylation in the tricarboxylic acid (TCA) cycle of almost all organs and tissues and 3) consumption of additional hydrogen in pyruvate-based cytosolic gluconeogenesis mainly in liver and kidney [4,7,9], reversing the Warburg effect in glucometabolic disorders [10,11]. The post-pyruvate metabolic profile was previously illustrated [9-11]. Oral pyruvate in Pyr-ORS multifaceted protection of organ metabolism and function above was supported by many previous studies with intravenous (IV) pyruvate and further evidenced by pyruvate peritoneal resuscitation in shock resuscitation of rats [10,12,13]. It is worthy of noting that pyruvate of sodium salt in animal shock resuscitation displays superiorities to anions in current medical fluids and commercial beverages: chloride, bicarbonate, lactate, acetate, citrate, phosphate and gluconate in the correction of hypoxic lactic acidosis and improvement of glucose metabolic disorders [7,10,11]. Although malate exerts in these respects [14], it still cannot protect red blood cells (RBCs) without aerobic metabolism, compared to pyruvate [15]. Alternatively, oral ingestion of Pyr-ORS and simple SP also clearly provided ergogenic effects on protection of multiorgan (brain, eye and kidney) function against aberrant glucose metabolisms in diabetic cataract, retinopathy and nephropathy and aging [11,16-19].

3. PYRUVATE PROTECTION AGAINST MULTIORGAN DYSFUNCTION IN HUMANS

Albeit non-FDA approved and no studies on Pyr-ORS yet in the clinical setting, clinical investigations of IV pyruvate even started in the 1930s. In 12% concentration, 18.8g sodium pyruvate was intravenously injected for exploring its metabolism in 7 psychologic patients [20]. About 2 decades late, 18 non-diabetic subjects and 19 diabetes patients were infused with IV10.0g (10% in 100 ml) SP over one hour period to exam pyruvate metabolism, accompanied with another mimetic report [21]. In 1996, the first clinical therapeutic investigation of pyruvate effects on chronic liver diseases in 10 patients was reported with a large dose of IV pyruvate (54.0-86.4g/d for 10 days) and followed by additional case reports, showing promising clinical and pathological improvements, clinical tolerance and safety without toxic adverse effects [22,23]. Further, the Lancet first released a clinical study on 8 patients subjected with dilated cardiomyopathy in 1999. A total of 4.58g pyruvate was infused in 30 min via the coronary artery per patient, leading to an immediate significant improvement of cardiac function [24]. Subsequent studies were followed with a similar approach in 9 patients subjected to congestive heart failure and 8 patients with acute myocardial infarction with cardiac shock, resulting in promising outcomes [25]. Furthermore, a small dose of pyruvate in cardioplegia was also demonstrated with a robust cardio-protection against surgical cardiac arrest in 15 patients underwent bypass surgery [26]. The only side effect is vascular pain in locations injected with hypertonic pyruvate solutions [21]. Pyruvate is rapidly metabolized, *in vivo*, in both animals and humans: the peak level in blood after pyruvate infusion can be down close to normal in about 30 min [21,27], suggesting its rapid systemic metabolism. However, the pyruvate effects can prolong for at least several hours probably due to its stimulation of HIF-1α activity [6].

On the other hand, oral ingestion of a large amount of SP products (30-60g/d for 7-10d) showed significant therapeutic effects in treatment of 6 Type 1 diabetic patients and 1 mitochondrial diabetic

patient (0.5 g/kg thrice daily for 10 months) with a remarkable reduction of total daily dose of insulin injection due to hypoglycemia and the stimulation of insulin secretion [28,29]. Interestingly, oral pyruvate with 0.3 g/kg/d for 3-6 month also increased fasting insulin secretion in 10 non-diabetic children [30]. Further, a large dose with long-term oral pyruvate therapy indicates the clinical effectiveness on mitochondrial dysfunction in dozens of patients [31]. Despite the small size of patients in each report, all the data released strongly suggest that oral pyruvate protects systemic metabolism and multiorgan function in absence of clinical adverse effects except gastrointestinal irritation. The clinical safety is further reinforced with several IV pyruvate loading tests (SP 10.0g over 4 min or 0.5g/kg over 10 min) with a well clinical tolerance and acceptance in several dozens of young and adult patients during past decades [32,33].

In all, extensive and intensive studies in animals and humans, both *in vitro* and *in vivo*, explicitly illustrate the pyruvate superiority in various routes of its administration, including IV infusion, peritoneal injection, oral taking and inhalation, with a diverse range of doses in past decades [22,26,34-36]. Therefore, pyruvate beneficial protection of cell/organ metabolism, function and survival is an acknowledged concept in the biomedical area to date.

4. PYRUVATE POTENTIAL ADVANTAGES IN BEVERAGES

Pyruvate superior biomedical features in medical fluids: The biomedical features demonstrated from a huge of studies above substantiate that pyruvate is a specific anion that holds pharmacological beneficial properties: increase of hypoxia tolerance and redox potentials, exertion of antioxidative stress and anti-inflammation, correction of hypoxic lactic acidosis and protection of mitochondrial function and against cellular apoptosis [11,17,34-38]. These characteristics are unparallel with current anions in medical fluids. Therefore, pyruvate-enriched fluids, such as pyruvate saline ([Na^+] 154 mM, [Cl^-] 104 mM, [Pyr^-] 50 mM), pyruvate Ringer's solution ([Pyr^-] 28 mM), Pyr-ORS (sodium pyruvate 3.5g/L) and hypertonic pyruvate saline (NaPyr 0.5-1.0 M) as well as pyruvate-based peritoneal dialysis solution ([Pyr^-] 40 mM) revealed the superiority in fluid resuscitation and dialysis to current commercial fluids [7,15,37,39]. Pyruvate-enriched fluids in both crystalloids and colloids will be not only volume expanders, but also therapeutic agents to directly improve organ metabolism and function additionally as a nutritional therapy, compared to current fluids basically as a volume expander only [10,40], in critically ill patients.

Pyruvate-enriched ORS as medicine and functional beverage: Pyr-ORS as the prototype (the formula that consists of sodium, chloride, potassium, pyruvate as a superior alkalizer and glucose can be improved by adding favorable components, if needed) is an innovative medicine as well as pyruvate-enriched functional beverages as a phenotype of drinks. Even though Pyr-ORS has not yet been employed in clinical settings, in Critical Medicine better outcomes of its rehydration of children diarrhea and cholera as well as burn injury would be convincible, relative to WHO-ORS (Ceralyte®90: US Cera products, or Pedialyte: US product) [41]. In addition, in oral peri-operative fluid management Pyr-ORS may be more beneficial, compared to lactate-based OS1® (Japan product) [42]. In Par/enteral Nutrition, additions of pyruvate as a nutritional ingredient may enhance the clinical efficient of current products. In Disaster Medicine, Pyr-ORS-based fluids can provide a feasible approach in a large scale of prehospital rescue scene, like earthquake and terrorist attack, without sufficient medical supply and just use as a novel functional drink to win the golden window for saving lives. In Sports and Altitude Medicine, Pyr-ORS or pyruvate-enriched beverages may show its advantages predominantly because of improving hypoxia tolerance, energy metabolism and lactic acidosis reversal in strenuous exercises and high attitude hypoxia. Although the famous drink like Red-Bull (taurine and caffeine) is popular, it is still controversial to enhance endurance performance [43,44]. It is well known that alkalizers like bicarbonate can improve endurance performance by affecting buffering capacity and lactic acidosis may be a critical factor in limiting performance in exhaustive sports [45,46]. Pyruvate-enriched beverages may improve endurance performance and prevent from mountain sickness responses and heatstroke by eliminating severe metabolic acidosis and improving energy metabolism. In Diabetes, a beverage based on Pyr-ORS formula may prevent and treat diabetes and its organ complication in a large population [11,16-18]. Most likely, pyruvate-enriched fluids are favorable in Geriatric Medicine and Pyr-ORS as a beverage may be helpful in anti-aging, as demonstrated in the protection against acute or chronic brain injury like traumatic brain injury and

Alzheimer's disease [8,19,47,48]. Interestingly, it is recently proposed that oral pyruvate in ORS, like melatonin, potentially facilitates the prophylaxis and intervention of Covid-19 and other severe viral infections with or without specific anti-virus agents [49]. The pyruvate clinical application was recently encouraged in shock therapy and is potentially another important advance in medical history [50].

Notably, exogenous pyruvate can spontaneously provide NAD^+ on the equal molecular basis via the spontaneous LDH reductive reaction with free of energy throughout the whole body in anoxia. It is a well acknowledged discovery that NAD^+, a molecule of youth, is essential for most enzymatic reactions associated with diseases and aging [51,52]. At least, a clinical trial with nicotinamide riboside (NR, a precursor of NAD^+) in patients with Covid-19 infection is recruiting, indicating that pyruvate in Pyr-ORS has potential modulation of immune function in fighting Covid-19 [49]. Nevertheless, preliminary investigations, *in vitro*, displayed that pyruvate more protected cell survival than equimolar NAD^+ in selected insults and cell lines [53,54]. Pyruvate, at least, is theoretically better in the reactivation of PDH activity and correction of hypoxic lactic acidosis, oxidative stress and hypo/hyperglycemia than equimolar NAD^+ though direct comparable evidence is lack, *in vivo*, yet [52]. Currently, pyruvate as an ingredient is not included in almost all commercial beverages in exception of a couple of drinks (Hansen's Slimdown™ and Pyru Forces™) with unfavorable formula, so that both show no ergogenic effects [55]. Although taurine, which is considered a semi-essential amino acid with insulin-like activation, has recently been indicated with supplemental effects in endurance exercises [56], it holds no property to improve the PDH activity and correct hypoxic lactic acidosis, as pyruvate expected in animal shock resuscitation. To compare taurine with pyruvate in exhaustive exercises may be attractive. Therefore, those with Pyr-ORS as a prototype of functional pyruvate-enriched beverages are innovative efficient products for both diseases and health, such as protection against critical illness and diabetes and service as healthy aging in a population [3-8,11,50,52].

5. FEASIBILITY OF PYRUVATE-ENRICHED BEVERAGES

Clinical data above of several hundred patients suffered from various diseases including chronic cirrhosis suggest its clinical safety. Early animal acute toxicity tests showed that the LD_{50} of oral pyruvate is 10.0g/kg in rats [22]. However, a large dose of oral pyruvate is gastrointestinal irritative, such as flatulence and diarrhea [22,27], which is prevalently unacceptable if used in a population, while a single dose less than 25g neither raises blood pyruvate levels, nor functions well [57,58]. On the contrast, a small amount of oral pyruvate in Pry-ORS (3.5g/L) robustly enhances pyruvate levels in plasma and exerts functionally [3-8]. Hence, the improvement of pyruvate supplementation regime is essential by a small dose of pyruvate together with sufficient glucose in Pyr-ORS formula, which is optimal for intestinal absorption of water and salts along with pyruvate, according to the physiology of intestinal epithelium with 'Na^+-glucose cotransporter' that is exactly the basis for WHO-ORS development, as functioned in shock resuscitation [3,4,7]. Prospectively, most of current beverages (commonly containing salts and glucose) may create a new brand with the pyruvate addition.

Although pyruvate dimers (para-pyruvate) spontaneously generated in pyruvate aqueous solutions at room temperature are cytotoxic, *in vitro*, no toxic side effects, *in vivo*, have been appeared in humans to date [50]. Enteral pyruvate in a relatively small dose as Pyr-ORS even containing a trace of para-pyruvate should be acceptable in clinical scenarios, not to mention the fact that IV pyruvate products were applied in patients suffered from parenchymatous diseases, which contained around 1.0% para-pyruvate, at the time several decades ago. In fact, appropriate acidic pyruvate solutions are long-term stable at room temperature with the patent protection [50]. Accordingly, commercial pyruvate-enriched beverages based on Pyr-ORS formula are feasible in the near future.

Calcium pyruvate is available in food supplement markets. However, the calcium salt is poor dissolvable in water and malabsorptive from intestines. Studies in exercises with oral calcium pyruvate indicate its ineffectiveness in humans [57-59]. However, the rise of its dissolvability in water by sufficiently acidic pH adjusting may raise the possibility to partially replace sodium salt of pyruvate in the pyruvate-enriched beverage products. It is worthy of note that ethyl pyruvate (EP) is not allowed to replace SP here. Albeit numerous studies, including oral EP, showed its pharmacological effects as SP on various animal models in the last decade [60], EP did not correct severe acidosis because of $[H^+]$ generation after its hydrolysis with or without the esterase, separating pyruvate moiety, whereas

accumulated blood lactate was eliminated in septic rats [61]. Importantly, EP does not work in humans [50]. Interestingly, recent findings showed that oral lactate favored oxidative metabolism of endurance training in mouse muscle, but a pyruvate counterpart was not compared [62]. The LDH reaction is bidirectional with preferable lactate generation; exogenous pyruvate reduction is more favorable with the LDH (A isoenzyme) than the lactate oxidation with the LDH (B isoenzyme) in cytosol, so that lactate infusion raises less blood pyruvate than blood lactate rises after equimolar pyruvate infusion [9]. Despite both pyruvate and lactate as energy substrates, they are quite opposite in the last step of glycolysis, acid-base balance, redox potential and oxidative stress by the LDH reaction and pyruvate oxidizes with a less oxygen consumption rate, compared to lactate. The impact of ingredients in functional drinks on metabolic and physiological adaptations has been concerned in endurance training [63], but their clinical implications have been less examined in a young and adult population [64]. Clinical trials with pyruvate-enriched fluids are urgently warranted in critical care patients and Pyr-ORS based functional beverages require comparative controlled tests in anti-diabetes/aging and exercise performance.

6. CONCLUSION

Pyruvate owns superior biomedical and pharmacological characteristics to anions in current medical fluids and beverages in cell metabolism and function. The pyruvate-enriched beverage based on Pyr-ORS formula will be an innovative nutritional product as a health drink as well as a medicine when needed. Further studies and clinical trials are warranted.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Biography of author(s)

Zhou, Fang-Qiang
Fresenius Medical Care, Dialysis Centers in Chicago, IL, USA.

Research and Academic Experience: He graduated from Shanghai Medical College, Fudan University, Shanghai, in 1965. He participated in the first clinical research case of successful cadaver kidney transplantation and clinical studies on dialysis with domestic products of dialysis solutions in China in the 1970s.
In 1986, he as a Nephrologist learned in Brigham and Women's Hospital, Harvard University, Boston, MA as a visiting scholar for two years. He, then, worked as a research associate at renal division in Loyola University Medical Centre, Chicago, IL. He as a team member first found in 1995 that pyruvate is superior to lactate in peritoneal dialysis solutions in protection of human neutrophils producing superoxide for prevention of peritoneal infection, which is closely associated with pyruvate sustention of a near normal range of neutrophils' intracellular pH. Based on this novel finding, he proposed an idea that pyruvate may be more beneficial than anions e.g. chloride, acetate, lactate and citrate in current medical fluids. Then, he independently investigated many animal models with shock by resuscitation with pyruvate in comparison with current anions, confirming the pyruvate superiority in fluids therapy. Since 2008, he also found that pyruvate significantly protects diabetic retinopathy in rats and first demonstrated that oral pyruvate prevents the progression of diabetic nephropathy in diabetic mice in 2020. In 2012, he first discovered that pyruvate in oral rehydration salt (ORS) is more beneficial than WHO-ORS in organ metabolism and function in shock resuscitation. He recently recommended that oral pyruvate is also anti-aging/diabetes as a functional beverage.

Research Area: His Research Area includes Pyruvate in Dialysis, Shock Resuscitation, Fluid Therapy, Diabetes and Healthy Aging.

Number of Published Papers: He has Over 50 research articles in the national and international journals.

Any Other Remarkable Point: He has 3 Patents on pyruvate issued by USA and China.

DISCLAIMER
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Chapter 7
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Correction of Skeletal Class II Malocclusion – An Accelerated Osteogenic Orthodontic Approach

A. Arif Yezdani [a*#]

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ABSTRACT

Selective alveolar decortication and periodontal augmentation with a bone graft were the two procedures used for the correction of the skeletal Class II malocclusion in the case reported. A 25-year-old male patient presented with a skeletal Class II malocclusion with increased bi-maxillary dentoalveolar protrusion, increased overjet, deep bite and imbricated & rotated mandibular incisors with bilateral presence of supernumerary teeth in the maxillary right and left premolar region. Extraction of the supernumerary tooth in the maxillary right and left premolar region as also the impacted UL5 was done. Pre-adjusted edgewise appliance, Roth's prescription (0.022 x 0.028-inch slot) was strapped up and a week later full thickness labial and lingual flaps were reflected in the maxillary and mandibular arches. Circumscribed corticotomy cuts were done and subsequently augmented with a bone graft. Orthodontic treatment was commenced immediately after surgery and orthodontic adjustments were carried out every 2 weeks. The entire orthodontic treatment was completed in 9 months. The primary objective was to evaluate whether selective alveolar decortication could accelerate orthodontic tooth movement and reduce the duration of orthodontic treatment. It was observed that regional acceleratory phenomenon, triggered by alveolar decortication was responsible for the rapid correction of the malocclusion and augmentation with the bone graft further provided adequate bone volume for housing the teeth. This combined procedure might probably contribute to the decrease in the possibility of the tell-tale relapse.

Keywords: Supernumerary premolar; decortications; bone graft; fixed appliance therapy; orthodontic tooth movement.

1. INTRODUCTION

Invasive or non-invasive modalities of treatment to accelerate orthodontic tooth movement has been reported by many authors in the literature [1-4]. Adult or late adolescent patients requiring orthodontic correction of malocclusion desire short treatment periods due to social and psychological reasons which in the opinion of certain authors is possible only with surgical procedures [5,6]. Orthopedist Harold Frost termed the cascade of physiologic healing events following surgical wounding of cortical bone the Regional Acceleratory Phenomenon (RAP) [7,8]. This phenomena is taken advantage of in bringing about rapid orthodontic tooth movement which together with periodontal augmentation with bone graft increases the alveolar bone housing as also corrects alveolar bone dehiscences and fenestrations [9]. The bone graft provides additional bone support, resulting in less tendency for relapse [10]. It also improves the posture of the lip in certain conditions. Animal experiments too have demonstrated an increase in apposition and resorption of alveolar spongiosa adjacent to corticotomy cuts [11].

Hence, in the case reported, circumscribed corticotomy cuts and augmentation with bone graft was carried out to increase the rate of orthodontic tooth movement and shorten the period of orthodontic treatment.

[#]Professor and Director;
[a] Department of Orthodontics and Dentofacial Orthopedics, Bharath Institute of Higher Education and Research, Sree Balaji Dental College and Hospital, Narayanapuram, Pallikaranai, Chennai-600100, Tamilnadu, India.
**Corresponding author: E-mail: arifyezdani@yahoo.com;*

2. CASE REPORT

A male patient aged 25 years presented with forward placement of maxillary anteriors and crowded mandibular anteriors with bilateral supernumerary premolar teeth in maxillary dental arch.

2.1 Extra-oral Assessment

The patient had a mesoprosopic face, convex profile, posterior divergence, incompetent lips, clinical low mandibular plane angle, incompetent lips and excessive maxillary incisor display on smiling, with no signs of temporomandibular joint dysfunction (Figs.1a-c).

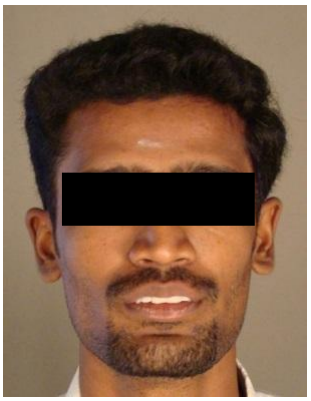

Fig. 1a. Pre treatment extra-oral - Frontal

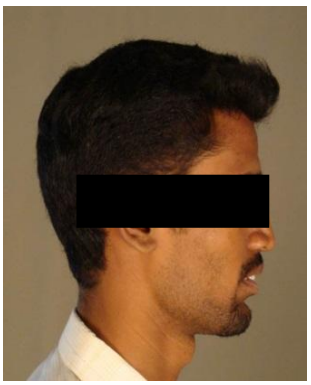

Fig. 1b. Pre treatment extra-oral - Profile

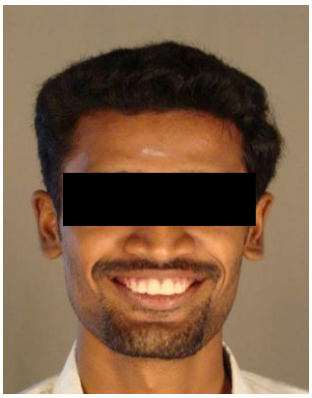

Fig. 1c. Pre treatment extra-oral - Smiling

2.2 Intra-oral Assessment

Oral hygiene was satisfactory. Maxillary arch was U-shaped, asymmetrical with a supernumerary premolar present bilaterally between UR4 and UR5 and UL4 and UL5, an impacted UL5 and proclined maxillary incisors. Mandibular arch was U-shaped, asymmetrical with proclined, rotated and imbricated mandibular incisors.

In occlusion, increased overjet and deep bite was observed. The maxillary dental midline was shifted to the left by 1mm and on left side the molar relation was Class II and on the right it was Class I while the canine relation was end-on bilaterally with UL5 and UL6 in mild palatal cross-bite (Fig.1d-h).

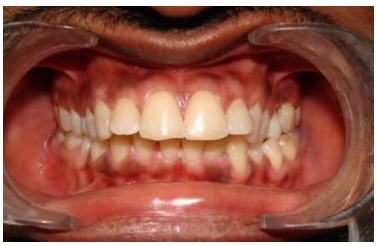

Fig. 1d. Pre treatment intra-oral - Frontal

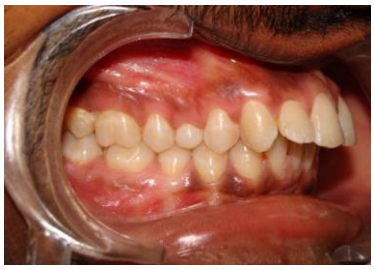

Fig. 1e. Pre treatment intra-oral - Right

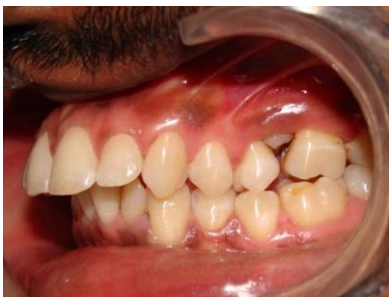

Fig. 1f. Pre treatment intra-oral - Left

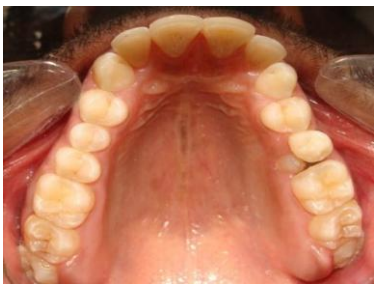

Fig. 1g. Pre treatment intra-oral - Upper occlusal

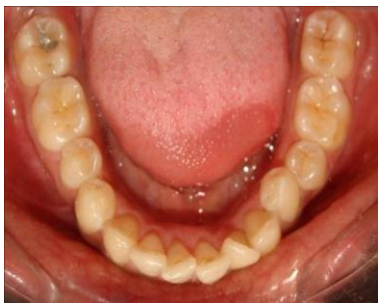

Fig. 1h. Pre treatment intra-oral - Lower occlusal

2.3 Radiographic Assessment

The panoramic radiograph confirmed the presence of all permanent teeth and normal alveolar bone levels except around impacted UL5. Impacted LR8 and LL8 with partially erupted UR8 and UL8 was also observed (Fig. 2).

Lateral cephalometric analysis revealed a skeletal Class II pattern with an orthognathic maxilla and retrognathic mandible with a low mandibular plane angle, severely proclined maxillary incisors and moderately proclined mandibular incisors (Fig.3).

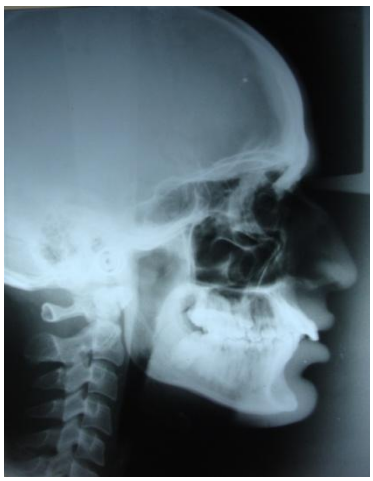

Fig. 2. Pre treatment panoramic radiograph

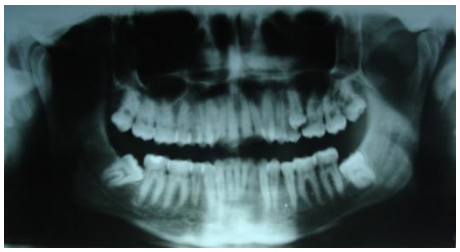

Fig. 3. Pre treatment lateral cephalometric radiograph

3. AIMS AND OBJECTIVES

1. Improve oral hygiene.
2. Improve facial profile and achieve lip competence.
3. Correction of protruded maxillary and mandibular teeth.

4. Correction of imbricated and rotated mandibular teeth.
5. Correction of end-on canine relationship.

The patient opted for the corticotomy procedure as he preferred the orthodontic treatment to be completed within a short period of time. Extraction of supernumerary in the maxillary right and left premolar region and impacted UL5 was done. Roth's prescription Pre Adjusted Edgewise Appliance Therapy (0.022 x 0.028-inch slot), (3M Unitek, Monrovia, CA, USA) with upper and lower 0.014-inch nickel titanium archwires alongwith transpalatal anchorage was strapped a week prior to the surgical procedure.

The patient underwent decortication of the alveolar bone and grafting with Grabio Glascera bone graft. (DORTHOM^{TM} MEDI DENTS PVT.LTD.), Coimbatore, Tamil Nadu, India). Grabio Glascera (GG) are bioactive, ceramic, composite, porous granules made up of :- 50% Bioactive Glass (BG) and 50% Hydroxyapatite (HA) mixture available in a particle size of 0.15 - 0.50 mm.

Orthodontic adjustments were carried out at 2 week intervals.

3.1 Surgical Procedure

Under local anaesthesia, labial and lingual sulcular incisions were made and full thickness mucoperiosteal flaps were elevated without disturbing the neurovascular bundles and the genioglossus attachment. A long shank surgical fissure bur was used to make the corticotomy cuts barely into the medullary bone on labial and lingual aspects of maxillary and mandibular anterior teeth alongside the roots with a scalloped cut slightly above the apices of the teeth. One to two mL of platelet rich plasma from the patient's blood was mixed with GG granules and placed over the decorticated area. The flaps were then returned to their original position and sutured with one interrupted loop 3/0 suture, interproximally (Fig. 4a-b, Fig. 5a-b, Fig. 6a-c).

Fig. 4a. Circumscibing corticotomy cuts - maxillary labial view

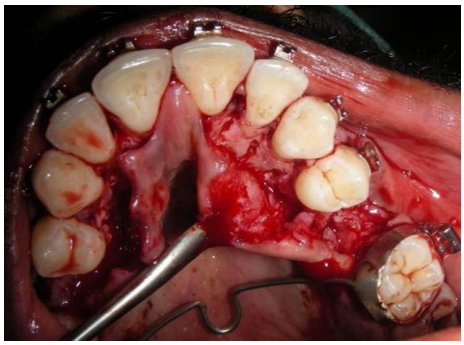

Fig. 4b. Circumscibing corticotomy cuts - maxillary palatal view

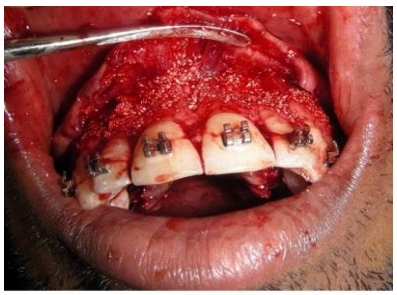

Fig. 5a. Alveolar augmentation with Grabio Glascera bone graft - maxillary anterior view

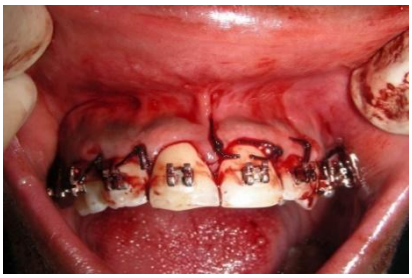

Fig. 5b. Suturing

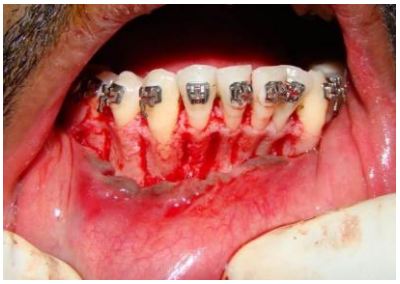

Fig. 6a. Circumscibing corticotomy cuts - mandibular anterior view

Fig.6b. Alveolar augmentation with Grabio Glascera graft - mandibular anterior view

Fig. 6c. Suturing

Amoxycillin 500mg t.id./week and anti-inflammatory drugs t.i.d/week were prescribed. An antiseptic mouthwash was advised. Two weeks later the sutures were removed and non-steroidal anti-inflammatory drugs were asked to be discontinued as they would interfere with orthodontic tooth movement.

3.2 Orthodontic Procedure

The orthodontic treatment was commenced immediately after suture removal. Initial aligning and levelling was done with upper and lower 0.014-inch nickel titanium archwires, upgraded to 0.016-inch nickel titanium archwires. Extraction space closure for the maxillary dental arch was done with 9mm nickel titanium closed coil springs attached to crimpable hooks on 0.017 x 0.025-inch stainless steel archwires. Detailing and finishing was done with upper and lower 0.019 x 0.025–inch stainless steel archwires (Fig.7a-b).

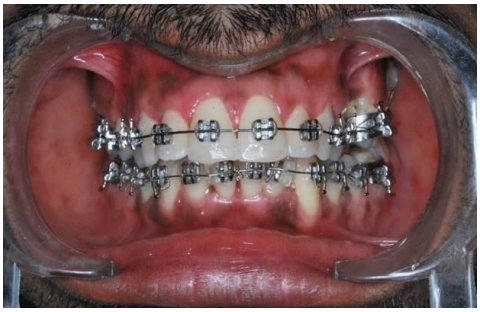

Fig. 7a. Orthodontic treatment – Frontal intraoral view

Fig. 7b. Orthodontic treatment - Left intraoral view

4. DISCUSSION

The patient showed remarkable improvement in his soft tissue profile and smile esthetics. The bi-maxillary dentoalveolar protrusion, increased overjet, rotated and imbricated mandibular incisors was corrected and a Class I canine relation was achieved in nine months (Fig.8a-e). The rapid correction of the malocclusion was most probably attributed to the RAP phenomena triggered by the corticotomy procedure which caused an increase in the osteoclastic activity of the bone. The resultant osteopenia was responsible for the rapid tooth movement that was observed. Corticotomy with bone augmentation increased the volume of the bone and maximized the metabolic response during orthodontic treatment in concert with what has been reported in literature [12]. Care was also taken to thin the bone on the distal aspect of the maxillary first premolar roots during the corticotomy procedure.

In the case reported full thickness mucoperiosteal flaps have been used. On the other hand, if one desires to minimize the invasive surgical corticotomy procedure, the flapless piezocision technique alongwith hard or soft tissue grafting via the tunneling procedure as advocated by Dibart *et al* could also be carried out as this procedure too enhances the periodontium considerably and reduces the duration of orthodontic treatment time [13-15]. Computer guided surgery with a translucent, rigid, 3D computer-assisted piezocision guide with buccal slots for precision in location, angulation and depth could also be used to avoid root injury and mitigate surgical complications [16-18].

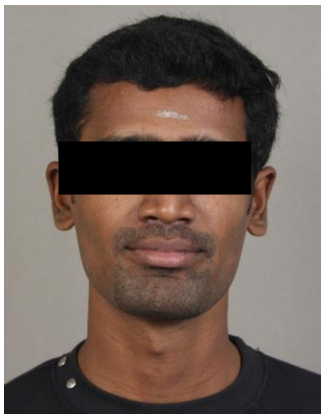

Fig. 8a. Post treatment extra-oral - Frontal

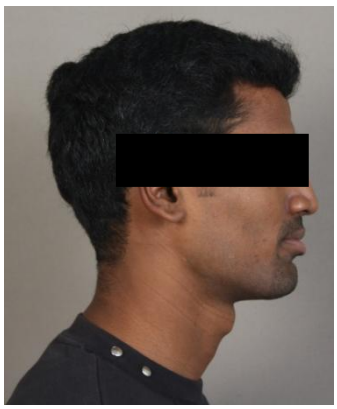

Fig. 8b. Post treatment extra-oral - Profile

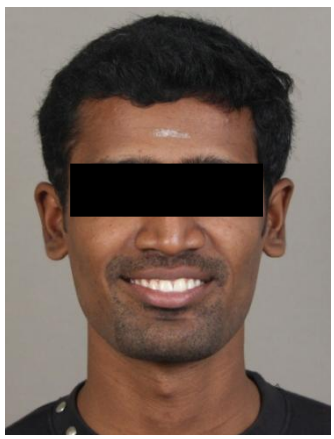

Fig. 8c. Post treatment extra-oral - Smiling

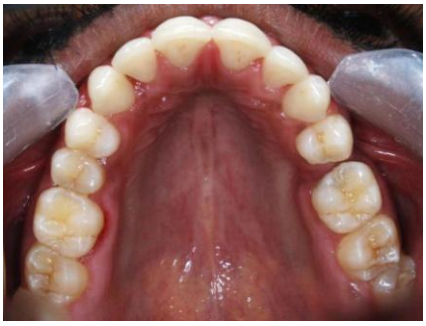

Fig. 8d. Post treatment intra-oral - Upper occlusal

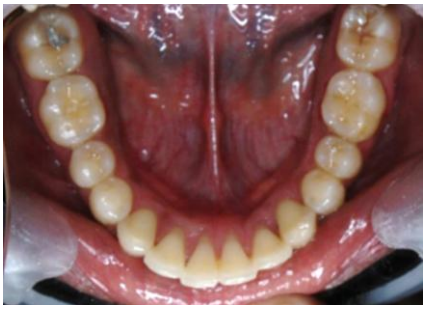

Fig. 8e. Post treatment intra-oral - Lower occlusal

No loss of tooth vitality, discoloration or pain was observed in tune with what has been reported in literature [19-21]. In conventional orthodontics, due to prolonged treatment duration or inadvertent application of heavy forces root resorption is observed [22]. In the case reported no root resorption was observed because of bone matrix transportation and reduced density of the bone due to osteopenia created by the corticotomy procedure. The patient was seen every 2 weeks throughout the phases of aligning, levelling, space closure, finishing and detailing to take advantage of RAP.

The periodontal alveolar augmentation with the GG bone graft increased the bone volume thereby reducing the incidence of relapse, a common occurrence, often observed when orthodontic treatment is done without bone grafting. This procedure is also useful for thinner mandibular cortices which are at increased risk for relapse subsequent to dental decrowding [23]. Fixed lingual retainers were given for both maxillary and mandibular dental arches. The rapid correction of the malocclusion was made possible because of the cortiocotomy facilitated procedure which also reinforces a claim made in literature that results are more stable with minimal risk of complications with faster orthodontic treatment after a corticotomy procedure [24].

Corticotomy facilitated orthodontics greatly contributed to the completion of the correction of the malocclusion in one-third to three-fourths the time required for conventional orthodontics.

5. CONCLUSION

The corticotomy facilitated orthodontics with selective alveolar decortication speedied up the correction of the malocclusion in just about nine months. The alveolar augmentation with the bone graft provided an increased bone volume to house the dentition. This created a stable and conducive environment to mitigate any occurrence of the tell-tale relapse.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Biography of author(s)

Dr. A. Arif Yezdani (MDS, FAGE, FWFO)
Department of Orthodontics and Dentofacial Orthopedics, Bharath Institute of Higher Education and Research, Sree Balaji Dental College and Hospital, Narayanapuram, Pallikaranai, Chennai-600100, Tamilnadu, India.

He has 32 years of teaching, clinical and research experience to his credit. His research areas of interest include Accelerated Osteogenic Orthodontics, Fixed Functional appliances, Temporary Anchorage Devices and Biologic skeletal maturity indicators in Orthodontics and Dentofacial Orthopedics. He has presented various scientific papers both in national and international conferences, symposiums, workshops & CDE programmes and published numerous publications in peer-reviewed national and international journals. Presently, he is an advisory board member of Indian Journal of Multidisciplinary Dentistry. He has also authored a book "Facial Esthetics – An Overview" published in 2012, by Lambert Academic Publishing GmbH & Co.,Saarbrucken, Germany and contributed Chapters 11, 12, 13 & 14 as Corresponding author in the book, "Challenges in Disease and Health Research – Vol. 4", published on 09 Oct, 2020, by Book Publisher International. ISBN-13 (15). 978-93-90431-79-3 (Print). 978-93-90431-84-7 (e-book) as also contributed a chapter in the book, "Highlights on Medicine and Medical Research – Vol 2"FIRST EDITION 2021, ISBN 978-93-90768-26-4 (Print), ISBN 978-93-90768-27-1 (eBook), DOI: 10.9734/bpi/hmmr/v2, published by Book Publisher International on 13 Feb, 2021.
He has worked as a Consultant and Specialist in prestigious Specialist Dental Centres of Ministry of Health, Kingdom of Saudi Arabia. In 1989, he was awarded "The President's Rolling Trophy" and Certificate for "Best Clinical Paper Presentation" at the 24th Indian Orthodontic Conference, held at Trivandrum, India. Was also awarded the 3rd prize in the "Clinical Innovation Category", at the 45th Indian Orthodontic Conference held in 2010 at Mangalore, India. Was conferred the "Best Teacher" award in 2014 by the Vice-Chancellor of Bharath University, Chennai, India. Won the best Poster Award at the 19th American Dental Congress at Phoenix, Arizona, USA and the "Best Dental Educator Award" (International Award of Excellence Dental Awards 2018) at the World Dental and Oral Health Congress held in London, UK and numerous other commendation awards, plaques & trophy for convening 185 scientific clinical society meetings in Sree Balaji Dental College and Hospital, Chennai, India.

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Chapter 8
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Assessment of Urinary Schistosomiasis Distribution among School Children at Elkeriab and Tayba ELkababish Villages, Sudan

Osama Hafiz Elshazali [a*], Qutoof Hashim Taha [b] and Abubakr Ibrahim Ahmed [b]

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ABSTRACT

Introduction: Schistosoma infection is one of the main infections in the tropics and sub tropics; from a global public perspective Schistosoma is the most significant water based disease. Only second to malaria among parasitic diseases with regard to the number of people infected and those at risk. The reported incidence of *S. haematobium* in Sudan is between 0-20%.
Aim: The aim of the study was to estimate the distribution of urinary schistosomiasis among school children in East Nile locality, Khartoum State and to identify the risk factors relating to *S. haematobium* infections
Methodology: This is a descriptive cross sectional study, we screened all the school age children from the two villages of Elkeriab and Tayba Elkababish in the East Nile locality, Khartoum state for *S. haematobium*, by examining the urine, using wet preparation and filtration technique.
Results: 1205 school children were screened for *S. haematobium*, 105 (8.7%) tested positive for Schistosoma, the infection rate was higher in Tayba Elkabish compared to Elkeriab (p value = 0.01) and in male more than females (p value = 0.01).
Conclusion: *S. haematobium* infection is still a common infection in school age children in rural areas especially within irrigated schemes, mainly affecting males. Schistosoma control programs have reduced the prevalence of *S. haematobium* infection but still there is a long road towards the eradication of Schistosoma.

Keywords: Children; Schistosoma haematobium; urinary Schistosomiasis; Sudan.

1. BACKGROUND

Schistosomiasis; also known as bilharzia is a parasitic disease prevalent in tropical and subtropical regions, caused by trematodes of the subclass Digenea, super family Schistosomatoidea and genus *Schistosoma*. More than 15 species of *Schistosoma* have been reported to cause infections in humans, but the major agents in causing human infections are S*. mansoni, S. heamatobium* and *S.japonicum* [1].

From a global public perspective Schistosoma is the most significant water-based disease. Schistosomiasis is ranked second only to malaria among the parasitic diseases with regard to the number of people infected and those at risk [2]. According to WHO, Schistosomiasis is endemic in 76 countries and territories, about 95% of the cases are in Sub Sahara Africa [2] there is a significant association between poverty and schistosomiasis; infection is higher in countries with limited health resources [3]. Schistosomiasis mainly affects school-age children, adolescent and young adults [4].

[a] *Department of Paediatric and Child Health, University of Khartoum, Sudan.*
[b] *Department of Microbiology, National Ribat University, Sudan.*
**Corresponding author: E-mail: o.elshazali@uofk.edu;*

In 2009, the World Health Organization had estimated that 239 million people were infected with Schistosoma [5]. Globally there are 150,000 deaths per year that are attributed to Schistosoma infection [6]. Schistosoma is primarily a disease of tropic and subtropic regions, this reflects the distribution of their intermediate host species of snails [1]. Recently outbreaks of Schistosoma were reported in other areas not known to have schistosoma e.g. Europe [7-9] with outbreaks seen in France and Spain.

It has become a serious public health and socio-economic issue in Sudan [10,11]. The reported prevalence rate of *S. haematobium* in Sudan is between 0-20% [2,7,8]. Schistosoma is widely prevalent in Sudan; it is estimated that there are around five million people requiring treatment whom are mostly children. Schistosomiasis mainly affects poor and rural communities, particularly agricultural populations. Inadequate hygiene and contact with infected water make children especially vulnerable to infection [12].

According to the WHO, the number of people in Sudan treated for the disease in 2011 was 2,281,000 and those who were in actual need for treatment were 5,820,000 [13] however, this number has increased especially after the spread of the disease to other regions that are not considered to be inhabited with the disease such as Khartoum state, Northern Kurdufan and some parts of Southern Darfur [14] due to the expansion in water resource development and increased population movement [15,16].

WHO recommends preventive chemotherapy (PC) consisting of periodic administration of Praziquantel for schistosomiasis as a short-term measure for the control of morbidity associated with schistosomiasis [17]. PC for schistosomiasis is required in 52 countries for a total of 219.9 million people; 60% of which are school-age children.

The goal of WHO is to treat at least 75% of school age children in all schistosomiasis-endemic countries by 2020. In 2017, 98.7 million people (81.8 million school age children and 16.9 million adults) received PC for schistosomiasis. The preventive chemotherapy cover in Sudan was reported to be lower than that of WHO's goal in the same year [17].

El-Selit irrigation scheme was established in the late 1980s in the East Nile locality of Khartoum State; from the early 1990s, a new focus of *S. haematobium* has been identified in the area [18]. El-Selit is irrigated by surface canals coming from the Blue Nile river. Living in close proximity of dams or surface irrigation schemes is one of the highest risk factors for Schistosomiasia [2]. Elkeriab and Tayba Elkababish villages are in the locality of El-Selit scheme.

2. OBJECTIVES

This study is aimed at estimating the distribution of urinary schistosomiasis among school children in East Nile locality, Khartoum State and identifying the risk factors relating to *S. haematobium* infections.

3. METHODS

This was a descriptive cross-sectional study conducted among primary-schools children in Elkeriab and Tayba El kababish villages. All the primary-school children in the two villages were tested for *Schistosoma heamatobium* ova in urine samples, using wet preparation and the filtration technique.

Inclusion Criteria: all the children who attended the primary schools in these two villages were included in the study. Children who were known to have Schistosoma, or received Schistosoma treatment in the last six months prior to the study were excluded from the study.

For the detection of *Schistosoma haematobium* ova we used a reusable monofilament polyamide (Nytrel) filter to filter the urine samples; as described by Mott et al. [19] we used this method as it is recommended by the World Health Organization [20] for the qualitative and quantitative diagnosis of *S. haematobium* infection. This method has been used extensively in field studies on the

epidemiology and control of urinary schistosomiasis. We did not use molecular methods for detecting S. *haematobium* [21] which are superior to urine microscopy as it is not available in Sudan.

Statistical Analysis Statistical Package for the Social Sciences (SPSS) (IBM. Armonk NY) Version 22 was used. Students; t-test were used to identify statistically significant differences.

Ethical consideration: The study was approved by the ethics committee of Khartoum State Ministry of Health. School administration officials in the locality, school headmasters and teachers were informed about the study. Informed consents were obtained from the families of all the children who participated in the study.

4. RESULTS

From the 1205 school children who were examined for evidence of *S. haematobium* infections in the two villages: 105(8.7%) school children showed evidence for *S. haematobium* infection (Table 1).

The infection rate among boys was significantly higher than when compared to girls, 103 out of the 616(16.7%) boys examined showed evidence of *S. haematobium infection*, while only 2 girls out of the 589 (0.3%) showed evidence of *S. haematobium* infection (p value = 0.01) (Table 2).

There was a significant difference in the distribution of the disease between the two villages: in Elkeriab village, 45 cases (5.4%) out of the 830 school children examined showed evidence of *S. haematobium* infection. In Tayba Elkababish, 60 cases (16%) out of the 375 school children examined showed evidence of *S. haematobium* infection (p Value = 0.01) (Table 3).

There was a significant difference in the age distribution of *S. haematobium* infection; the prevalence of the disease according to age group was 6 out of 382(1.5%), 33 out of 317(10.4%), 40 out of 272 (14.7%) and 26 out of 234 (11.1%) in the age groups 6-8 years, 8-10 years, 10-12 years, 12-14 years respectively **(**p value =0.04) (Table 4).

Table 1. Distribution of Urinary schistosomiasis in Tayba Elkababish and Elkeriab Schools

Village	Number examined	Tested Positive for *S. haematobium*
Elkeriab (Boys)	405	60 (14.8%)
Elkeriab (Girls)	425	0 (0%)
Tayba Elkakbish (Boys)	211	43 (20.3%)
Tayba Elkakbish (Girls)	164	2 (1.2%)
Total	1205	105 (8.7%)

Table 2. Distribution of Urinary Schistosomiasis among schools children according to Sex

Sex	Number examined	Tested Positive for *S. haematobium*
Male	616	103(16.7%)
Female	589	2(0.3%)
Total	1205	105(8.7%)

(p value =0.01)

Table 3. Distribution of Urinary Schistosomiasis according to the Village

		Urinary schistosomiasis		Total
		Positive	Negative	
	Elkeriab Village	45	785	830
	Tayba Elkakbish	60	315	375
Total		105	1100	1205

(p value = 0.01)

Table 4. Distribution of Urinary Schistosomiasis infection according to age

Age Group (Years)	Positive	Negative	Total
6 - < 8	6 (1.5%)	376	382
8 - <10	33 (10.4%)	284	317
10 - < 12	40 (14.7%)	232	272
12 -<14	26 (11.1%)	208	234
	105	1100	1205

(p value = 0.04)

5. DISCUSSION

The total prevalence rate of *S. haematobium* infection is 8.7 %, this is lower than the majority of the previous studies. A study by Ismaiel et al*.* [22] in the White Nile state showed a 45% prevalence of *S. haematobium*, whereas a study by Ahmed et al*.* [23] in the River Nile state showed a prevalence of 51.4% of *S. haematobium* infection, study from South Darfur by Deribe et al*.* [14] showed a prevalence of 56%.

Historically in Sudan Schistosmiasis was only known in Gezira irrigation scheme [2] but recently it has been identified in other areas including in the El-Selit irrigation scheme [15,18].

A study by Abdien [24] examined 1426 residents in the same area, including children, It found a total *S. haematobium* prevalence of 23.7% with 39.6% prevalence in school children. A study by Mohamed et al*.* [18] in Elkeriab village examined 346 children found 97 children (28%) who were infected with *S. haematobium*. Comparing with these two studies, it seems the prevalence of *S. haematobium* infection in this locality is decreasing; this could be attributed to the success of the implemented measures.

In this study there was a significant difference in the prevalence between the two villages; the prevalence rate was found to be 5.4% in Elkeriab and 16% in Tayba Elkabish. Interesting, the study by Abdien [24] showed a higher *S. haematobium* prevalence in Elkeriab (22.9%) compared to Tayba Elkabish (11.7%), the reasoning for this difference may be due to the fact that their study included adults and children; In our study, only school children were included; Tayba Elkabish school is very close to a *S. haematobium* infected water canals.

According to gender, the distribution of the *Schistosoma heamatobium* infection showed a significant difference between boys and girls. The prevalence was higher among boys (16.7%) than in girls (0.3%).

A Study in the same area [24] showed statistically different prevalence between males and females; male prevalence was 30.7% while female prevalence was 14.9%. Similar to our study, most of schistosomiasis studies from Sudan showed that infection was found more frequently among males than in females [13,19,20]. This could be attributed to boys being more exposed to activities that involve contact with infested waters; the cultural and social beliefs state that females are not allowed to participate in swimming activities, especially in the public areas. This finding is consistent with a study from South Africa which showed a difference in the attitudes and practices on urinary schistosomiasis between females and males [25].

The highest age group to be affected is 10 -12 year olds with a prevalence of 14.7%, while the lowest is 6-8 year olds with a prevalence of 1.5%; 6 – 8 year olds are new to the school and are relatively shy and hence do not have the confidence to take part in water based activities.

6. CONCLUSION

S. haematobium infection is still a common infection in school-aged children in rural and irrigated schemes, especially affecting males.

Schistosoma control programs have reduced the prevalence of *S. haematobium* infection but there remains a lot to be desired when concerned with the eradication of Schistosoma. Health education should be stressed to all children in schools to increase the awareness of the disease and the dangers of water-contact activities especially to males. Other measures should also be implemented and monitored such as removing snail hosts in endemic area, mass screening, large scale treatment with Praziquantel [26] as well as permanent improvement in water supply and sanitation [27].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 9
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NCIC FPC Filing Sequence Formula

A. J. Washington [a*]

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ABSTRACT

The NCIC FPC Filing Sequence Formula can be used in a physiological study on the combination of fingerprint patterns and their frequency for each digit. It was designed as applicable to genetics or any other discipline that encompasses the phenomenon of human physical structure. Theoretically, fingerprint classifications are listed chronologically according to their appearance upon the person of individuals; an advanced computational formula for educational institutions and the criminal justice system. Conclusions can be drawn from any research project that is developed with the implementation of the calculation methods provided in this article.

Keywords: NCIC FPC filing sequence; physiological study; chronological order.

1. INTRODUCTION

The NCIC FPC contains 20 characters, each pair of characters represents one digit beginning with the right thumb as the No. 1 digit and ending with the left little finger as the No. 10 digit; the left thumb is then No. 6. For every consecutive pair of characters, one code can be assigned out of a possible 114 codes (from PI to XX). This means that there are actually only 10 individual segments for the NCIC FPC code, with each one maintaining a pair of the 20 characters to represent an individual code [1,2] (Fig. 1). The use of fingerprints for identification purposes is based upon distinctive ridge outlines which appear on the bulbs on the inside of the end joints of the fingers and thumbs. These ridges have definite contours and appear in several general pattern types, each with general and specific variations of the pattern, dependent on the shape and relationship of the ridges [3-5].

The following is a formula for filing the NCIC FPC into a sequential order which may be used in a physiological study of the combination of fingerprint patterns and their frequency for each digit. The NCIC FPC is arranged into a chronological order according to its appearance upon the person of individuals. As a result, there is genetic and evolutionary value in the NCIC FPC Filing Sequence Formula

Let (X) equal the number of assigned to the NCIC FPC code (Table 1). Each of the ten segments, with the exception of segment #1, must be assigned a decimal number which shall be added to the assigned number for the given NCIC FPC Code.

This adding of the decimal number to the assigned number for the NCIC FPC code must be done to establish a unique numerical value to each segment of the ten-segment unit. Segment number 1, however, need not be assigned a decimal value because the other nine segments maintain an identity distinguished from it. (The reason for choosing #1 segment as the one which shall not be assigned a decimal value is because #1 segment is the only segment which can provide us with the lowest possible number in the calculation if no decimal was to be added to it.) It can be noted that in this way no two or more segments can provide the same exact number. Furthermore, no two NCIC FPC codes can provide the same filing number, even if the original code appeared in a reverse sequence (Table 2).

[a] Fingerprint Geometric Analysis, Post Office Box 165, Bronx, New York 10451-0165, USA.
**Corresponding author: E-mail: director@dermatoglyphics.com;*

Table 1. Let X equal the number of assigned to the NCIC FPC code

Digit	1	2	3	4	5	6	7	8	9	10
Number Assigned (X)	X	X.00001	X.00002	X.00003	X.00004	X.00005	X.00006	X.00007	X.00008	X.00009

Table 2. Adding of the decimal number to the assigned number for the NCIC FPC code must be done to establish a unique numerical value to each segment of the ten-segment unit.

NCIC FPC code	14	XX	AA	CO	04	SR	60	TT	DM	10
Number Assigned to the Given Code	2.6	11.4	11.2	0.6	1.6	11.3	7.1	11.1	0.8	2.2
Decimal added to the Assigned Number	2.6	11.40001	11.20002	0.60003	1.60004	11.30005	7.10006	11.10007	0.80008	2.20009

After the decimal number is added to the assigned number for the NCIC FPC code, multiplication takes place. That is, #1 segment times #2 segment and that product multiplied by #3 segment, and so on until #10 segment has been included in the multiplication. The end product shall provide the filing location for the given NCIC FPC code.

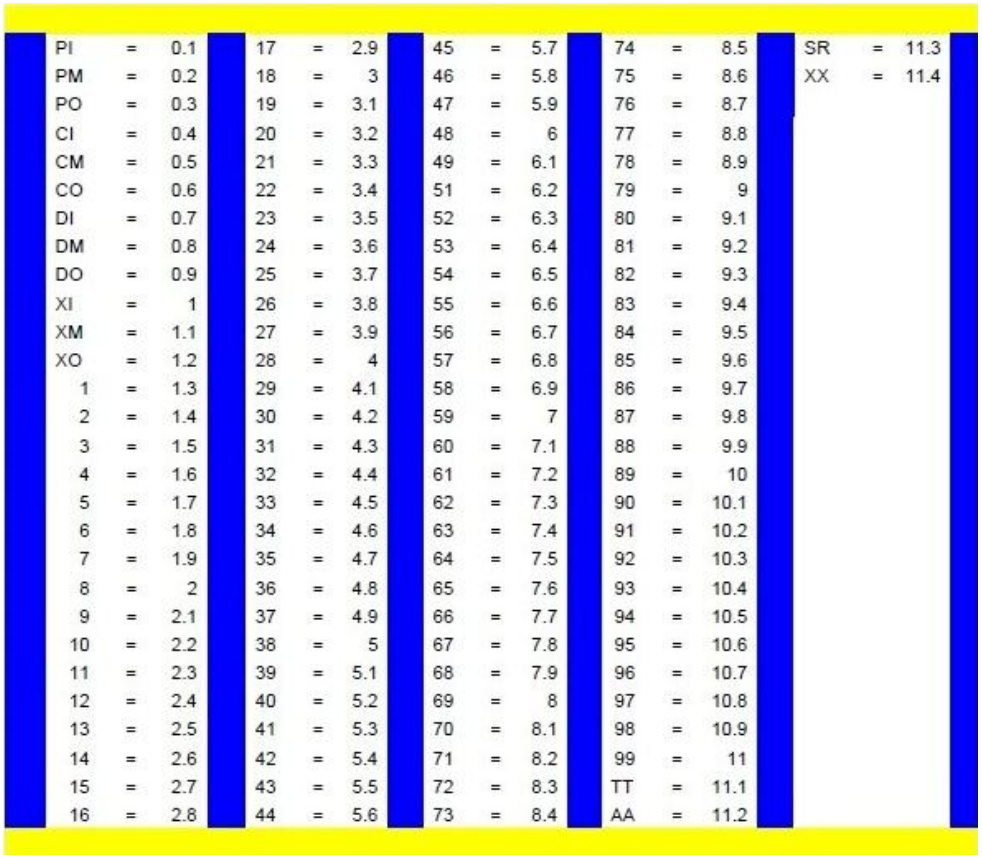

Fig. 1. Each code for the NCIC FPC is assigned a number of the 114 possible.

When there are ten segments and 114 possible codes for each segment the total number of combinations is 3.707221314118566e+20.

2. EXAMPLE

(2.6) X (11.40001) X (11.20002) X (0.60003) X (1.60004) X (11.30005)

X (7.10006) X (11.10007) X (0.80008) X (2.20009) = 499623.8317. In conclusion, the NCIC FPC code, 14XXAACO04SR60TTDM10 would be filed as 499623.8317 between 1.00451E-10 and 37,073,676,543.

Click here for statistical data on female NCIC FPC frequencies. Click here for statistical data on male NCIC FPC frequencies. Click here to determine the NCIC FPC filing number.

Click here to calculate the percent frequency of a pattern using the NCIC FPC.

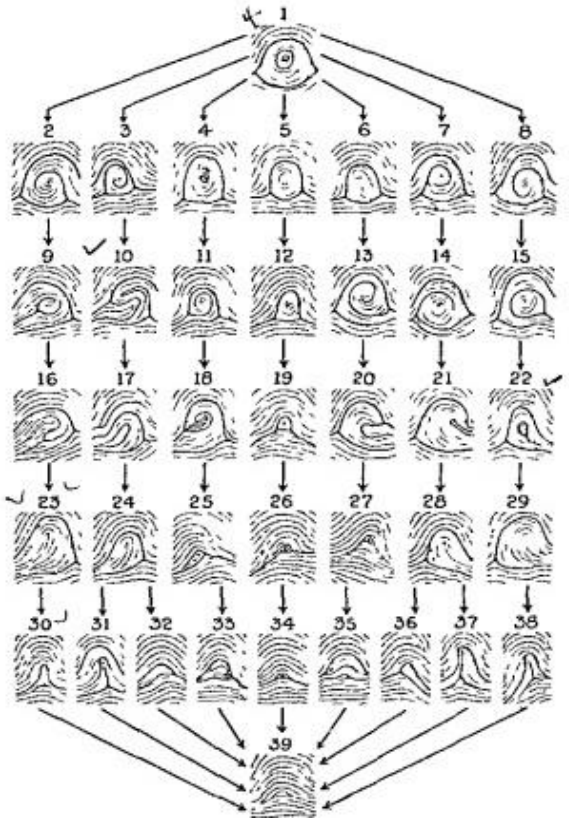

Fig. 2. Evolution of fingerprint patterns in chronological order
Finger Prints Palms and Soles by Harold Cummins and Charles Midlo (1943) showing fingerprint patterns in chronological order (page # 62)

3. CONCLUSION

The NCIC FPC can be looked-on as a universal language, all law enforcement agencies and other entities understand it. The filing sequence formula was created because computers should also be networked in the implementation of this strategical function, with its useful possibilities in an all scientific community. In short, it was created so that we can globally share the interpretations of the same fingerprint pattern discoveries.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Biography of author(s)

Mr. A. J. Washington
Fingerprint Geometric Analysis, Post Office Box 165, Bronx, New York 10451-0165, USA.

His experience in the performance of finger print identification encompasses research and study of the dermatoglyphic configurations. He provided instruction on the identification of fingerprints for the City of New York Department of Correction Training Academy; which included lectures in that discipline. During 1988, he assisted in the composition of a lesson plan on fingerprint interpretation. By 1989, he was known within the department to hold an interest in this area. It was in that year and the subsequent that the Department of Correction City of New York authorized my attendance into the FBI fingerprint classes. From 1979 to the present, he has been conducting independent research on the combination of fingerprint patterns and their frequency for each digit. In reference to the filing of ten set fingerprint record cards, he is familiar with the Henry System of Fingerprint Classification and Filing. However, he has originally noted that the classification formula derived after an examination of the fingerprints varies according to jurisdictional venue. Notwithstanding, all jurisdictions communicate and utilize the National Crime Information Center Fingerprint Classification (NCIC FPC) in the same manner. This was the reason for the development of the NCIC FPC Filing Sequence Formula in 1981, which was later published by the International Association for Identification in February of 1983 and in 2017 was revised and improved for publication in the journal of Medical and Clinical Archives. The aforementioned is a new innovation of the formula that would be beneficial to the law enforcement, academic and medical communities so that we can globally share the interpretations of the same fingerprint pattern discoveries. In addition, he created the Fingerprint Diagonal Reverse Sequence Arrangement which is the central theme of my theory in Sacred Geometry, a fingerprint formula which asserts its foundation on the dimensions of the perimeter of the Great Pyramid Khufu in Giza Egypt, which reflects time and space in its construction. It provides a way in which individuals can work together in harmony by team development according to fingerprint codes. His Research Interests on the correlation that exist between the elevation of the mental manifestation and the phenomenon of the dermal ridge arrangements. His interest in the association between diseases and the configuration of the friction ridge formations is also paramount.

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Chapter 10
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Study on Colonoscopic Polypectomy in Children

Tanveer Akhtar [a], K. L. Venkatesh [a*], Anand Alladi [a] and Siddappa [a]

DOI: 10.9734/bpi/idmmr/v5/15372D

ABSTRACT

Colonoscopic polypectomy is one of the current methods in pediatric age group. The diagnosis and treatment of juvenile polyps requires a combination of obtaining accurate history, digital rectal examination and colonoscopy. Juvenile polyps can be due to hereditary, genetic, hamartomatous malformation.

Materials and Methods: From November 2008 to December 2010, 30 patients presenting with recurrent bleeding per rectum, underwent digital rectal examination, colonoscopy and colonoscopy removal of polyps using snare.

Results: 26 cases showed solitary polyp. 4 children showed multiple polyps. In 14 cases polyp was found in the posterior wall within 8 cms of the anal verge (rectum).In 16 children it was found to be beyond 8 cms.

Conclusion: Colonoscopy in all children with h/o bleeding per rectum after DRE and proper preparation helps for resection of polyp and visualization of the proximal colon for its multiplicity.

Keywords: Juvenile polyp; bleeding per rectum; colonoscopy.

1. INTRODUCTION

Colonoscopy polypectomy is one of the current methods in pediatric age group. The majority of polyps in the pediatric population are simple juvenile polyps and have no premalignant potential unless occurring as part of a polyposis syndrome. The majority are pedunculated with a moderate-to-long stalk and removal is performed largely as the polyps are causing symptoms, such as rectal bleeding, iron deficiency anemia or potentially intussusceptions [1,2]. The diagnosis and treatment of juvenile polyps requires a combination of obtaining accurate history, digital rectal examination and colonoscopy.

The shift of juvenile polyps to the more proximal colon and the concern for the presence of juvenile polyposis (>5 polyps), with its increased risk of malignancy, mandates that the entire colon be surveyed [3].

Children with juvenile polyposis and adenomatous changes are more likely to have right-sided colonic polyps [4].

2. MATERIALS AND METHODS

From November 2008 to December 2010, 30 patients presenting with recurrent bleeding per rectum, underwent digital rectal examination, colonoscopy and colonoscopic removal of polyps by snare.

In all the cases there was *no* family history of juvenile polyposis.

Age ranged from 1 to 15 years. Digital rectal examination detected 14 children with polyps. In the remaining 16 children polyp was diagnosed at colonoscopy.

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[a] *Department of Paediatric Surgery, VaniVilas Hospital, Bangalore Medical College and Research Institute Bangalore, Karnataka, India.*
**Corresponding author: E-mail: drvenkatbh@yahoo.co.in;*

13 were male children and 17 female children: Colonoscopy was done under General Anesthesia with propofol induction followed by inhalational anesthesia without endotracheal intubation.

The double channel Olympus colonoscopy was used in all cases for polypectomy. The polyps were removed by high frequency blended coagulation current. All the resected polyps sent for histopathology examination. All the patients were observed for 24 hours and none had any complication like bleeding, ulceration or perforation.

3. RESULTS

26 cases showed solitary polyp. 4 children showed multiple polyps. In 14 cases polyp was found in the posterior wall within 8 cms of the anal verge (rectum). In 16 children it was found to be beyond 8 cms. For 11 patients it was found in the recto sigmoid region. For 4 patients it was found in the sigmoid region. For 1patient it was found in the transverse colon. In 26 patients it was pedunculated, and sessile in 4 patients. HPE revealed juvenile polyp in 29 patients, lymphomatous in 1 patient. Size of the polyp varied from 1.5 cm to 3 cm.

All the patients were electively admitted the previous day of the procedure. Peglec preparation is done with 25 ml/kg/hr till stools are clear. Peglec was given through N G tube for those children who could not drink. Maintenance I V fluid were put.

Age group	Number of children
1 to3 years	2
4 to 8 years	22
>8 years	6

2. DISCUSSION

Horrileno [5] fist used the tern juvenile polyp in 1957. Most polyps of the colorectum are benign and result from hamartoma of the mucosa or lymphoid hyperplasia of the sub mucosa.

Juvenile polyps can be due to hereditary, genetic, hamartomatous malformation. They are as a result of a structural rearrangement of the mucosa secondary to an inflammatory process.

Polyps are the most frequent cause of rectal bleeding in toddlers and preschoolers 2 to 5 years of age. Juvenile polyps are most common (>80%), followed by lymphoid (15%) and adenomatous (3%) [6].

The distinction between the commonly occurring isolated juvenile polyps, which are benign, and the rare juvenile polyposis syndromes, which may be malignant has become increasingly important [7-8].

Jass [9] has proposed the following criteria for increased risk of cancer in children with polyps.

1. > 5 juvenile polyps
2. Polyps throughout gastrointestinal tract
3. Any number of polyps associated with a family history of juvenile polyposis.

3. SNARE POLYPECTOMY [10]

Snare is a self contained metal ring that is opened over the polyp and then closed entrapping polyp tissue for resection by closing the ring. Polyp is captured in the snare; the snare plastic sheath should be advanced moving the polyp away from the scope tip if electrocautery is to be used to avoid electrical damage to the scope. When snaring a pedunculated polyp, the snare should be placed about half way up the stalk, so that after cutting, a stalk remnant is left which can be grabbed or clipped if hemorrhage occurs. The polyp is pulled away from its base into the lumen tenting the colon wall to avoid burning the adjacent deep colon layers [11]. If the snare is too tight prior to

electrocautery application, it could result in inadvertent cold cutting the polyp, resulting in bleeding from the stalk or in the snare becoming entrapped into coagulated tissue in the stalk [12].

4. CONCLUSION

Colonoscopy in all children with h/o bleeding per rectum after DRE and proper preparation helps for resection of polyp and visualization of the proximal colon for its multiplicity. Children with juvenile polyposis and adenomatous changes are more likely to have right –sided colonic polyps. Therefore all polyps should be removed and undergo histological evaluation. Complications after colonoscopic removal of polyps are rare.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

K. L. Venkatesh
Department of Paediatric Surgery, VaniVilas Hospital, Bangalore Medical College and Research Institute Bangalore, Karnataka, India.

Biography in author available in this link: https://www.bookpi.org/wp-content/uploads/2021/12/2021_BP_15372D.pdf

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Chapter 11
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Assessment of Combined Surgical Approach in a Late Case of Orbital Cellulitis

Akshay Jawahirlal Bhandari [a≡*], Somen Misra [bφ], Pratik Gogri [c#] and Neeta Misra [b†]

DOI: 10.9734/bpi/idmmr/v5/2415C

ABSTRACT

Orbital cellulitis refers to infection of the ocular adnexal structures posterior to the orbital septum. The infection most commonly originates from the sinuses. Mucopyoceles are infected cysts of mucous content that affect the paranasal sinuses most commonly the frontal sinus. Many of these lesions have an intraorbital extension causing vision threatening ocular complication like orbital cellulitis and intracranial extension causing life threatening neurological complication like meningitis. We treated a 12-year-old female patient of acute orbital cellulitis secondary to frontoethmoidal mucopyocele with combined surgical approach- transnasal endoscopic and open surgical drainage.

Keywords: Orbital cellulitis; endoscopic marsupialization; anterior orbitotomy.

1. INTRODUCTION

Orbital cellulitis is a potentially life-threatening but uncommon ophthalmic emergency characterised by infection of the soft tissues behind the orbital septum [1]. As such, rapid diagnosis and prompt initiation of therapy, medical and/or surgical, are important in order to minimize complications and optimize outcomes. Mucocele is a chronic, expanding, mucosa-lined lesion of the paranasal sinus characterized by mucous retention that can become infected forming a mucopyocele [2]. A mucopyocele when extends into the orbit, can present as acute orbital cellulitis, as was the case in our patient [3].

The management of orbital cellulitis is primarily medical with prompt initiation of intravenous antibiotics. Surgical intervention should be considered in patients who fail to respond or deteriorate on medical therapy, display worsening visual function/pupillary changes, or develop an orbital abscess, particularly in those cases in which the primary cause requires surgery too. However it is recommended that the treatment approach should be case based [4]. We describe a case of late presentation of acute orbital cellulitis subsequent to a frontoethmoidal mucopyocele extending into the orbit treated with a combined conventional external orbitotomy and newer endoscopic approach.

2. CASE REPORT

A 12-year-old female patient presented with progressive left eyelids swelling since 1 week. There was associated acute pain, redness along with downward and outward protrusion of the left eyeball. Patient complained of diplopia and mild diminution of vision in the left eye. Also there was history of fever with chills since 10 days and intermittent frontal headache since last 2 years. There was no

[≡] *Associate professor;*
[φ] *Professor & Head;*
[#] *Cornea Consultant;*
[†] *Professor;*
[a] *Department of Ophthalmology, Dr. Balasaheb Vikhe Patil Rural Medical College, Pravara Institute of Medical Sciences, Loni, India.*
[b] *Department of Ophthalmology, AIIMS, Raipur, India.*
[c] *LVPEI, Hyderabad, India.*
**Corresponding author: E-mail: kiwis143@gmail.com;*

history of projectile vomiting, nasal discharge or toothache. On local examination, there was fluctuant, erythematous, non-pulsatile, tender swelling in the upper eyelid of the left eye along with ptosis. The left eyeball was pushed inferiorly and laterally causing a proptosis of 26mm (Fig. 1). Vision was 6/12 in the left eye and ocular movements were painful and restricted in all gazes. On fundus examination, there was blurring of disc margins along with venous dilatation and tortuosity in the left eye. All the findings in the right eye were within normal limits. There was history of left sided endoscopic frontal sinus surgery 3 months back. A clinical diagnosis of acute orbital cellulitis was made.

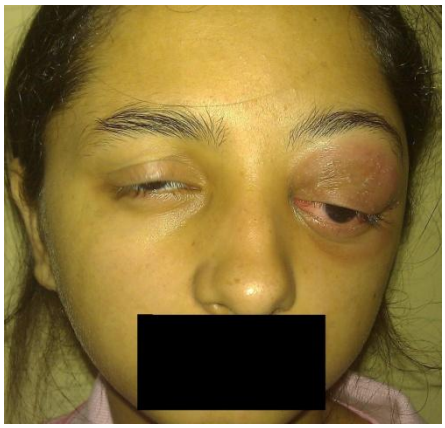

Fig. 1. Clinical photograph of the patient showing left eyelid swelling, eryhthema, ptosis and left eye downward outward proptosis

On contrast-enhanced CT scan, there was a large, expansile, cystic lesion arising from the left frontal and ethmoid air cells extending into left retrobulbar space destroying the lamina papyracea, posterior wall of frontal sinus and eroding the floor of the anterior cranial fossa (Fig. 2). MRI scan suggested that the lesion was bi-loculated, the medial one in close proximity with the left frontal lobe of the brain and the lateral loculus extending into the orbit causing proptosis of the left eyeball (Fig. 3).

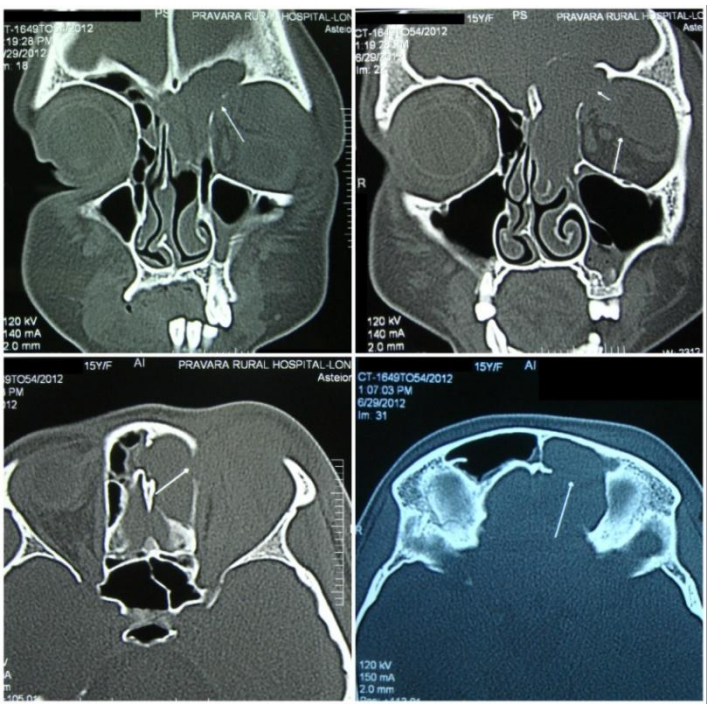

Fig. 2. CT scan images showing homogenous isodense well defined cystic lesion eroding the lamina papyracea, posterior frontal sinus wall extending into the left retrobulbar space

We started the patient on intravenous amoxicillin and clavulanate potassium combination, amikacin and metronidazole along with anti-inflammatory and pain killers. In spite of starting the patient on intravenous antibiotics and anti-inflammatory, her visual acuity deteriorated to 6/36 in the left eye and there was colour vision defect on testing with Ishihara's charts. There was worsening of proptosis to Hertel value of 30mm. Fundoscopy revealed choroidal folds and indentation of the superior hemisphere of the retina. The patient was planned for surgery. Firstly, endoscopic drainage of the medial loculus of the mucocoele was done along with marsupialization by the axillary flap technique. Subsequently, the external orbitotomy was done using the Benedict incision for complete drainage and removal of the lateral orbital loculus along with the mucosa. A Rains frontal sinus stent was used for stenting the frontal sinus during marsupialization and silicon drains were placed in the external orbitotomy incision. Both were removed subsequently after 12 weeks and 1 week respectively. Postoperatively, the patient received intravenous antibiotics to which she responded very well with steady decrease in proptosis, swelling and fever over the next seven days. Eyelid edema subsided fully after two weeks of treatment (Fig. 4) with free and full movement of eyeball in all directions of gaze and normal colour vision along with normal visual acuity in the left eye. There were no choroidal folds on fundoscopy.

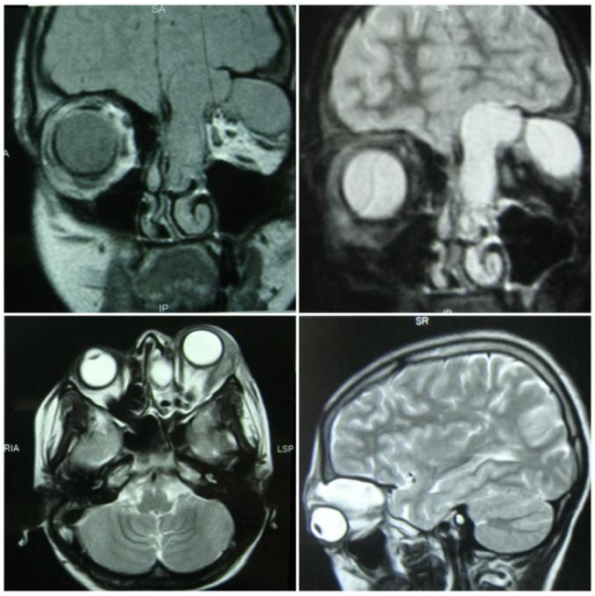

Fig. 3. MRI Orbit suggestive of a bi-loculated lesion which is iso-hypointense on T1W images and hyperintense on T2W images with characteristic rim enhancement on post-contrast study. The mass is seen eroding the superomedial wall of the left orbit displacing the eyeball laterally and downwards, and extending to anterior cranial fossa

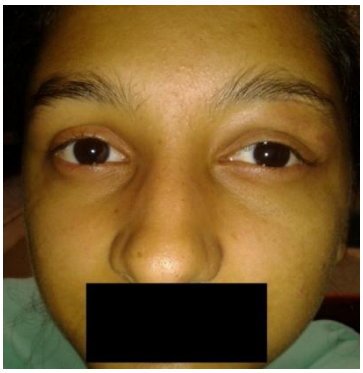

Fig. 4. Subsidence of eyelid edema, proptosis and ptosis 2 weeks after surgery

3. DISCUSSION AND CONCLUSION

Orbital cellulitis describes infections that involve the tissues posterior to the orbital septum within the bony orbit [5]. Orbital cellulitis affects all age groups but is more common in the adolescent population. The most frequent cause of orbital cellulitis is secondary extension of infection from the paranasal sinuses, particularly from the ethmoid sinus given the thin medial orbital wall. Other notable causes of orbital cellulitis include trauma with associated orbital fracture or foreign body, dacryocystitis, dental infections, and untreated preseptal cellulitis [6].

Mucoceles of the paranasal sinus are slowly expanding lesions which consist of accumulation of mucus and epithelial debris in the mucosa of the sinus subsequent to obstruction of the ostium of the sinus. They affect most commonly the frontal sinus. If there is acute infection of mucocele, leading to mucopyocele, there is higher likelihood of complications mainly orbital or intracranial [7]. In our case the frontoethmoidal mucopyocele had extended into the orbit causing acute orbital cellulitis and abscess formation. The globe itself was compressed superomedially, resulting in the development of chorioretinal striae. A progressive optic neuropathy from compression of the orbital portion of the optic nerve occurred as the mucocele expanded posteriorly to compress posterior orbital structures [8].

Given the potential for significant complications, intravenous antibiotics should be started promptly for all cases of orbital cellulitis [9]. Surgical drainage is considered in case of non-response to medical treatment, subperiosteal or orbital abscess formation, or presence of signs of optic neuropathy. The surgical drainage can be endoscopic alone, open drainage or combination of open and endoscopic drainage [10,11,12]. In our case, there were two abscess cavities with one abscess extending medially upwards towards the roof of the orbit and the other one spreading laterally and posteriorly into the retrobulbar space. Also there was history of previous sinus surgery. Because of the failure to medical therapy, worsening visual function, and presence of two large orbital abscesses, surgical intervention was done in our patient. We used the combined external and endoscopic approach to get a wide drainage of marsupialization of the medial abscess via the transnasal endoscopic approach and adequate excision of the lateral abscess via the external orbitotomy approach. The combined approach gave us an added advantage of treating the sinus pathology along with the abscess drainage, thus reducing the chances of recurrence.

Ours is a case of very late presentation of acute orbital cellulitis having two separate orbital abscesses subsequent to frontoethmoidal mucopyocele. The nasal abscess was easily accessible through the nasal endoscopic method but the temporal one could not be drained through that route as it was a thick encapsulated loculus which was beyond the reach of the endoscopic approach. The uniqueness of this case is very late presentation, two separate abscess loculi which were treated successfully with two simultaneous different surgical approaches- transnasal endoscopic and external orbitotomy, resulting in complete recovery. In spite of being a relatively uncommon late presentation of frontoethmoidal mucopyocele, orbital cellulitis remains a potentially sight and life threatening infection that requires careful examination and prompt treatment. Through this article, we are laying emphasis on the fact that orbital abscess can have varied presentations depending upon the extent and complexity of the lesion, and hence, conventional preferred surgical approaches cannot be applied in all such cases. An individualized therapeutic approach should be undertaken for each case.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

Dr. Akshay Jawahirlal Bhandari
Department of Ophthalmology, Dr. Balasaheb Vikhe Patil Rural Medical College, Pravara Institute of Medical Sciences, Loni, India.

He is working as an Associate professor in department of ophthalmology in Dr. Balasaheb Vikhe Patil Rural Medical College, Loni. He did his MBBS and MS from Dr. Balasaheb Vikhe Patil Rural Medical College Loni. Phaco training from Venu institute of ophthalmology Delhi. He has published several research articles in reputed journals which are available in Google scholar.

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Chapter 12
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A Propos Cascade Stomach – A Commentary

Daniel Benharroch [a*] and Roberto Umansky [b]

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ABSTRACT

The clinician does not usually deny the diagnosis of a cascade stomach based on imaging. A specific treatment is rarely necessary, with the exception of initiating therapy for reflux symptoms or dyspepsia. As a result, gastroenterologists are rarely interested in cascade stomach. Occasionally, further features are described in a patient presenting the radiological upper gastroenterological series of this condition. It remains to prove that such unusual symptoms represent coincidental, but non-specific features of the ailment. A patient diagnosed with a cascade stomach, who exhibited heterogeneous upper abdominal complaints, the majority of which are not associated, as a rule, with this condition, is herein reported.

Keywords: Cascade stomach; reflux symptoms; dyspepsia; dysphagia; AAD.

1. INTRODUCTION

A cascade stomach is mostly diagnosed using an upper gastrointestinal series. A contrast material (barium) is swallowed by the fasting patient and is traced by fluoroscopy, to disclose the esophagus, stomach, and duodenum. This "variant" stomach pathophysiology bears several forms [1]. However, the demonstration of an enhanced angulation with an air-fluid level encompassing the stomach, proximally to the angulus, is required [2]. Moreover, the fundus, often almost empty, or showing an additional air-fluid level, will exhibit parallel linear tracks of the contrast material, that highlights the unmarked gastric folds – the "cup and spill" (Fig. 1a & 1b) [3]. Cascade stomach is well known to radiologists but its clinical spectrum is not well researched and there is much speculation about its pathological basis. There is limited data on the treatment of this condition in the literature [4,5,6].

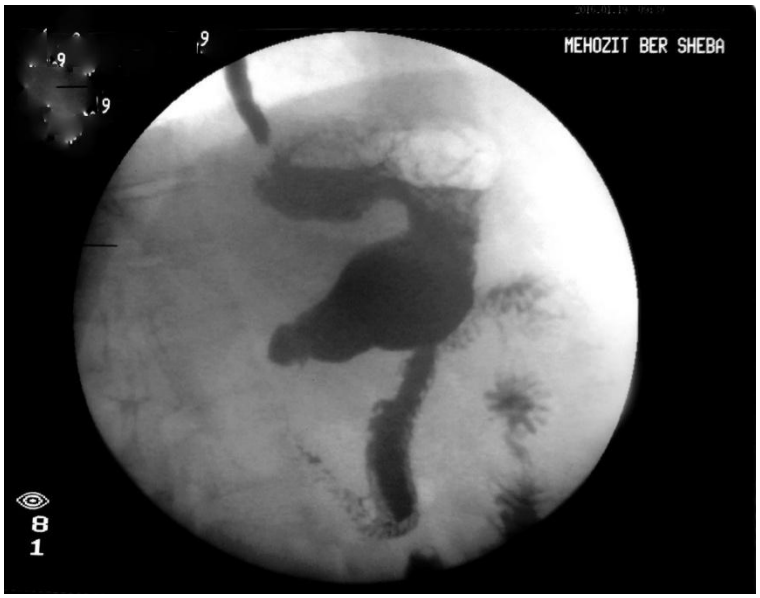
The symptoms, generally considered to accompany this condition, are scarce and have included mainly dyspepsia and gastroesophageal reflux features [3]. Dysphagia has also been rarely described [7]. Additional studies have noted further characteristics, mentioned as "obscure" by one author [8]. There should be a way to confirm the association of these "atypical" symptoms with the disease entity. If the indefinite symptoms are shown to belong formally with the cascade stomach, one might not assess this relationship as incidental [8]. Modes of therapy, both medical and surgical have been previously suggested. However, is treatment justified in these cases [4]? A patient with this disease entity is presently reported.

2. CASE PRESENTATION

Following the recurrence of ischio-rectal abscesses, this 24-year-old soldier was submitted to an upper gastrointestinal series. The radiological exam did not display the existence of an anorectal fistula, as evidence against the alleged Crohn's disease. Instead, it highlighted the presence of a cascade stomach (Fig. 2).

[a] *Department of Pathology, Soroka University Medical Centre, and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel.*
[b] *Department of Psychiatry, Independent Physician, Retired. Beer-Sheva, Israel.*
**Corresponding author: E-mail: danielbenharroch1@gmail.com;*

The subject had complained of epigastric pain, after meals and of heartburns, from the age of 16, but for a long period of time, was neither diagnosed, nor treated. Progressively, the symptoms evolved, to crystalize into a gastroesophageal reflux. At the age of 46, the patient was submitted to a urease test, which disclosed a H. pylori infestation. Otherwise, the gastroscopy was unremarkable.



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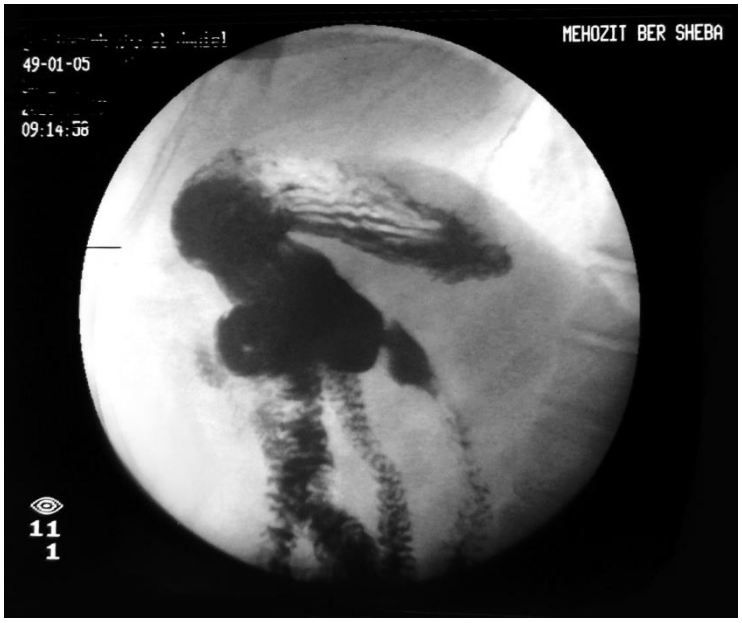

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Fig. 1. The patient's upper gastrointestinal fluoroscopy discloses an increased angulation with an air-fluid level at the gastric fundus (Fig. 1a). Note the underlining of the folds (by negative contrast) in the gastric body, suggesting a waterfall (Fig. 1b)

Soon after treatment with three agents for the H. pylori infection, and following persistent and annoying aerophagia and meteorism, lactose intolerance was diagnosed. Several gastroscopies had shown normal features, except, intermittently, for evidence of a mild gastroesophageal reflux. Two of the exams revealed pre-pyloric, low grade intestinal metaplasia.

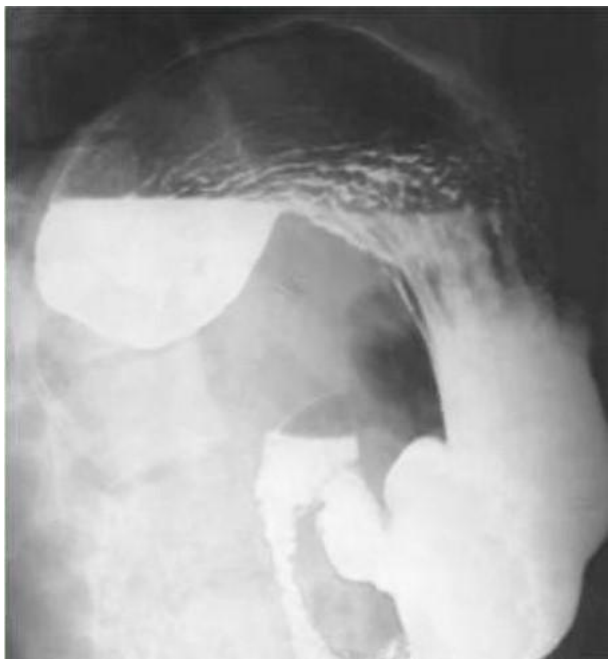

Fig. 2. Cascade stomach – an air-fluid level and an apparent cataract are evident (Borrowed from a poster)

The individual had led a 'relatively' comfortable life, till the age of 71. By now, he had started complaining of dysphagia, with difficulty in swallowing mainly water. He also described short runs of hiccup, especially at raising from bed, and a few minutes after meals, in addition to belching.

A number of investigations were ordered, in addition, several of which were GI-related. A few were directed toward musculoskeletal symptoms. Except for the suspicion of distal esophageal spasm (DES), all were about normal.

A cardiologist discussed a mild to moderate aortic regurgitation, as an isolated finding, at the completion of his investigations. An ORL exam was within normal limits. Nevertheless, the patient was referred for esophageal manometry. The manipulation raised the suspicion of a distal esophageal spasm (DES) (Fig. 3). The patient was prescribed esomeprazole, 20 mg/d for a month, as his symptoms did not bear the severity usually attributed to achalasia. It was felt that the various ailments, might not all derive directly from the cascade stomach; especially, not after a long symptomless period had evolved.

Due to a discrepancy, by which the patient described a swallowing difficulty localized to the proximal part of the esophagus, the gastroenterologist consulted again the patient's files. This physician noted that, at 16 years of age, the subject had been diagnosed with pulmonary tuberculosis. Among the three medications he was prescribed for this infection, for 9 months, para-amino-salicylic acid (PAS) was provided in the form of 12 tablets, to be swallowed every morning.

The gastroenterologist had also disclosed that, recently, the patient had been prescribed aripiprazole 5mg, once daily for the maintenance treatment of a long standing, otherwise well managed, schizoaffective disorder. At the lecture of the package insert, he noticed further that the medication contained 26 mg lactose per dose; that it might cause difficulties in swallowing, in addition to abdominal and gastric discomfort, weight gain, increased salivation, as well as rapid breathing with a difficult respiration. The leaflet mentioned among others, sequels for special attention, difficulty in swallowing with occasional inhalation of fluids and food, and potentially, subsequent pneumonia. Most of the above were comprised among the symptoms the patient had reported, at one time or other, even though the dose of aripiprazole was the lowest possibly accounted for.

The patient's psychiatrist has searched persistently for an appropriate, new generation anti-psychotic drug, but, using trial and error, he met time and again, with unacceptable adverse effects. A search for a more admissible candidate drug is ongoing.

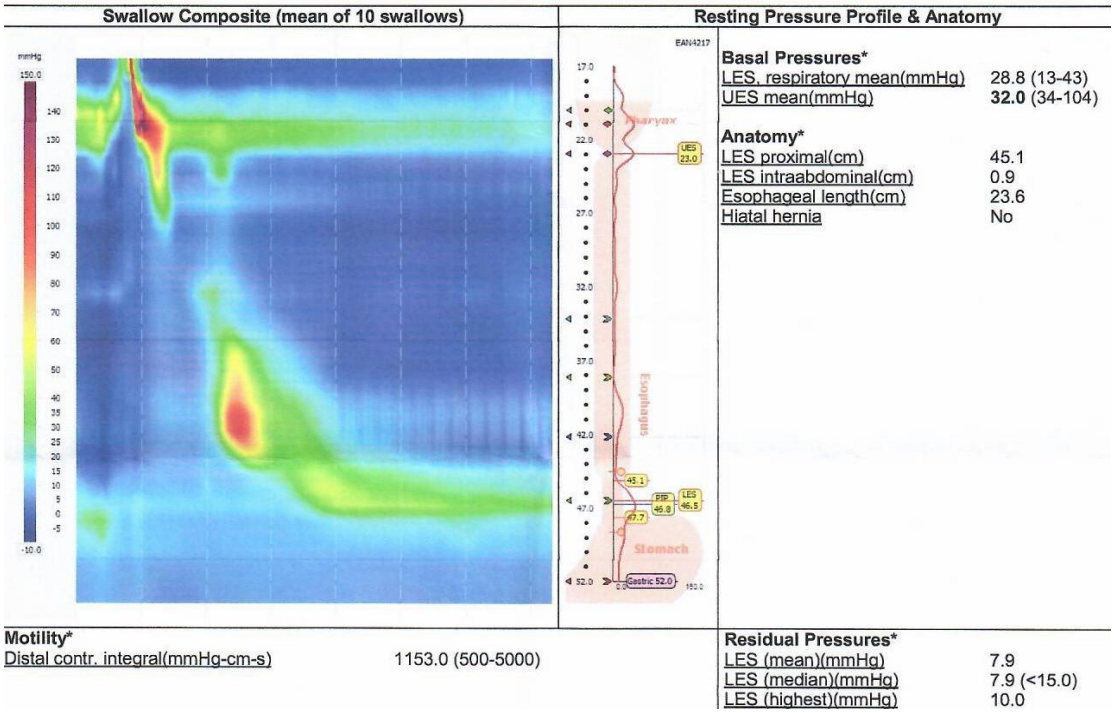

Fig. 3. The patient's manometry examination rising suspicion for distal esophageal spasm

3. DISCUSSION

A 71-year-old man is presented with a complex gastrointestinal array of symptoms, forty-seven years after the incidental diagnosis of cascade stomach, during which time he had suffered of heartburn and of intermittent epigastric pain only. Endoscopy had not been contributory [9].

Among his past maladies, tuberculosis at age 16 and a schizoaffective, bipolar disorder, diagnosed at age 28, and appropriately managed, were accounted for.

The patient's saga rises two main queries: primarily, was the cascade stomach, idiopathic, or perhaps, a cascade-like defect consequent to the massive and extended PAS therapy at 16, age marking the outset of his GIT complaints? No indication of a causal effect between PAS and a gastric pathology of any type has been retrieved, although this medication, especially if administered in the form of tablets, had not been without consequences [10]. This drug is now used in a liquid form, both per os or by injection, including for drug resistant tuberculosis.

Moreover, a cascade stomach is usually described in conjunction with reflux symptoms or with dyspepsia and rarely with dysphagia. Anyway, what could be the cause of the clustering of new GIT ailments, many years after the diagnosis of the cascade defect?

An expensive array of studies, including CT scans, a MRI, and an esophageal manometry, were interpreted as normal or near normal.

The culprit might well be identified with regard to the second query!

The second issue concerns the severe sequels due to aripiprazole, which afflicted the present patient. These complications might result in distress, in an unknown proportion of psychotic patients [11]. Do

the (atypical) antipsychotic medications (AADs) alleviate, in any way the suffering of these individuals, or do they make things worse for them. The poor compliance shown in more than a fifth of the patients in some series, as well as their dropping-out of follow-up, may favor this opinion [12-14]. However, psychotic patients often fail to report the serious sequels of the AADs. Their physicians, having performed their duty, by the book, are relieved, at least temporarily, from their responsibility.

4. CONCLUSION

This report has highlighted two sets of evidence, the first being that cascade stomach is still a poorly understood condition and that all the symptoms that may accompany it, are not necessarily an integral part of the disorder. Moreover, we have confirmed a truism: that administration of the AADs to patients with schizoaffective disorders, may significantly ameliorate their condition. However, the price, in terms of complications, might be so high, that their compliance drops by more than 20%, often to the point of their abandoning the treatment and at times, the follow-up as well.

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The patient has given his approval to the report of his medical history.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

Daniel Benharroch
Department of Pathology, Soroka University Medical Centre, and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel.

Research and Academic Experience: He has 40 years of Experience.

Research Area: His Research area includes Hematopathology, Measles virus and Cancer.

Number of Published Papers: He has 137 research articles in the national and international journals.

Special Award: Head of Hematopathology Unit.

Any Other Remarkable Point: He is a Professor Emeritus, Ben Gurion University of the Negev, Beer Sheva, Israel.

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Chapter 13
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‘From Theory and Practice’ of Retention and Resistance form of Tooth Preparations for All Ceramic Restorations: A Digital Imaging Study

Alex M. Muruppel [a], Joyce Thomas [b*], Sudeep Saratchandran [a], Dinesh Narendran [a], Sheeba Gladstone [a] and Milen M. Rajeev [c]

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ABSTRACT

Background: Retention and resistance forms are the characteristics of a preparation that prevent castings from becoming uncemented, debonded, or cement failures, which are one of the top three reasons for the replacement of castings
Aim: This study aims to discern an actually achieved degree of taper in a clinical situation, and the textbook recommended degree of taper. Furthermore, it also assessed how resistance and retention form correlate with the degree of taper of tooth preparation.
Materials and Methods: Scanned digital images of 114 die preparations for all ceramic restorations (n = 114) were collected from a dental laboratory. All the images were also analyzed digi- tally using Adobe Photoshop® software to analyze the degree of taper (angle of convergence) of each preparation and then applied the Zuckerman’s circle, and the Lewis perpendicular methods were used to measure the resistance form.
Results: For the current study, the overall average degree of taper was found to be 20.9° (range, 2–80°), which is more than what is recommended by most previous studies and also sharply greater than the textbook ideal of 3 to 6°. Mean degree of taper for maxillary was 17.56° (anterior—10.50°, posterior—23.7°), and for mandibular teeth, it was 25.22° (anterior—15°, posterior—28.45°). Out of the 64 analyzed images of maxillary teeth, 61 presented resistance form, while 3 were without it. Out of the 50 mandibular teeth analyzed, 38 possessed resistance form, whereas 12 were without. All the anterior teeth showed resistance form irrespective of the arch.
Conclusion: The degree of taper showed a significant relation- ship with resistance and retention form, which was inversely proportional to each other. The recommended “degree of taper” is not always the clinically achievable as advocated in textbooks, as it is modified by various factors in the actual clinical situation.
Clinical Significance: The study provides scientific background regarding the relationship between the degree of taper with resistance and retention form, and the relationship was found to be inversely proportional to each other. The recommended “degree of taper” is not always the clinically achievable as advocated in textbooks, and it is modified by various modifying or limiting factors in the actual clinical situation.

Keywords: All ceramic; degree of taper; resistance form; retention form; tooth preparations.

1. INTRODUCTION

Traditionally, physicians have used resistance form to determine preparation design criteria, ensuring the clinical effectiveness of cemented cast restorations. Resistance form is defined as the features of a tooth preparation that enhance the stability of a restoration and resist dislodgment along an axis

[a] *Department of Prosthodontics and Implantology, PMS College of Dental Science & Research, Thiruvananthapuram Kerala, India.*
[b] *Department of Prosthodontics, Al Azhar Dental College Thodupuzha, Kerala, India.*
[c] *Department of Prosthodontics, Mar Baselios Dental College Ernakulam, Kerala, India.*
**Corresponding author: E-mail: abhinavjoyce@gmail.com;*

other than the path of placement [1,2]. While retention form counteracts tensile stress, resistance form counteracts shearing stress. All tooth preparations require the incorporation of the certain design features, such as parallelism, length, and surface area that are mainly associated with retention and resistance along with other factors, such as axial surface, groove, box, and pinholes to prevent the dislodgment of the restoration by functional stresses [3] (Figs. 1 & 2).

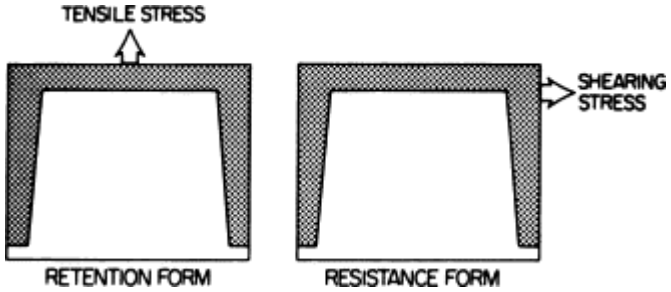


Fig. 1. Comparison of retention and resistance form with functional stress

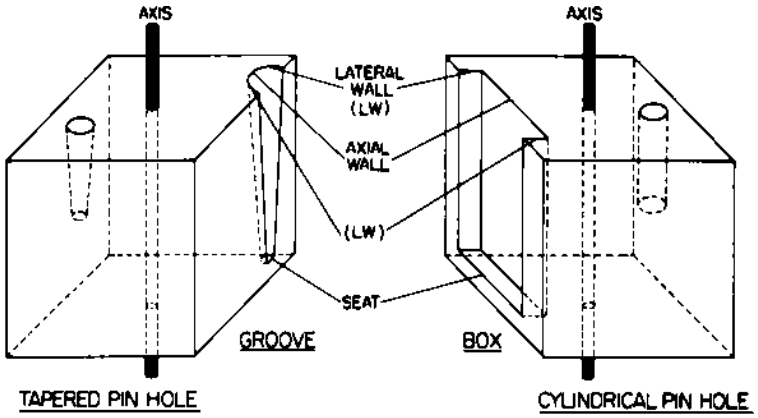


Fig. 2. Secondary retentive features

All-ceramic systems have expanded the range of restorative treatment options significantly; at the same time, their handling has been simplified substantially. The use of lithium disilicate glass-ceramic- and zirconium oxide-based frameworks along with an identical veneering ceramic enables the dental care professional to cover almost all indications in fixed prosthodontics while achieving the same esthetic results [4]. The fit of metal ceramic frameworks may change after the application of veneering ceramic because of frame-work design, type of alloy, shrinkage of the ceramic during firing, and different coefficients of thermal expansion for ceramic and alloy [5].

The most retentive preparation is theoretically represented as the one with parallel walls (zero taper). Nevertheless, some of the earlier studies found that some degree of axial wall taper is inevitable and necessary to ensure complete seating of casting, though retention and resistance decreased. Although 6° is widely accepted as the gold standard and criterion of taper for full veneer crown preparation, studies show the actual taper of most preparations to be <12° [6-10]. Tylman said that to develop frictional resistance, the near parallelism of axial walls in tooth preparations is necessary, which meant a 2 to 5° taper, an arbitrary figure advocated earlier for cast inlay preparation [11,12]. Jørgensen's [13] study on the relationship between retention and convergence angle in cemented veneer crowns related taper to retention and determined maximum retention with a 5° taper [10]. A subsequent study on factors influencing the retention of cemented gold castings by Kaufman et al. [11] identified that the 5° taper is the point at which the most significant increase in retention occurs [14]. However, Smith et al8 found that the overall mean taper for full veneer crown preparation in preclinical prosthodontics exceeded his targeted criterion of 12°; however, he stated that a 12° criterion is more realistic than a 6° criterion for full veneer crown preparations.

Retention and resistance forms are the characteristics of a preparation that prevent castings from becoming uncemented, debonded, or cement failures, which are one of the top three reasons for the replacement of castings [15,16]. Tier et al. [14] study on dislodged crowns and retainers revealed that there was a fundamental relation between clinical success or failure and the all-or-none nature of resistance form. They proved dislodged crowns came almost exclusively from preparations with tapers that did not provide resistance form and over 95% of all castings that failed by becoming uncemented lacked resistance form [15,17,18].

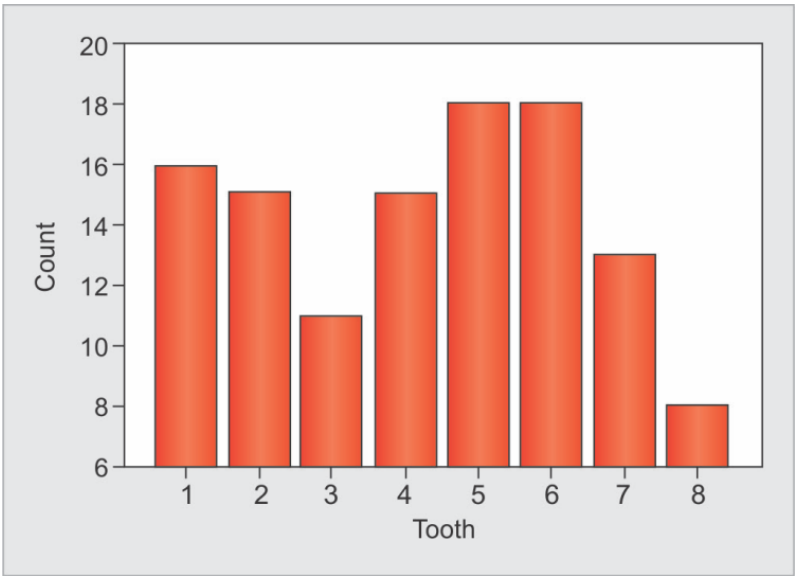
Furthermore, Walton [15] evaluated the modes of failure and the influence of various clinical characteristics on the outcome of the metal–ceramic fixed dental prosthesis and observed that 13% of them failed due to loss of retention [19].

This study aims to discern an actually achieved degree of taper in a clinical situation, and the textbook recommended degree of taper. Furthermore, it also assessed how resistance and retention form correlate with the degree of taper of tooth preparation.

2. MATERIALS AND METHODS

Scanned digital impressions of die preparations used for all ceramic restorations were collected from a dental laboratory. Images included die preparations of all ceramic single crowns (79 preparations) and bridge (35 preparations) restorations only. All the available images (n = 114) from the laboratory were included in the study sample (Graph 1). Samples were grouped into maxillary and mandibular, and again subgrouped, based on tooth type, for making intergroup as well as intra group comparisons (Table 1).

All the available images were analyzed with the help of Adobe Photoshop CS6® photo editing software. To find out the degree of taper of each image, two straight lines extended from base of the preparation in a coronal direction along the mesial and distal margins of the preparation until they meet at a common point (Fig. 1). The resultant angle was measured for each preparation and recorded as the "degree of taper" or "degree of convergence" (Tables 2 and 3).



Graph 1. Distribution of samples into subgroups

Table 1. Sample distribution

Maxillary teeth			Mandibular teeth		
Tooth		**Total**	**Tooth**		**Total**
Incisors	Central incisor	12	Incisors	Central incisor	4
	Lateral incisor	11		Lateral incisor	4
Canine		7	Canine		4
Premolars	First premolar	8	Premolars	First premolar	7
	Second premolar	12		Second premolar	6
Molars	First molar	6	Molars	First molar	12
	Second molar	4		Second molar	9
	Third molar	4		Third molar	4
Total		64	Total		50

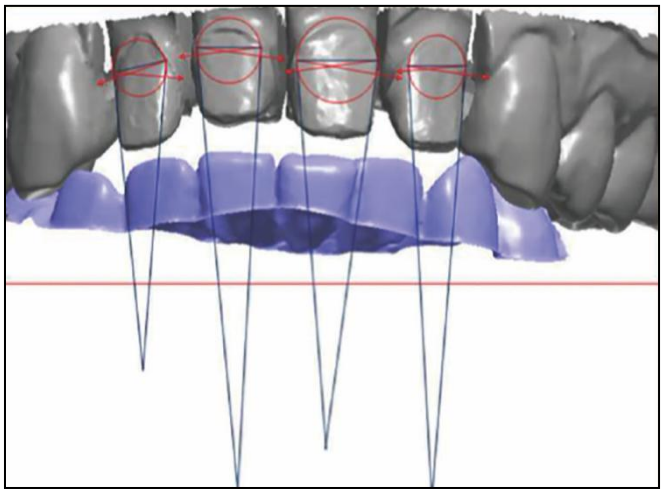


Fig. 3. Analysis of the degree of taper and resistance using adobe photoshop

To assess the retention and resistance form by Zuckerman's method,16,18 a circle is extended around each preparation with the width of the base of preparation taken as diameter. The point at which the circle inter- sects the mesial or distal margin (extended line or taper line) of the preparation indicates the demarcating point between areas with and without retention and resistance form (Fig. 3). Points of the preparation outside of the circle have resistance form. Whereas, when the point of intersection is on the extended line above or occlusal to the top of the preparation, the preparation lacks resistance form (Tables 2 and 3).

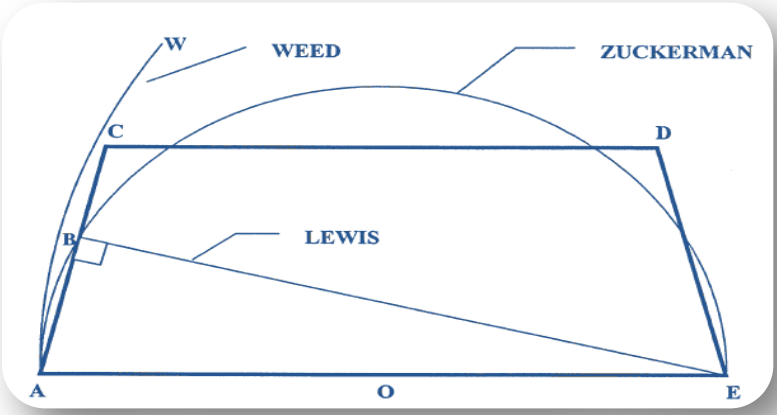


Fig. 4. Zuckerman's circle method & Lewis perpendicular method- schematic representation

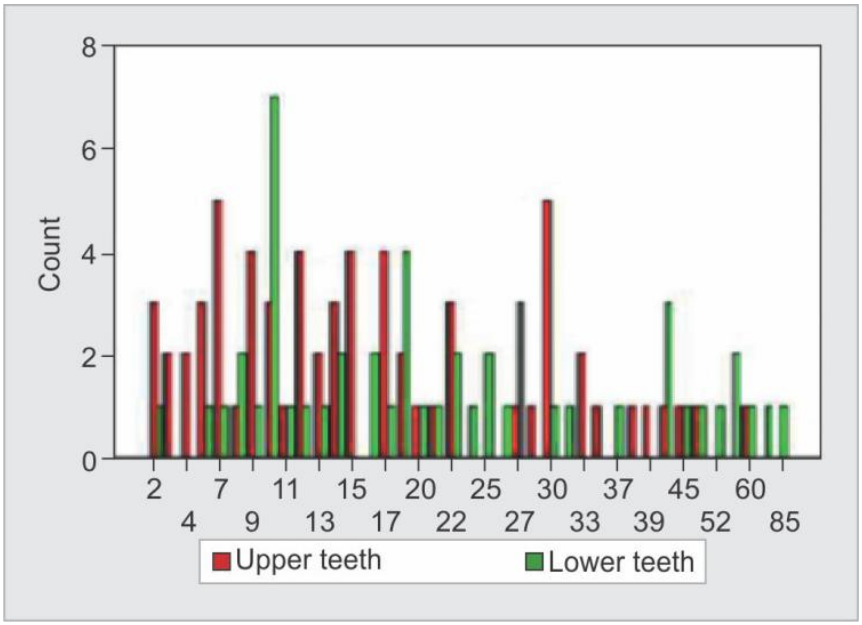
In the Lewis perpendicular method,16,18 the dividing point between the resistive and nonresistive sections of a preparation wall is taken as the point of intersection with the perpendicular line from the center of rotation on the opposing margin (Graphs 2 and 3). All points occlusal to the point of the intersection have resistance form, and all points gingival to it. Both the methods provide the same point of intersection and, thereby, the same results. Based on these measurements, preparations were recorded as preparations with or without resistance form (Tables 2 and 3).

Table 2. Degree of taper and resistance form—maxillary teeth

Tooth	Degree of taper	Resistance form
CI-1	9	Yes
CI-2	12	Yes
CI-3	12	Yes
CI-4	14	Yes
CI-5	2	Yes
CI-6	13	Yes
CI-7	17	Yes
CI-8	9	Yes
CI-9	2	Yes
CI-10	7	Yes
CI-11	9	Yes
CI-12	6	Yes
LI-1	10	Yes
LI-2	8	Yes
LI-3	3	Yes
LI-4	3	Yes
LI-5	6	Yes
LI-6	11	Yes
LI-7	14	Yes
LI-8	7	Yes
LI-9	18	Yes
LI-10	6	Yes
LI-11	17	Yes
C-1	14	Yes
C-2	12	Yes
C-3	13	Yes
C-4	33	Yes
C-5	12	Yes
C-6	9	Yes
C-7	7	Yes
IPM-1	30	Yes
IPM-2	7	Yes
IPM-3	38	Yes
IPM-4	4	Yes
IPM-5	15	Yes
IPM-6	40	Yes
IPM-7	21	Yes
IPM-8	4	Yes
IIPM-1	20	Yes
IIPM-2	17	Yes
IIPM-3	30	Yes
IIPM-4	30	Yes
IIPM-5	10	Yes
IIPM-6	17	Yes
IIPM-7	30	Yes
IIPM-8	60	No
IIPM-9	7	Yes

Tooth	Degree of taper	Resistance form
IIPM-10	39	No
IIPM-11	30	Yes
IIPM-12	10	Yes
IM-1	22	Yes
IM-2	18	Yes
IM-3	36	Yes
IM-4	15	Yes
IM-5	28	Yes
IM-6	15	Yes
IIM-1	33	Yes
IIM-2	27	Yes
IIM-3	2	Yes
IIM-4	22	Yes
IIIM-1	15	Yes
IIIM-2	45	No
IIIM-3	50	No
IIIM-4	22	Yes

CI: Central incisor; LI: Lateral incisor; C: Canine; IPM: First premolar; IIPM: Second premolar; IM: First molar; IIM: Second molar; IIIM: Third molar



Graph 2. Degree of taper comparisons of upper and lower teeth

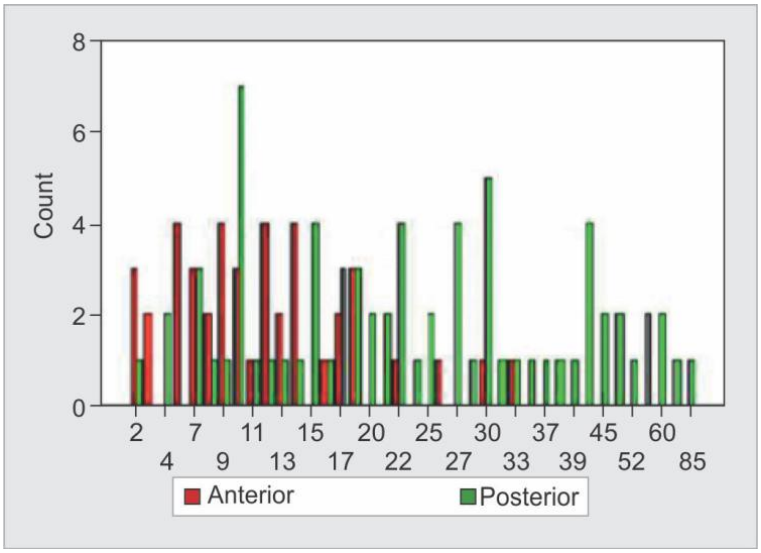
Collected data were analyzed using Statistical Package for the Social Sciences for Windows 7, and descriptive tables and cross-tabs were prepared. A variance ratio test carried out (F-test) before comparing the degree of taper between maxillary and mandibular teeth as the standard deviations for these groups were unequal.

Modified t-test applied for the analysis and it showed a significant difference between these groups with t = 2.49 and p = 0.015. Similar tests used for anterior–posterior teeth comparison within maxillary (t = −5.090 and p = 0.0001) and mandibular (t = −6.89 and p = −0.002) groups also showed significant difference in degree of taper. Kruskal– Wallis one-way analysis of variance test for comparing the degree of taper among different teeth also showed the highly significant difference in taper with p = 0.0001.

Table 3. Degree of taper and resistance form—mandibular teeth

Tooth	Degree of taper	Resistance form
CI-1	10	Yes
CI-2	6	Yes
CI-3	16	Yes
CI-4	18	Yes
LI-1	14	Yes
LI-2	10	Yes
LI-3	8	Yes
LI-4	2	Yes
C-1	22	Yes
C-2	30	Yes
C-3	26	Yes
C-4	18	Yes
IPM-1	37	No
IPM-2	10	Yes
IPM-3	16	Yes
IPM-4	7	Yes
IPM-5	9	Yes
IPM-6	10	Yes
IPM-7	8	Yes
IIPM-1	17	Yes
IIPM-2	14	Yes
IIPM-3	12	Yes
IIPM-4	11	Yes
IIPM-5	21	Yes
IIPM-6	20	Yes
IM-1	27	Yes
IM-2	27	Yes
IM-3	40	No
IM-4	10	Yes
IM-5	60	No
IM-6	32	Yes
IM-7	52	No
IM-8	56	No
IM-9	85	No
IM-10	40	No
IM-11	25	Yes
IM-12	56	No
IIM-1	77	No
IIM-2	27	Yes
IIM-3	25	Yes
IIM-4	18	Yes
IIM-5	45	No
IIM-6	22	Yes
IIM-7	10	Yes
IIM-8	40	No
IIM-9	10	Yes
IIIM-1	24	Yes
IIIM-2	13	Yes
IIIM-3	50	No
IIIM-4	18	Yes

CI: Central incisor; LI: Lateral incisor; C: Canine; IPM: First premolar; IIPM: Second premolar; IM: First molar; IIM: Second molar; IIIM: Third molar



Graph 3. Degree of taper comparisons of anterior and posterior teeth

3. RESULTS

Collected data analyzed using SPSS for windows 7, descriptive tables and cross tabs were prepared. A variance ratio test carried out (F test) before comparing the degree of taper between maxillary & mandibular teeth as the standard deviations for these groups were unequal.

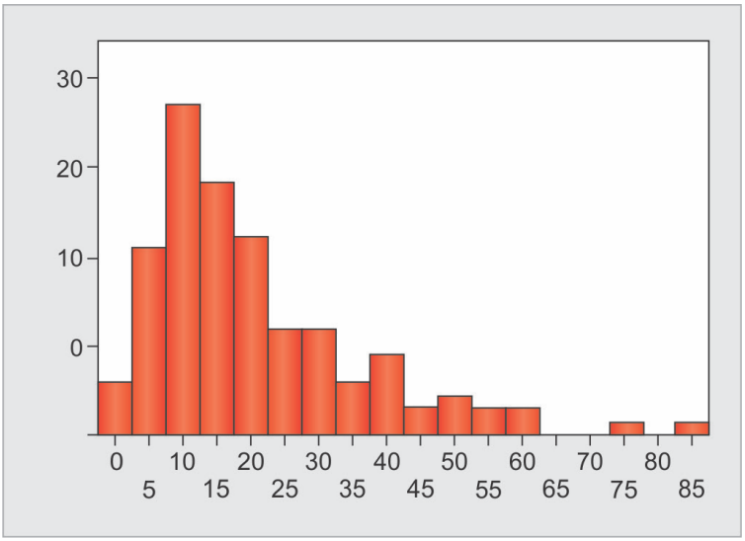
Modified T – test was applied for the analysis showed significant difference between these groups with t value 2.49 and p value 0.015. Similar tests were used for anterior posterior teeth comparison within maxillary (t value-5.090 and p value 0.0001) and mandibular (t value-6.89 and p value-0.002) groups also showed significant difference in degree of taper. Kruskal- Wallis one way ANOVA test for comparing the degree of taper among different teeth also showed highly significant difference in taper with p value 0.0001.

For the present study the overall average (mean) degree of taper is 20.9º (range- 2ºto 80º). The mean degree of taper for maxillary was 17.56º (Anteror-10.50º, Posterior-23.7º). For mandibular teeth mean degree of taper was 25.22º (Anteror-15º, Posterior-28.45º) respectively.

Chi square test used for comparing resistance form showed highly significant differences between maxillary and mandibular teeth (p value-0.007) & also between anterior and posterior teeth (p value-0.001 (Graphs 2 and 3).). Out of the 64 analyzed images of tooth preparations of maxillary teeth 60 preparations (93.8%) were found to have sufficient resistance form and 4 were found to be without it. Tooth preparations without resistance and retention form were from the posterior teeth group (Tables 2 and 3).

Out of 50 mandibular tooth preparations analyzed 38 were found to be with resistance form (76%) and 12 were found to be without resistance form. As observed in the maxillary arch those preparations without resistance and retention form were again from the posterior (77.8%) teeth group.

As observed in the study majority of the tooth preparations which had a degree of taper ≥35º showed lack of resistance & retention form but a few preparations even with 40º of convergence angle showed sufficient retention & resistance form. All the anterior teeth showed resistance form irrespective of the arch (100%). Significantly the maximum number preparations without retention & resistance form were posteriors with Ist molar group having 61.1% resistance form followed by IIIrd molar group (63.5%), IInd molar group (76.9%),IInd premolar group (88.9%) and Ist premolar group(93.3%).



Graph 4. Distribution of degree of taper

4. DISCUSSION

In the literature, there are many studies available that evaluate the resistance and retention form, but most of them are based on measurements on die preparations and simulated models.5,7,13-19 In the era of digital diagnostic aids, digital imaging, and computer-aided design (CAD)/ computer-aided manufacturing (CAM)-based restorative treatments, the study on resistance and retention form based on measurements on digital impressions of clinical tooth preparations, to best of our knowledge, have very rarely been undertaken. Apart from that, the study also utilized two methods simultaneously to assess the resistance and retention form to provide an unbiased result.

Mean degree of taper (20.9°) observed in the present study is more than what was recommended by most previous studies and also greater than the textbook ideal of 3 to 6°.8 All the anterior teeth showed resistance form irrespective of the arch, which may be attributed to reasons, such as increased accessibility and greater preparation height to base width ratio.

Trier et al12 described about the concept of limiting taper, as it has been described as a boundary between tapers that do and those that do not provide resistance form for preparation. Their study revealed a relationship between clinical success or failure and the all-or-none nature of resistance form as the dislodged crowns come almost exclusively from preparations with tapers that did not provide resistance form. Ayad et al. [19] confirmed through his study that tooth preparation taper and type of luting cement had a direct effect on the bond strength of complete metal crowns [20]. In a computer-aided evaluation of preparations for CAD/CAM-fabricated all-ceramic crowns done by Güth et al. [21] they concluded that mean convergence angle was determined to be 26.7°, which was much higher than the recommended taper and similar to the results of the present study.

In an interesting study by Tiu et al. [22] they presented a coordinate geometry method of capturing and evaluating crown preparation geometry in which they used limiting taper theory based on the mathematical formula described by Parker et al. [23,24]. If H is the occlusion cervical dimension, B is the base of preparation, and T1 is the average taper, then the limiting taper is T (H/B) = ½/sin (H/B). If T1 is greater than this value, the preparation has no resistance form. However, none of the preparations had total occlusal convergence values close to 12°, which are the current manufacturer guidelines [23,24]. Criteria related to the permissible degree of taper of preparations in current Prosthodontic Textbooks, such as Rosenstiel et al. [20] and Shillingburg et al. [25] recommend that the taper should be within the range of 2 to 6°. However, various studies conducted by different authors, such as Olm and Silness, [3] Weed and Baez, [26] Noonan and Goldfogel, [6] Güth et al. [21] and Tiu et al. [22] show that in a clinical setting, the degree of taper ranges from 12 to 26.7° [8,9,10,27,28]. This also justifies the mean degree of taper observed in the present study, which is

20.9° Even though as a foundation principle of fixed prosthodontics, 2 to 6° has been taught for years as a standard for preparation taper, unfortunately, it is not consistent with reality. Owen states, "Most teeth are prepared with tapers in excess of 12° and still function adequately [22]".

It is not known what retentive figure is the minimum required clinically. An explanation for the inability of retention to provide a minimum standard is that it is a continuous function of taper. There is no exact demarcation to separate acceptable taper from unacceptable taper as it differs in different clinical situations. The most that can be deduced is that less taper gives better retention [22].

The essential principle in determining the success of crowns and permanent partial dentures has been resistance form. Loss of cement bonding without damaging the abutment or crown is one of the most common modes of clinical crown failure. It is widely assumed that if sufficient resistance form exists, the cement will not be subjected to excessive tensile and/or shear stresses, lowering the risk of crown dislodgement owing to cement failure. Resistance form can be regarded as the accumulation of multiple resistance areas on the preparation surface that are placed in compression and that prevent rotational displacement of the crown when it is subjected to lateral forces. Factors like magnitude and direction of the dislodging force, geometry of the tooth preparation, and physical properties of the luting agent will be the deciding elements for adequate resistance to dislodgenment. Among these factors, magnitude and direction of the dislodging force are fundamental patient factors that the dentist may not be able to appropriately manage [2,29].

The diameter for every preparation is controlled somewhat by tooth size. However, length and occlusal convergence are subject to modification.25 Before crown preparations are made, factors, such as length, diameter, and occlusal convergence angle must be evaluated. In general, these factors have been considered in relation to retention form only. It has been reported that an occlusal convergence angle of 16° or less provides adequate resistance form for a complete cast crown 3.5 mm long.25 Hence, further studies are necessitated to conclusively decide on how to develop a guideline, which is more reliable and more efficient to assess the resistance and retention form. Clinically achievable taper is also affected by multiple factors like dexterity and experience of the dentist, accesses available for tooth preparation because of the tooth location, angulations, crowding, restricted mouth opening, tongue and cheek interference, anatomical limitations amount or remaining tooth structure etc.

One limitation of the assessment methods used in the present study is that it can only be used in straight-walled preparations and also that the resistance and retention form are assessed from point-to-point rather than from any one aspect of the tooth. However, this study has been able to establish that the morphological factors of individual teeth and tooth position in the arch have an overall bearing on the retention and resistance form as evaluated by Zuckerman's and Lewis perpendicular methods. Indication for the next study is to extend the analysis to the entire preparation, to determine an average taper value that guarantees that the entire preparation is resistive and, therefore, clinically sound. For the average dimensions of each tooth, molars to incisors, values can be calculated as guidelines for preparation taper.

5. CONCLUSION

Within a few mentioned limitations, the study can conclude that:

- There is a significant relationship between the degree of taper with resistance and retention form, and the relationship was inversely proportional to each other.
- The recommended "degree of taper" is not always the clinically achievable as advocated in textbooks, as it is modified by various subjective and objective factors in the actual clinical situation.
- Optimal retention and resistance form for extra coronal restorations should be established or assessed based on the inclusive analysis that takes into consideration the geometric configuration of tooth preparations, including aspects, such as, convergence, surface area of the preparations, internal surface roughness of castings, auxiliary grooves, tooth surface preparation, and type of cement used and not exclusively any one of them.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 14
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Study on Nasal Parameters of Medical Students (n=61) in South Indians: A Clinical Analysis

Mukherjee Ankita Atin [a*] and Shivaam Kesarwaani [b]

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ABSTRACT

Background: The shape of the nose indicates the ethnicity, race, age and sex. Both genetic and environmental factors influence the shape of the nose. Anthropometric parameters vary with age, sex, and ethnic background, and several authors have attempted to document the normative values which may serve as references.
Materials and Methods: This study includes measurement of different parameters of nose among 61 South Indian medical students (34 females;27 males) which were statistically analysed.
Results: 1) Morphological width of the nose -3.3cm (males) and 2.9 cm(females).2) Columella Length -1.8 cm (males) and 1.6 cm(females).3) Columella Width -0.59 cm(males) and 0.57 cm(females).4) Alar Width -0.52 cm (males) and 0.45 cm (females). 5).The most common type of cheek alar groove was cheek type followed by labial and tube type in both males and females.
Conclusion: All the measurements can be used for evaluation of nasal deformity, treatment planning and post surgical evaluation of the correction achieved during rhinoplasty.

Keywords: Alar width; columella length; columella width; cheek alar groove.

1. INTRODUCTION

The shape of the nose indicates the ethnicity, race, age, and sex. Anthropometric parameters vary with age, sex, and ethnic background, and several authors have attempted to document the normative values which may serve as references. The meaning of the word “aesthetics” is to sense and it expresses tastes and values of every culture. Nose is a pyramidal structure located in the midline of the mid-face and attached to the facial skeleton made up of bones, cartilages, muscles, and soft tissue [1]. During racial evolution, nose shows a high degree of variation [2]. Both genetic and environmental factors influence the shape of the nose [3,4]. For beautification of the nose, the evaluation should be based on anthropometric considerations which are related to individual’s relationship with society and its racial and ethnic considerations. There are many studies that analysed anthropometric nasal parameters among different regions of Indian population [5,1,2,6]. This study is carried out among males and females of South India which will help in understanding the idea of a perfect nose among South Indian population. Analysing these variations will also help in the treatment planning as well as post operative evaluation of the nose in question. The face is divided into three equal portions by four horizontal lines [7].

- **Glabella-** Bony triangular area on frontal bone between the supraorbital ridges [7].
- **Nasion-** Junction of upper end of suture between nasal bones with frontal bones [7].
- **Rhinion-** The lower end of suture between the nasal bones [7].
- **Subnasal-** Point at the nasal spine where the nasal septum merges with upper lip in the mid saggital plane [7].
- **Frankfort line-** A line along intraorbital border and tragus [7].
- **Gnathion-** Lowest point in the midline of chin [7].

[a] *Department of ENT and HNS, College of Medicine and JNM Hospital, Kalyani, West Bengal, India.*
[b] *Department of ENT and HNS, Calcutta National Medical College, Kolkata, West Bengal, India.*
**Corresponding author: E-mail: ankita.atin@gmail.com;*

2. MATERIALS AND METHODS

After obtaining Institutional ethics committee clearance and consent from 61 South Indian Medical students (34 females and 27 males) aged between 21 and 25 years, procedure was explained to them. Pictures of basal view, frontal view, profile view of nose of the students were taken methodically. Measurements using Vernier calipers were taken and statistically analysed using Pearson's Chi Square Test. Students who have undergone previous nasal surgeries and having nasal deformities or trauma were not included in the study.

The measurements were documented by photography. The photographic set up consisted of Canon SX610 HS Digital camera with camera effective pixels of approx. 20.2 megapixels. Aspect ratio 4:3. All images are taken under uniform illumination. The subjects were asked to sit against a dark coloured backdrop and were asked to look straight into the camera in natural head position with facial muscles relaxed. All the photographs were taken at a distance of 5 feet for a sharp image.

The parameters measured were-

1. **Morphological width of the nose -** The maximum length between the most lateral point in the curved base line of each ala [8].
2. **Columella length-** The distance between subnasal and highest point of columella [8].
3. **Columella width-** The distance between midpoints of columella [8].
4. **Alar width-** The distance between the midportion of the alae where the thickness of each ala is measured [8].
5. **Cheek alar groove-** The shape and resiliency of the nostril and the posterior half of the alar side walls depend on dense fibro-fatty connective tissue. The variations of the cheek alar groove are-a) Cheek type, b) Labial type, c) Tube type [9].

3. RESULTS

The mean values of different nasal parameters are enlisted below:

Morphological width of the Nose: a) Among males, the mean morphological width of nose was 3.3cm and it varied from 2.94cm to 3.66 cm (Table 1). Out of 27 males,20(74.07%) are within the range,4(14.81%) more than the range and 3(11.11%) less than the range. b)The mean morphological width of nose among females was 2.9 cm and it varied from 2.67cm to 3.13 cm (Table 2). Out of 34,24(70.59%) lie within the range,6(17.65%) more than the range and 4(11.75%) less than the range.

Columella Length: a) Among males, the mean columella length was 1.8cm and it varied from 1.35 cm to 2.25 cm (Table 1). Out of 27,20 (74.07%) lie the range,3(11.11%) more than the range and 4(14.81%) less than the range. b)The mean columella length among females was 1.6 cm and it varied from 1.37 cm to 1.83 cm (Table 2).Out of 34,26(76.47%) lie within the range,5(14.70%) more than the range and 3(8.82%) less than the range.

Columella Width: a) Among males, the mean columella width was 0.59cm and i varied from 0.47 cm to 0.71 cm (Table 1).

Out of 27, 21(77.77%) lie the range,2(7.41%) more than the range and 4(14.81%) less than the range. b)The mean columella width among females was 0.57 cm and it varied from 0.48 cm to 0.66 cm (Table 2).Out of 34,25(73.53%) lie within the range,6(17.65%) more than the range and 3(8.82%) less than the range.

Alar width: a) Among males, the mean alar width was 0.52cm and it varied from 0.42 cm to 0.62 cm (Table 1). Out of 27,18(66.66%) lie the range,2(7.41%) more than the range and 7(25.92%) less than the range. b)The mean alar width among females was 0.45 cm and it varied from 0.38 cm to 0.52 cm (Table 2). Out of 34, 29 (85.29%) lie within the range, 2 (5.88%) more than the range and 3(8.82%) less than the range.

Cheek Alar Groove: The most common type of cheek alar groove was found to be cheek type followed by labial and tube type in both males and females. In males, out of 27, 15(55.55%) had cheek type, 9(33.33%) had labial type and 3(11.11%) tube type. In females, out of 34, 20(58.82%) had cheek type, 13(38.23%) had labial type and 1(2.94%) had tube type (Table 3).

Studying variations of nose gives us an idea about the variations of anthropometric aspects of nose between males and females.

4. DISCUSSION

The nose balances the facial appearance .The shape of the nose differs with ethnicity,race, age, and sex. This study is aimed to describe the differences in various nasal anthropometric measurements among South Indian males and females.

The mean morphological width of nose for males was found to be 3.3±0.36cm (Table 1) which is more than females (2.9±0.23cm) by 13% approximately (Table 2). This was similar to the study conducted by Ahmet Uzhun et al. [8] (3.3 cm) in Turkish males but shorter than Afro– American [10] (4.3cm), Chinese [11] (3.9cm), Japanese [12] (3.6cm),Canadian–Caucasian [13] (3.6cm),African [14] (4.5cm), Afro–Caucasian noses [14] (3.9 cm) and Afro-Indians [14] (4.2 cm) by 38%, 25%, 16%, 16%, 45%, 25% and 35% respectively.

Table 1. Mean with standard deviation of different nasal parameters in males

Nasal parameters	Mean	Standard deviation	Minimum	Maximum
Morphological width of nose(cm)	3.3	0.36	2.94	3.66
Columella Length(cm)	1.8	0.45	1.35	2.25
Columella Width(cm)	0.59	0.12	0.47	0.71
Alar Width(cm)	0.52	0.10	0.42	0.62

Table 2. Mean with standard deviation of different nasal parameters in females

Nasal Parameters	Mean	Standard deviation	Minimum	Maximum
Morphological width of nose(cm)	2.9	0.23	2.67	3.13
Columella Length(cm)	1.6	0.23	1.37	1.83
Columella Width(cm)	0.57	0.09	0.48	0.66
Alar Width(cm)	0.45	0.07	0.38	0.52

Table 3. Frequency of cheek alar groove in males and females

Gender	Cheek	Labial	Tube	Total
Males	15(55.55%)	9(33.33%)	3(11.11%)	27
Females	20(58.82%)	13(38.23%)	1(2.94%)	34
Total	35	22	4	61

The mean columella length for males was found to be 1.8±0.45 cm (Table 1) which is more than females (1.6±0.23cm) (Table 2) by 12.5% approximately. This was more than the Turkish men [8] (0.9 cm), Chinese [11] (1.1cm), Caucasian [10] (1.1 cm) and Afro–American [10] (1 cm), Afro–Indians [14] (0.8cm) but shorter than Japanese [10] (2.2cm) by 47%, 35%, 35%, 41%, 52% and 29% respectively.

The mean columella width for males was found to be 0.59±0.12 cm (Table 1) which is more than females (0.57±0.09cm) (Table 2) by 3% approximately. This is greater than what was found by Ahmet Uzhun et al. [8] (0.5 cm) by 8%.

The mean alar width for males was found to be 0.52±0.10 cm (Table 1) which is more than females (0.45±0.07 cm) (Table 2) by 13%. This is similar to what was found by Ahmet Uzhun et al. [8] (0.48cm).

The most common type of cheek alar groove was cheek type followed by labial and tube type in both males and females (Table 3).

5. SUMMARY

The shape of the nose is a signature indicating the ethnicity ,race, age, and sex. Anthropometric parameters vary with age, sex, and ethnic background, and several authors have attempted to document normative values which may serve as references.

This study includes measurement of different parameters of nose among 61 South Indian medical students (34 females; 27 males) using vernier calliper and was statistically analysed.

The means of various parameters were- 1) Morphological width of the nose -3.3cm (males) and 2.9 cm (females).2) Columella length -1.8 cm(males) and 1.6 cm(females).3) Columella Width -0.59 cm(males) and 0.57 cm(females).4) Alar Width -0.52 cm(males) and 0.45 cm (females). 5).The most common type of cheek alar groove was cheek type followed by labial and tube type in both males and females.

All the measurements can be used for evaluation of nasal deformity, treatment planning and post surgical evaluation of the correction achieved during rhinoplasty.

6. LIMITATIONS OF THE STUDY

1. Low study population.
2. Medical students often do not represent all young people.

7. CONCLUSION

The variations between males and females in same geographical locations are due to the genetic makeup. The normal values will help in evaluating nasal deformities and will help us mapping the results of rhinoplasty in South Indians.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

Mukherjee Ankita Atin
Department of ENT and HNS, College of Medicine and JNM Hospital, Kalyani, West Bengal, India.

She is an expert surgeon & specialist in ENT & Head-Neck Surgery. She has done her double specialisation in field of ENT & Head-Neck Surgery(DNB,DLO) And has special interest in endoscopic scarless ENT surgeries. Completing her DNB from College of Medicine & J.N.M. Hospital, She is posted as senior resident surgeon at same institution. She has keen interest in scientific research. She has around 8 clinical research papers, published in renowned international journals. She has a vision to expand the horizons of ENT in field of Endoscopic Skull Base Surgeries. She sets an inspiring example by benefitting huge number of medical students with her style of teachings, courtesy, motivation and the knowledge.

Shivaam Kesarwaani
Department of ENT and HNS, Calcutta National Medical College, Kolkata, West Bengal, India.

He Is a highly ambitious author, teacher, motivational guide, one of the emerging ENT, and Head-Neck Surgeon. He is praised for his teachings through his youtube channel across country. This has helped medical aspirants & has now around 25,000 doctor's subscriptions and of over 35 Lakhs minutes of views by aspirants across the country. Completing his MS from Calcutta National Medical College and Hospital, he is currently posted as senior resident surgeon at same institution. He has around 15 clinical research papers published in renowned international journals. His book "Medendo" dedicated to NEET PG preparations is creating sensations. He sets an inspiring example by benefitting huge number of medical students with his style of teachings, courtesy, motivation and the knowledge. YOUTUBE CHANNEL – "NEET PG By Dr.Shivaam Kesarwaani" https://www.youtube.com/channel/UCg9Y3L2nxA74DYpKSXGN-Bw

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Chapter 15
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Reliability and Validity of the Functional Assessment of Currently Employed Technology Scale (FACETS)

Charles M. Lepkowsky [a*] and Stephan Arndt [b]

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ABSTRACT

Introduction: Health protocols have not included technology as a specific area of assessment or treatment. The Functional Assessment of Currently Employed Technology Scale (FACETS) was created for this purpose. FACETS is a ten-item questionnaire that assesses five functional domains. The current study was carried out to determine the validity and reliability of FACETS.
Methods: Using 423 previously deidentified FACETS forms from clinical records, analyses such as Cronbach's alpha, McDonald's omega, confidence intervals for alpha and omega, multiple group factor analysis, Fleming's index of scale fit, and differential item (domain) function were carried out (DIF).
Results: Internal consistency and factor validity for the 10 FACETS items and intra-domain correlations were high. The factor scale fit index calculated by Fleming indicated an excellent fit. With the exception of one domain, all domains contain enough unique information to produce differential item functioning.
Discussion and Conclusions: For the five domains, FACETS demonstrated high internal consistency reliability, strong general factor validity, and strong factor validity.

Keywords: Functional assessment of currently employed technology scale; FACETS; reliability; validity; internal consistency; domains.

1. INTRODUCTION

Technology as a specific area of assessment or treatment has not been included in protocols for health professionals [1]. The majority of research into the acceptance and use of new technologies has come from the information technology sector [2-14]. Several instruments that assess a person's perception of their own proficiency with various technologies have been developed [15-21]. The studies and models described above assess factors determining a person's decision to use specific technologies, or self-perceived proficiency in using specific technologies, but none of them functionally assesses the frequency with which the person employs commonplace current information technologies in a way that informs individualized treatment planning, and directs choice of media for communicating with a specific patient to facilitate better treatment outcomes and higher satisfaction ratings by patients and providers of care. The Functional Assessment of Currently Employed Technology Scale (FACETS, Appendix 1) was designed specifically to meet those previously unaddressed needs.

The FACETS questionnaire consists of 10 questions, two in each of 5 functional domains: Home, Social, E-commerce, Health Care, and Technical. Each question has 6 optional answers that characterize the respondent's frequency employing a specific type of information technology. The scores for the two questions in each functional domain are added to produce a subtotal for that domain. The five domain subtotal scores are then added to produce an overall total score.

[a] Independent Practice, 1143 Deer Trail Lane, Solvang, CA 93463, USA.
[b] Carver College of Medicine, University of Iowa, Iowa Consortium for Substance Abuse Research and Evaluation, USA.
**Corresponding author: E-mail: clepkowsky@gmail.com;*

Higher scores suggest more frequent utilization of technologies across domains. There are no foreseen risks or benefits associated with completing FACETS. Some updates in this area are available elsewhere and may find attention of the readers [22-24]. The current study was conducted to establish validity & reliability for FACETS.

2. METHODS

423 completed FACETS forms were randomly selected using pre-existing deidentified records originally collected for clinical purposes. Respondents varied in age, ethnicity, socio-economic status, household income, and educational level. No control group was applicable. The Santa Barbara Cottage Health Hospital Review Board granted a waiver for the current data.

2.1 Statistical Analysis

The distribution of FACETS scores was markedly non-normally distributed. Consequently, nonparametric statistical tests were used when possible. Cronbach's alpha coefficient was used to assess internal consistency. Additionally, calculated McDonald's omega [25] was calculated. Confidence intervals for alpha and omega were found using bootstrap resampling (5,000 iterations). Item statistics were also derived including the alpha if each item was removed with corrected item total correlations. To address the factor validity of the five domains, multiple group factor analysis, a quasi-confirmatory method, was used [26,27]. This method uses a weighting matrix to pre-define the factors, in this case the five domains. The weights used the item standard deviations to simulate actually summing the items to construct the domains. An oblique extraction allowed the domains to remain correlated. Fleming's index of scale fit is reported [28]. Differential item (domain) function (DIF) was assessed using partial correlations. Each domain was correlated with age while controlling for the sum of the other domains. Statistical analyses were performed using STATA 15MP and R.

3. RESULTS

A description of the sample is shown in Table 1. The mean age in the sample was 54.58 (sd = 18.42). The youngest respondent was 18 years of age while the oldest was 95 years old. The sample was predominantly female and had an income between $50,000 and $100,000. The most frequently cited education was a Bachelor's degree. Over 90% had home access to a computer and to the internet.

Table 1. Sample demographics

Trait	Number of respondents	% of sample
Gender		
Male	173	40.9
Female	250	59.1
Race/Ethnicity		
Hispanic	50	11.99
African American	5	1.2
Asian	11	2.64
Other	351	84.17
Income		
<$25,000	8	1.9
<$50,000	71	16.9
<$100,000	154	36.67
<$150,000	98	23.33
>$150,000	89	21.19
Education		
N/A	4	0.96
High School	80	19.14
Some college	97	23.21
AA	8	1.91
Bachelor's	180	43.06
Post graduate	49	11.72

Trait	Number of respondents	% of sample
Access to computer		
Yes	386	92.79
No	30	7.21
Access to High-speed Internet		
Yes	388	93.49
No	27	6.51

Internal consistency for the 10 items was high, Cronbach's alpha = 0.95 (95% CI: 0.94 – 0.96). Similarly, omega was 0.95 (95% CI: 0.94 – 0.96). These high internal consistency values suggest a strong general factor underlying the FACETS score. Alpha is not increased by removing any item and all of the corrected item total correlations are moderate to high, indicating some item redundancy between the items and the large general factor. Item statistics appear in Table 2.

Table 2. Data by item, SD, alpha and corrected item total correlations if item removed

Item numb	Mean	SD	Alpha if Item removed	Corrected item total correlation
1	4.32	1.50	0.95	0.73
2	4.19	1.54	0.95	0.75
3	4.35	1.63	0.95	0.68
4	4.07	1.77	0.95	0.69
5	3.11	2.17	0.94	0.89
6	3.13	2.16	0.94	0.87
7	2.63	2.14	0.94	0.86
8	2.58	2.13	0.94	0.85
9	3.14	2.21	0.94	0.80
10	2.74	2.03	0.95	0.79

Table 3 shows the factor structure of the five domain scores.

Table 3. Factor structure for five domain scores

Item Number	Domain 1	Domain 2	Domain 3	Domain 4	Domain 5
1	0.99	0.83	0.55	0.50	0.52
2	0.99	0.80	0.60	0.54	0.55
3	0.82	0.97	0.55	0.47	0.45
4	0.79	0.97	0.56	0.53	0.42
5	0.58	0.57	0.99	0.87	0.75
6	0.58	0.56	0.99	0.85	0.74
7	0.53	0.51	0.87	1.00	0.76
8	0.53	0.51	0.85	1.00	0.75
9	0.55	0.46	0.75	0.75	0.99
10	0.52	0.43	0.74	0.75	0.99

All of the items were correlated with all of the domains due to the large general factor. However, the domain specific correlations were near 1.0. Communalities were all 0.95 or higher. The domain intercorrelations ranged from 0.51 (Domain 2 and 4) to 0.83 (Domain 1 and 2). The factor solution accounted for 97.87% of the total variance and the root mean squared residual was less than 0.01, indicating good fit. Fleming's factor scale fit index was 0.99 overall and greater than 0.97 for each domain, also indicating excellent fit.

Differential item (domain) functioning (DIF) was also investigated regarding age of the respondent. Partial correlations were calculated between age and each domain, controlling for the sum of the remaining domains. If the relationship of age was constant across all domains, controlling for the remaining domains should make the partial correlation go to near zero. Only Domain 1 did not show DIF (partial $r = 0.06$, $p > 0.21$). Domains 2, 3, 4, and 5 all showed differential correlations with the

partial correlations ranging from -0.17 (Domain 2) to -0.35 (Domain 3; p's < 0.001). These analyses were repeated using partial Spearman correlations with nearly identical results. The partial Spearman correlations ranged from -0.23 (Domain 2) to -0.50 (Domain 3; p's < 0.001). Thus, while there is a strong general factor, all but one domain contain sufficient unique information to produce differential item functioning.

4. DISCUSSION AND CONCLUSIONS

There are two main findings to this study. One finding is both the large alpha and omega coefficients indicate very high internal consistency reliability for FACETS. The other finding is that FACETS demonstrated strong factor validity for the five domains, in addition to the strong general factor. This finding suggests that both the overall score (summing all of the items) and the five individual domain scores can offer meaningful values.

Additional analyses indicated that domains demonstrated differential item functioning with regard to dependent variables. While this can cause some issues for instruments thought to have a general factor, it adds weight to the validity of the domains. The DIF also supports the need for considering the domains separately, and further confirms the validity of the five domain factor solution.

This study has several strengths as well as limitations. The strengths include a large sample size to generate accurate estimates for the internal consistency coefficients and the factor solution. Another strength was the broad ranges for age, education, and income. However, this was also a convenience sample in a clinical setting, which may limit the generalizability to the general public.

Overall, the high internal consistency reliability and strong factor validity suggest that FACETS has value for determining not only an individual's overall frequency of IT use, but also for determining in which technology domains the individual has greater or lesser frequency of IT use. FACETS also appears effective for determining differences between groups, not only in general frequency of IT use, but within specific IT usage domains. FACETS has demonstrated value in a clinical setting, but further research is recommended using FACETS with a general population. Longitudinal studies using FACETS may also be of value for understanding age, gender, and other differences over time.

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APPENDIX 1

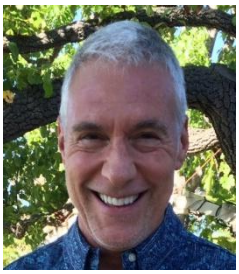
Functional Assessment of Currently Employed Technology Scale (FACETS)

Age: _____ ○Male/○ Female ○ Hispanic ○ African American ○ Asian ○ Other
Household Income: ○< $25,000 ○< $50,000 ○< $100,000 ○< $150,000 ○ >$150,000
Degree: ○N/A ○High School ○Some college ○ AA ○ Bachelor's ○ Post graduate
Access to a computer at home? ○Yes/ ○No **Access to internet at home?** ○Yes/ ○No

Instructions: Check the response that most accurately completes each statement.

A.	**Home Domain**						
1.	I send email...	○ Never	○ A few times a year	○ A few times a month	○ Once a week	○ A few times a week	○ Daily
2.	I find, open & close files in my computer...	○ Never	○ A few times a year	○ A few times a month	○ Once a week	○ A few times a week	○ Daily
Home Domain Subtotal							
B.	**Social Domain**						
3.	I send text messages using a smart phone…	○ Never	○ A few times a year	○ A few times a month	○ Once a week	○ A few times a week	○ Daily
4.	I post on social media (e.g., facebook, twitter)…	○ Never	○ A few times a year	○ A few times a month	○ Once a week	○ A few times a week	○ Daily
Social Domain Subtotal							
C.	**E-Commerce Domain**						
5.	I manage my banking and credit card accounts online…	○ Never	○ Tried, but it didn't work	○ Got help but didn't work	○ Only with help	○ Can but prefer not to	○ Prefer to
6.	I pay bills and make purchases via the internet…	○ Never	○ Tried, but it didn't work	○ Got help but didn't work	○ Only with help	○ Can but prefer not to	○ Prefer to
E-Commerce Domain Subtotal							
D.	**Health Care Domain**						
7.	I communicate with my doctor or clinic online…	○ Never	○ Tried, but it didn't work	○ Got help but didn't work	○ Only with help	○ Can but prefer not to	○ Prefer to
8.	I communicate with my health insurance company online…	○ Never	○ Tried, but it didn't work	○ Got help but didn't work	○ Only with help	○ Can but prefer not to	○ Prefer to
Health Care Domain Subtotal							
E.	**Technical Domain**						
9.	I have installed components (monitors, speakers, mice)…	○ Never	○ Tried, but it didn't work	○ Got help but didn't work	○ Only with help	○ Myself, with difficulty	○ Myself easily
10.	I have reset a modem or router in my home…	○ Never	○ Tried, but it didn't work	○ Got help but didn't work	○ Only with help	○ Myself, with difficulty	○ Myself easily
Technical Domain Subtotal							
Total FACETS Score							

Biography of author(s)



Charles M. Lepkowsky
Independent Practice, 1143 Deer Trail Lane, Solvang, CA 93463, USA.

He has been a faculty member of graduate psychology programs at the University of California at Santa Barbara, Antioch University and the University of San Francisco, a member of the medical staff and department chair at local hospitals and an independently practicing psychologist since 1985. He has served as clinical and executive director for several non-profit counseling agencies and in regional, state and national psychological associations. He is active in patient and practice advocacy and legislative action work. His research interests include Geropsychology, Neurocognitive Disorders, Collaborative Care, Personality Disorders, Medical Psychotherapy, Technology, Epidemiology, and Person-Centered Approaches.

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Chapter 16
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Diagnosis and Management of Chronic BCR-ABL-Positive and BCR-ABL-Negative Myeloproliferative Neoplasms in Elderly Patients: An Approach towards Hematologic Oncology and Public Health

Vasile Musteata [a,b*] and Valentina Stratan [c]

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ABSTRACT

Background: Chronic myeloproliferative neoplams (CMN) are totally the most common chronic leukemias among the elderly persons in the structure of morbidity by hematologic malignancies with primary bone marrow involvement, being characterized in the advanced stages by a severe, relapsing evolution and unfavorable prognosis, with negative socio-economic impact.
Objectives of the Study: Evaluation of the diagnosis and management options in chronic BCR-ABL-positive and BCR-ABL-negative myeloproliferative neoplasms in elderly patients in order to upgrade an approach to hematologic oncology care.
Material and Methods: A clinico-analytical, descriptive, prospective-retrospective study was accomplished along with the narrative review of the international literature on the subject. The study included 91 elderly patients with chronic myeloid leukaemia (CML), primary myelofibrosis (PMF), and polycythemia vera (PV) who were followed and treated at the Institute of Oncology from 1995 to 2021. In regard to the impact scale, 29 relevant primary sources were distinguished and selected with a scientific, reproducible and transparent approach to the subject under discussion, followed by the data extraction and analysis.
Results: Thirty-four (37.3%) patients with PMF, 26 (28.6%) – with CML and 31 (34.1%) – with PV were diagnosed in the elderly age groups and followed up by our study. The age group of 60-69 years dominated in CML (22 cases or 84.6%), constituting 25 (80.6%) cases in PV, and 25 (73.5%) - in PMF. The one- and 5-year overall survival (OS) in CML patients aged greater than or equal to 60 years treated with tyrosine kinase inhibitors (TKIs) was 97.6% and 79.8%, being lower as compared with the same indicators in the totality of CML. In elderly PV patients the OS over one year constituted 100%, over 5 years – 93.5%, over 10 years – 76.4%, being lower within 5-10 years than those registered in all patients with PV (over one year – 100%, 5 years – 98.6%, 10 years – 85.9%). Although the relapse rate was lower in patients treated with busulfan as compared to those managed with hydroxycarbamide, there was no significant difference in the OS of the elderly PV patients undergoing chemotherapy with these antineoplastic agents. As stipulated in the recent bibliographic databases, a significant rate of patients with CMN experienced the reduced work hours, discontinued employment, and medical disability: PMF – 38%, 35%, 33%, and PV – 33%, 28%, and 15%, respectively.
Conclusions: The long-term results of treatment in elderly patients with CMN proved to be inferior to those in CMN totality because of the development of age-related diseases and vascular events on the account of the increased values of leukocytes and platelets. The targeted treatment with TKIs remains a first-line management option for CML patients of 60 years and more. In the elderly PV patients no significant difference was revealed in short- and long-term outcomes of chemotherapy with busulfan and hydroxycarbamide in combination with phlebotomy, being totally superior to those in PMF patients.

[a] *Discipline of Hematology, "Nicolae Testemitanu" State University of Medicine and Pharmacy, Republic of Moldova.*
[b] *Department of Hematology, Institute of Oncology Chisinau, Republic of Moldova.*
[c] *Laboratory of Immunology and Molecular Genetics, Institute of Oncology Chisinau, Republic of Moldova.*
**Corresponding author: E-mail: vasile.musteata@usmf.md;*

Keywords: Chronic myeloproliferative neoplasms; chronic myeloid leukemia; primary myelofibrosis; polycythemia vera; elderly patients; chemotherapy; survival; disability.

1. INTRODUCTION

Chronic myeloproliferative neoplasms (CMN) are totally the most common chronic leukemias among the elderly persons in the structure of morbidity by hematologic malignancies with the primary bone marrow involvement, being characterized in the advanced stages by a severe, relapsing evolution and unfavorable prognosis, with negative socio-economic impact [1,2,3,4,5,6]. The most common CMN are chronic myeloid leukaemia (CML), primary myelofibrosis (PMF), and polycythemia vera (PV).CML is a BCR-ABL-positive clonal neoplastic pathology of the hematopoietic system, which results from the malignant transformation of the pluripotent stem cell, while maintaining the ability of differentiation into all cell lineages [7,6,8,9]. CML morbidity increases with age, with a peak incidence between 40 and 65 years (median age – 53 years), indicating that the workable population is the most affected [10]. CML morbidity ranges between 1.0 and 2.0 cases per 100,000 people. The clinico-evolutional and hematologic patterns of CML comprise splenomegaly, myeloid hyperplasia of the bone marrow, hypercatabolic symptoms and progression to the acute phase (blast crisis) in refractory cases [7,9,10,11]. PMF represents a BCR-ABL-negative chronic myeloproliferative neoplasm, which derives from the clonal myeloid prolifetaion as a result of malignant transformation of the stem cell. The disease is manifested by splenomegaly, bone marrow fibrosis, anemia, extramedullary hematopoiesis, tendency to cachexia and blastic transformation. According to the majority of references, the incidence of PMF constitutes 0.7-1 case per 100000 of population [1,3,4]. In 67% of cases PMF is diagnosed in persons over 54 years old. PV is a BCR-ABL-negative clonal trilineage proliferation of the maligned hematopoietic stem cell, being characterized by the blood hyperviscosity and increased risk of thromboses. The estimated incidence of PV per 100000 of population ranges from 0.4 to 2.8 cases in Europe and from 0.8 to 1.3 cases in USA. The reported age median encompasses 65-70 years. The bone marrow is hypercellular and exhibits hyperplasia of myeloid, erythroid, and megakaryocyte lineages. Erithrocyte formation is predominantly increased. The symptoms and signs of PV can be attributed mainly to the expanded total blood volume and to the slowing blood flow as a result of increased blood viscosity. The latent thrombogenic condition develops. The arterial hypertension commonly occurs. PV and PMF are considered orphan diseases in the USA because they affect less than 200000 people regardless of the observational period [12]. Marketology researches demonstrated that in 2003 the prevalence per 100000 population of PV was 22 and PMF – 19. The development and increased prevalence of CMN in the elderlies correlates with the demographic aging process in the USA and the European Union [13], in which the rate of the population over 80 years old will triple with predictability between 2011 and 2060.

Patients across all CMN experience marked disease burden in terms of symptoms and negative effects on life quality, productivity, and daily living activities [14]. It is suggested an importance of having an updated and appropriate understanding of these burdens from a financial standpoint in order to improve the health and life quality of patients with CMN. Predominantly late diagnosis, increased degree of disability, morbidity and mortality indices in the age categories greater than or equal to 60 years [2,13] identify CMN as an actual issue of hematologic oncology and public health.

2. OBJECTIVES OF THE STUDY

The main aim of the study was the evaluation of the diagnosis and management options in chronic BCR-ABL-positive and BCR-ABL-negative myeloproliferative neoplasms in elderly patients in order to upgrade an approach to hematologic oncology care.

3. MATERIALS AND METHODS

A clinico-analytical, descriptive, prospective-retrospective study was accomplished along with the narrative review of the international literature on the topic. The study enrolled 91 elderly patients with different phases of CML, PMF and PV, who were followed up and treated at the PMSI Institute of Oncology in the period of 1995 – 2021. The following research methods were used: epidemiological, descriptive, comparative, clinical-analytical, and cohort statistics [15]. The type of CMN was identified

according to the Revised 2017 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues [16,17,18]. The diagnosis was proved by histopathological, cytological, cytogenetic and molecular examinations of the bone marrow and peripheral blood [1,5,7,8,9,18,19]. The quantitative real-time PCR was used with the aim to detemine the expression of the BCR-ABL chimeric gene p210 and p190 transcripts while proceeding CML diagnosis. Five transcription products (b2a2, b3a2, b2a3, b3a3 si e1a2) were analyzed by the usage of the quantitative PCR test [8]. The quantitative detection of JAK2 V617F mutation served as a major criterion in the diagnostically unasserted cases of PV and PMF. CML patients underwent TKI single-agent chemotherapy without serious side effects. The first-line treatment of PMF and PV patients included a single-agent conventional chemotherapy with busulfan and hydroxycarbamide. The accumulation of information for research was performed by analyzing the data provided by the international scientific sources and official statistics related to the above mentioned nosological entities. More than 50 reference bibliographic sources have been studied. In regard to the impact score, 29 relevant primary sources were distinguished and selected with a scientific, reproducible and transparent approach to the subject under discussion, followed by the data extraction and analysis. A copy of the data extraction sheet was initially obtained in order to minimize the error, listing all the elements that should be extracted from the primary studies. When doing the qualitative research, a narrative synthesis of data has been realized.

3. RESULTS AND DISCUSSION

Thirty-four (37.3%) patients with PMF, 26 (28.6%) – with CML and 31 (34.1%) – with PV were diagnosed in the elderly age groups and followed up by our study. The prefibrotic stage of PMF was confirmed in 15 (44.1%) cases, fibrotic stage – in 21 (55.9%). The diagnosis of CML was determined in the chronic phase in 24 (92.3%) patients and in the accelerated phase in 2 (7.7%) patients. In all cases PV was diagnosed in the erythremic stage: II A – in 27 (87.1%) patients, IIB – in 4 (12.9%). The age group of 60-69 years proved to be more numerous in CML (22 cases, or 84.6%), constituting 25 (80.6%) cases in PV, and 25 (73.5%) cases in PMF. The duration of the disease from the time of onset of the initial clinical symptoms to diagnosis ranged in PMF between 1.4-7 months (median – 3.7±0.63 months), in CML between 1.5-12 months (median – 2.1±0.37 months) and in PV between 1-7 months (median – 3.8±0.54 months). The clinical onset and addressability of patients with CML and PMF didn't not differ significantly, the absolute majority (over 90%) being consulted by a family doctor because of the appearance of fatigue, heaviness and then the pain in the left hypochondrium or in the left hemiabdomen. The majority of patients with PV (21 persons or 67.7%) went to the territorial medical institutions for the medical care in a case of stable hypertension or so-called "astheno-vegetative" syndrome. In 3 (9.7%) cases the diagnosis of PV followed after the treatment of myocardial infarction in cardiology wards. Two (6.5%) patients were hospitalized in neurology wards for emergency medical care with the diagnosis of ischemic stroke, in the course of which the diagnosis of PV was confirmed.

The references updates indicate, that the median life-span of CML patients under the conventional chemotherapy ranges between 4-5 years, exceeding 10 years in 30% of them. Although the allogeneic hematopoietic stem cell transplantation is considered in many instances as the most efficient curable option, with a potential of complete recovery, especially in cases refractory to TKIs, it remains currently inapplicable in the elderly CML patients [9,19,20]. Regardless the age, recombinant interferon α (IFN α-2b) preserves its role as a valid treatment option in chronic phase of CML, committing to the achievement of complete hematologic response in 81% of cases [11]. Under the treatment with IFN α-2b the major cytogenetic response may be obtained in 40% and complete cytogenetic response in 25-30% of CML patients. The overall 5-year survival of patients treated with IFN α-2b is rated at 57%, being superior to that one in patients managed with conventional chemotherapy (42%).

Our study showed no significant difference in the rate of complete clinical-hematological response (92.3% vs 92.8%) and complete molecular response (23.1% vs 24.7%) under TKIs medication between elderly patients and the totality of CML patients. The overall one- and 5-year survival in elderly patients treated with TKIs was 97.6% and 79.8% (Fig. 1), being comparable with the respective parameters in the totality of CML patients (98.5% and 87%, correspondingly). IFN α-2b was used in rare cases of resistance to conventional chemotherapy and TKIs, with partial response.

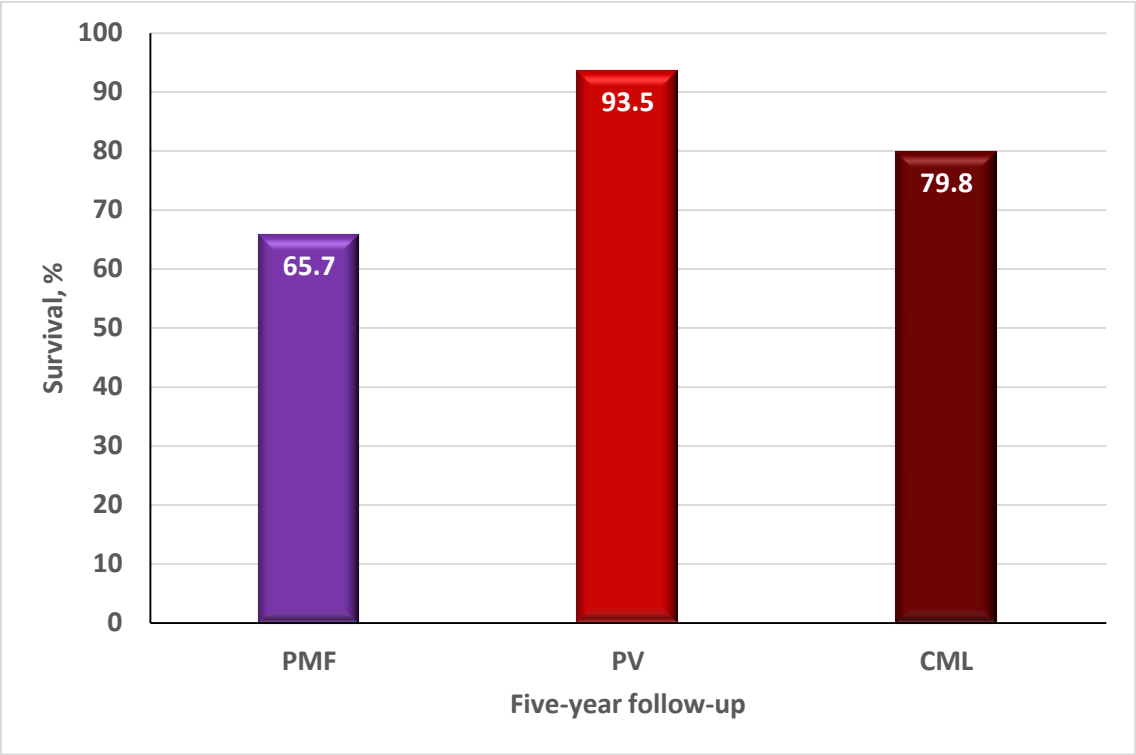

Fig. 1. Five-year survival of the elderly patients with CMN

Under the combination of chemotherapy and phlebotomies the clinico-hematological remission was achieved in all 31 patients with PV. The duration of response ranged from 3 to 9 months (median – 5.8 months). In all cases, the disease relapsed, with plethoric syndrome and thrombocytosis, which required the resumption of induction chemotherapy with busulfan, hydroxycarbamide, with regaining remissions. Fatal cases associated with the treatment and thromboembolic complications did not occur. In elderly patients the overall survival over one year constituted 100%, over 5 years – 93.5%, over 10 years – 76.4%, being lower than those registered in all patients with PV (over one year – 100%, 5 years – 98.6%, 10 years – 85.9%). Although the relapse rate was lower in patients treated with busulfan as compared to those managed with hydroxycarbamide, there was no significant difference in the overall survival of the elderly patients undergoing chemotherapy with these antineoplastic agents.

The recent bibliographic sources consider single-agent chemotherapy with hydroxycarbamide as the first-line treatment option in elderlies with PMF, associate with splenomegaly and thrombocytosis. Thalidomide or lenalidomide in combination with prednisolone, danazol may be administered in cases with marked symptoms, especially in those with splenomegaly and anemia. The patients with intermediary-2 risk, especially those with prognostically unfavorable mutations ASXL1, SRSF2 and age ≥ 65 years old, should be cosidered for treatment with JAK kinase inhibitors (ruxolitinib, etc.). In the majority of studies the survival median is estimated at 3,5-5 years, ranging from 1 year in some patients to decades in others. According to the international literature data, the survival in PMF (median – 5.9 years) remains inferior to the same indicator in the other BCR-ABL-negative CMN like PV (median – 13.5 years) and ET (median – 19.8 years) [21]. In the reported by us study, the rate of clinico-hematological responses (73.5%) and survival under busulfan and hydroxycarbamide treatment in patients with PMF were also lower than in PV and CML. The 5-year overall survival of elderly PMF patients, constituted 67% and proved to be inferior, if compared to the median 5.9-years survival in the totality of PMF patients, revealed by the majority of reports on subject [14,22,23]. Only three PMF patients, diagnosed in 2001, 2007 and 2009 respectively, continue follow-up till now. Generally, it is suggested that the new therapies may be recommended in CMN at any age without absolute contraindication. The selection of the personalized therapy for the certain patient is

mandatory [20,24]. In PMF and PV cases refractory or intolerant to hydroxycarbamide, COMFORT and RESPONSE studies showed the rate of 41.9-62% of disease control under the treatment with JAK inhibitors, as compared with best available therapy (0-19%). For these reasons, an accurate definition of diagnosis and prognostic stratification is required. Precision in CMN definition and prognostication is decisively useful for personalizing therapeutic approach [20].

The narrative review of the international experience was also performed in order to assess the financial burden of CMN on Public Health, A study on financial burden of CMN on patients was realized in the USA in 2014 [25]. The subjects who were diagnosed before 2013 and were 16 to 65 years of age at the time of diagnosis were eligible for this analysis (PMF – 85, PV – 172). Almost all patients (99%) had health insurance, primarily group commercial insurance through an employer (PMF – 46%, PV – 53%) and Medicare (PMF – 40%, PV – 34%). The mean 2013 household income of patients with PMF and PV were similar to each other (79800 USD, and 80200 USD, respectively) and slightly higher than the total 2013 USA mean household income of 75839 USD. A significant rate of patients in each CMN cohort reported that their disease led to reduced work hours, discontinued employment, and medical disability: PMF – 38%, 35%, 33%, and PV – 33%, 28%, and 15%, respectively. Patient demographics, such as age and health insurance status, were similar among patients who reported CMN-associated effects on employment and patients who did not proceed with such a report inside each CMN entity. In each CMN cohort, the mean percentage household income loss in patients with reduced work hours, discontinued employment, and medical disability were: PMF – 16%, 18%, 28%, and PV – 15%, 24%, 17%, respectively, compared with patients who did not experience any effects of their diagnosis on employment. Discontinued employment and medical disability, especially in elderly patients, tended to have a greater negative impact compared with reduced work hours across CMN [25,26].

We accomplished a literature review in order to pursue a relationship between myeloproliferative neoplasms and other hematologic malignancies. Among cases with CMN, there may be a higher rate of second malignancies before, concomitantly with, or after their primary diagnosis as compared with the general population [27,28]. In contrast with the Porpaczy et al. study, Pemmaraju N. et al. did not found the statistically significant difference in the incidence of a subsequent lymphoma diagnosis in patients with CMN when comparing those who received prior JAK inhibitor therapy and those who did not. However, the rate of lymphoma after CMN diagnosis in the last study proved to be much lower (9/1617 [0.56%]) than that reported by Porpaczy et al. (5.8% to 9.7%). An unusual association of non-Hodgkin lymphoma and BCR-ABL-negative CMN was reported in the rare pediatric newly-diagnosed cases [29].

4. CONCLUSIONS

- The long-term results of treatment in elderly patients with CMN proved to be inferior to those in CMN totality because of the development of age-related diseases and vascular events on the account of the increased values of leukocytes and platelets.
- The sluggish onset, gradual increase of hemoglobin, erythrocyte count and blood viscosity along with the reduced oncologic vigilance of primary care physicians may hasten the occurrence of thrombotic and vascular complications in the elderly PV patients.
- The targeted treatment with TKIs remains a first-line management option for CML patients of 60 years and more.
- In the elderly PV patients no significant difference was revealed in short- and long-term outcomes of chemotherapy with busulfan and hydroxycarbamide in combination with phlebotomy, being totally superior to those in PMF patients.
- The narrative analysis of the bibliographic sources shows that the patients with CMN, especially those elderly, may experience a sizeable unfavorable impact on their employment status, which in turn may be associated with the reduced annual household income.
- Prevention or backtracking discrete aspects of CMN, which negatively influence the individual productiveness, is acknowledged as an important ruling factor in the management of these hematologic malignancies.

- Elderly patients with CMN may experience a higher incidence of second hematologic malignancies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

Dr. Vasile Musteata
Discipline of Hematology, “Nicolae Testemitanu” State University of Medicine and Pharmacy, Republic of Moldova.
Department of Hematology, Institute of Oncology Chisinau, Republic of Moldova.

He is a licensed hematooncologist and associate professor at the University Discipline of Hematology, with a background of 20 years in clinical and translational researches in hematooncology. He completed his PhD in 2002 after the postgraduate studies at the “N. Testemitanu” State University of Medicine and Pharmacy from the Republic of Moldova. He serves as the studies chief at the University Discipline of Hematology, the main specialist of the Ministry of Health in the field of hematological malignancies, MAS responsible physician, ESMO, IACH and SOHO member, iCMLf representative for Moldova and the European LeukemiaNet member. In 2006 he was decorated the Award for Youth in the domain of Sciences, Technology and Literature on behalf of the Government of the Republic of Moldova. In 2002-2011 he served as the ESMO National Representative for Moldova. In 2012 he successfully completed the ERSAP Preceptorship Program in the field of hematologic malignancies at MD Anderson Cancer Center, Houston, Texas, USA. He is a principal investigator of the ARENSIA EM site at the Institute of Oncology. He published more than 150 articles and abstracts in the reputed journals and abstract books. He served as an editorial board member of the Journal of BUON, JHCR, JCTR.

Dr. Valentina Stratan
Laboratory of Immunology and Molecular Genetics, Institute of Oncology Chisinau, Republic of Moldova.

She serves as a head of the Immunology and Molecular Genetics Laboratory at the Institute of Oncology. She is a biologist, with a high expertise in immunogenetics. She is as an associate researcher, empowered scientific advisor (consultant) to doctoral theses, profile General Medicine, Specialty Oncology and Radiotherapy, as well as a member of the Board of Directors of the National Health Insurance Company. In 2015 shereceived the National Award 2015 for scientific work "The Theory of hereditary lung cancer". She took an active part at the following international conferences: "Actual problems of laboratory service. Standardization in clinical laboratory medicine. Modern Technologies ", "Actual problems of medicine in the laboratory. Implementation of new methods of quality management based on European standards". She attended the Republican Seminar "Current Issues on the implementation of quality management in laboratory medicine" and the training "Government public hospitals in the context of health reform: RM solutions", conducted by the Center for Health Policies and Studies in collaboration with Friedrich Ebert Stiftung, in the alternative and independent mechanism for public policy analysis. She is an author and co-author of over 70 scientific works: one monograph, one textbook (compendium), 20 scientific articles in the national journals, 10 scientific articles in the international journals, 35 collections of scientific articles and theses. She was a member of the Parliament of the Republic of Moldova, member of the Board of Experts for Health at the Interparliamentary Assembly of the CIS member states.

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Advanced Proposals to Improve Advanced Directives in Portugal

João Carlos Macedo [a#*]

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ABSTRACT

This article describes the current state of Advance Directives (living wills and/or attorney health care in the event of inability to express one) in Portugal. It investigates the reasons for the low number of registered advance directives and proposes two ways to increase citizens' adherence: Setting up a counselling in the national health system and the standard form.

Keywords: Advance directives; living will; attorney health care; counselling; proposal form.

1. ADVANCE DIRETIVES IN PORTUGAL

Advance directives (AD) are the ultimate expression of exercising autonomy while also respecting informed consent. This instrument originated in the United States in the mid-1970s, specifically with a human-rights lawyer named Luis Kutner. He began with the common law and constitutional law premises that "the law provides that a patient may not be subjected to treatment without his consent (…). The challenge was what to do about patients who no longer were capable of making health care decisions. He suggested that the individual should indicate in writing ahead of time the extent to which he or she would consent to treatment. He referred to the document as a "living will," "a declaration determining the termination of life," or a "testament permitting death,"among other names" [1].

So the AD provide an individual a means of expressing his or her wishes for medical treatment when he or she is no longer able to make those wishes known due to incapacity [2].

The disclosure of the AD has been making its way in the USA and only more recently started to be implemented in several European countries [3].

Only at the beginning of the 21st century was it possible for portuguese society to have access to this instrument of prospective autonomy Portugal ratified in 2001 the Convention for the protection of human rights and dignity of the human being with regard to the application of biology and medicine: convention on human rights and biomedicine (Oviedo Convention) [4]. Only then could the Portuguese write an AD under Article 9 which stated: " *Previously expressed wishes -The previously expressed wishes relating to a medical intervention by a patient who is not, at the time of the intervention, in a state to express his or her wishes shall be taken into account*". However, this written document was not binding on health professionals.

Finally, the advance directives were legalized in Portugal, in 2012, by Law nº. 25/2012 of 16 July [5]. According to the Portuguese AD Law, any citizen over eighteen years old and duly capable may declare in advance, and in a clear, conscious and informed manner, his or her wishes regarding medical care, by preparing an advance directive. These directives may take the form of a living will and a health care power of attorney [5]. The law states that AD can take two forms: living will (LW) and / or appointment of attorney health care (AHC). Also determines that AD can be revoked at any time and has five years of validity.

[#]*Adjunct Professor*
[a] *Nursing School, University of Minho, Portugal.*
**Corresponding author: E-mail: joaomacedo71@gmail.com;*

The AD can be written in free text or use an optional Ministry of Health form [6].

Citizens can declare that their advance directive is effective when they are in one or more of these situations:

- I have been diagnosed with terminal incurable disease
- Unconsciousness due to irreversible neurological or psychiatric illness complicated by complications respiratory, renal or cardiac;
- There are no expectations of recovery in the clinical evaluation by members of the medical team. caregiver according to state of the art;
- Other situations.
  Then the citizen can indicate some instructions to health professionals, like:
- Not undergo artificial supportive treatment of vital functions;
- Not to be subjected to futile treatment, useless or disproportionate in their clinical condition and in accordance with good professional practice, particularly with regard to basic life support measures and artificial feeding and hydration measures that only aim to retard the natural process of death;
- Receive adequate palliative care
- Not be subjected to treatments that are in the experimental phase;
- Authorize or refuse participation in scientific research programs or clinical trials;

Concerning the AHC, the portuguese legislation say that anyone can appoint one or more persons, giving them representative powers to decide on health care to receive, or not to receive, when unable to express their will personally and autonomously. The law state that AHC decisions must be respected by healthcare professionals. In case of conflict between the provisions set forth in the LW and the will of the AHC, the LW prevails.

Finally, in 2014 the Health Ministery created a database, a National Register of Advance Diretives [7] allowing citizens to have their AD accessible at any hospital in the country.

2. THE ADVANCE DIRECTIVE: NUMBERS AND CAUSES

According to the latest data, early 2020, Portugal has registered around 29000 AD. Women with 19 301 records, men with 10 046 [8].

Notwithstanding some national bibliography that was emerging for citizen consultation [9-11] the numbers reveal the poor adherence of the portuguese to the advance directives. There are certainly multiple factors contributing to this case. Being aware of the low numbers of AD in the portuguese population, here are some of the likely underlying causes:

1. We still continue to live in a death denial society; [12-13]
2. There is a need to promote education for death so that this subject can be discussed since childhood. [12- 13]. Talking about the end of life is something that is not in people's goals, much less make the decision to think about the care you want or refuse when you are in a situation where you can not communicate your wishes;
3. We still have a very paternalistic paradigm in health care. The exercise of autonomy is not yet properly developed in the practice of care; [14-15]
4. The population's health literacy level is low. There are fringes of the digital illiteracy population. In this regard, people have a hard time writing their directives; [16 -19]
5. Portuguese knowledge about autonomy in end-of-life care and particularly about AD is very low. Recent studies point to values of around 1%. [14,15,20,21].
6. The Health Ministry did not make a good promotion campaign, on the Internet and social networks and forgot about people who have digital illiteracy; [22].
7. Health professionals do not have adequate training for counseling; [23]
8. The form proposed by the Health Ministry is complex and unintuitive; [6]

9. The lack of an advance directives guide. A guide with explanations of advance directives would allow citizens to draw up the document with more information and awareness. In Spain, in the Andaluzia region, the Ministry of Health has developed a guide for AD that greatly facilitates citizens; [24].
10. The lack of obtaining counselling from a health professional in the national health system so that the citizen can prepare the AD.

3. INCREASE ADVANCE DIRECTIVES: COUNSELLING AND FORM PROPOSAL

3.1 Counselling: The nurses' role

One of the predominant factors in the lack of adherence of citizens to AD is related to AD literacy. From our perspective, it seems that the Portuguese health system does not provide a place where citizens can obtain the necessary advice to prepare their AD. Our contribution is towards the creation of a counselling consultation in the Community Care Unit of the Health Centres**.** These units are designed to "**…** provide health care and psychological and social support, at home and in the in situations of greater risk or physical and functional dependency or disease that requires close monitoring, and also act in health education in the integration of family support networks and in the implementation of mobile intervention units "[25].

These care units, which are part of primary health care, are mainly staffed by nurses. Nurses are a highly qualified professional group to provide counselling in end-of-life care. On the other hand, within the scope of health education that nurses develop in this community care unit, this task would be framed and would empower the citizens' literacy level in the exercise of autonomy in end-of-life care. In addition, this counselling in this primary health care unit is much more favourable to the preparation of a well-reflected AD, without pressure and mediated by a health professional. Not dismissing the idea of citizens being able to perform their AD in hospital or in a palliative care unit [23], we believe that reflection on end-of-life care and particularly on AD should be performed in a calm environment, without the expectation of a hospital admission. In this context, the offer of these counselling services in community care units with properly trained nurses is the most appropriate option to increase the levels of adherence to AD in Portugal.

In addition to this incremental measure, we believe that the citizen should have a more intuitive tool for drafting ADs. In this sense, we leave below a small contribution, a form proposal, which was inspired by various models existing in European countries. However, the biggest contribution was from the Spanish model, more specifically from Andaluzia [24].

3.2 Form proposal

Advance Directive

Under and for the purposes of Law No. 25/2012 of 16 July this document reflects my early manifestation of conscious, free and enlightened will with regard to caring that I wish to receive, or do not wish to receive, if for any reason unable to express my will personally and autonomously.

This document, which I subscribe to being of legal age and capable and not being prohibited or disabled by psychic anomaly, and unilateral by me and freely revocable at any time. I know that this document is valid for a period of five years.

Personal considerations on end of life values:

IDENTIFICATION

Name:_____
Doc.Ident.nº_____
Validity:_____Nationality:_____
Health nº: _____ Date of Birth: _____
Address:_____
Postal Code: _____ Country: _____Tel.:_____
E-mail: _____

I WANT TO APPOINT MY ATTORNEY HEALTH CARE

Name:_____
Ident Doc. ._____ No. _____ Validity: _____
Nationality: _____
Health n.º: _____ Date of birth_____
Address:_____
Postal Code: _____ Country: _____Tel.:_____
E-mail: _____
Signature of health care attorney :_____

CLINICAL SITUATION IN WHICH ADVANCE DIRECTIVES IS EFFECTIVE

When I find myself unable to express my will autonomously as result of my state of physical and / or mental health, and if one or more of the following conditions ocurr:

Mark with **X** the applicable assumptions below

- ☐ I have been diagnosed with terminal incurable disease
- ☐ There are no expectations of recovery in the clinical assessment by team members according to state of the art
- ☐ Severe and irreversible brain damage (irreversible coma, permanent vegetative state)
- ☐ Degenerative disease of the nervous system in advanced phase
- ☐ Degenerative disease of the neuromuscular system in advanced phase
- ☐ Immunodeficiency in advanced phase
- ☐ Other serious and irreversible diseases or conditions similar to those above

Another clinic situation:

HEALTH CARE INSTRUTIONS RECEIVING/NOT RECEIVING

I direct the health care providers to withhold and withdraw treatment that merely prolongs my dying. Thus, I express my clear and unambiguous desire to:

Mark with **X** the applicable assumptions below

- Invasive means of artificially supporting vital functions
  ☐ I want ☐ I refuse
- Cardiorespiratory resuscitation
  ☐ I want ☐ I refuse
- Parenteral feeding
  ☐ I want ☐ I refuse
- Feeding through gastrostomy

☐ I want ☐ I refuse
- Feeding by naso-gastric tube

☐ I want ☐ I refuse
- Intravenous hydration

☐ I want ☐ I refuse
- Renal clearance techniques (hemodialysis / peritoneal dialysis)

☐ I want ☐ I refuse
- Pain relief care and comfort for a serene death

☐ I want ☐ I refuse
- Palliative sedation

☐ I want ☐ I refuse

Another treatment:
Other personal considerations:

4. CONCLUSION

AD in Portugal is still a young tool in the exercise of citizen autonomy. Several factors are present for the lack of adherence of the Portuguese. There is a need to continue to investgate this phenomenon and try to find proposals that can increase the level of adherence to AD and, on the other hand, contribute to the respect self-determination and human dignity. Our contribution in this paper is in this direction: to have counselling in the preparation of ADs and also to present easier instruments for the completion of ADs.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Chapter 18
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Cause of Entrapment Syndromes in Gastrocnemius Muscle: A Prospective Study

Shinde Amol A. [a*#], Patel Dinesh K. [a#] and Bharambe Vaishaly K. [b†]

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ABSTRACT

Gastrocnemius injuries have been one of the leading causes of calf injuries. Medial head of gastrocnemius is more commonly involved. Accessory heads of gastrocnemius have given rise to entrapment syndromes in the popliteal fossa. 60 formalin embalmed lower limbs were meticulously dissected. Any variation in number of heads, origin, insertion and nerve supply of gastrocnemius muscle is noted. Popliteal artery entrapment syndrome has been reported due to aberrant course of popliteal artery or accessory heads of gastrocnemius or plantaris muscle compressing a normal popliteal artery. Bilateral variation is seen in 10% cadavers. Three (6.66%) and four (8.33%) headed gastrocnemius muscles are reported. Knowledge of additional heads of gastrocnemius is important while autografting and using imaging techniques for entrapment syndromes. During imaging exams for polpliteal entrapment syndromes and neurovascular compression, the radiologist will benefit from learning about the accessory heads of the gastrocnemius.

Keywords: Gastrocnemius tertius; accessory head of gastrocnemius; popliteal artery entrapment syndrome; calf injury; additional heads of gastrocnemius.

1. INTRODUCTION

The calf is made up of bipennate gastrocnemius and multipennate soleus muscles. Gastrocnemius has a medial head known as the capute mediale and a lateral head known as the capute laterale [1]. The medial head emerges from the posterior surface of the femur above the medial condyle, while the lateral head emerges above the lateral condyle. The gastrocnemius heads fuse to form a single belly, which, along with the soleus, forms the Achilles tendon. The Achilles tendon inserts on the calcaneum's posterior surface. The word gastrocnemius is derived from greek words gastro meaning stomach and kneme meaning leg. It is called “stomach of leg” referring to bulging of the calf [2].

Gastrocnemius is supplied by the tibial nerve. It is strong plantar flexor of foot. Soleus acts to initiate walking while gastrocnemius is muscle used in running.

The inferolateral and inferomedial boundaries of popliteal fossa are formed by gastrocnemius.

Close proximity to Tibial nerve and popliteal vessels in popliteal fossa makes any variant anatomy of gastrocnemius as a differential diagnosis in neurovascular compressions and entrapment syndromes [3].

Aim of this study: 1] To find the incidence of accessory heads of gastrocnemius. 2] To check for any variation in nerve supply.

[#]Associate Professor,
[†]Professor and HOD,
[a] Department of Anatomy, Dr. D. Y. Patil Medical College, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, Maharashtra, India.
[b]Department of Anatomy, Symbiosis Medical College for Women, Symbiosis International University, Lavale, Pune, Maharashtra, India.
**Corresponding author: E-mail: amol9267@gmail.com;*

2. RESULTS AND OBSERVATIONS

Methodology: 60 formalin embalmed lower limbs were meticulously dissected. Any variation in number of heads, origin, insertion and nerve supply of gastrocnemius muscle is noted. Length of the heads of gastrocnemius and Achilles tendon are measured with vernier caliper and measuring tape as applicable. Relation of the popliteal artery to the additional heads of gastrocnemius was noted.

Results: Additional head of gastrocnemius was seen in 9 (15%) lower limbs. 3 cadavers showed bilateral variation(10%). 2 right sided and one left sided lower limb showed unilateral variation. 4 heads of gastrocnemius (Fig. 3) were seen in 5 (8.34%) limbs. 2 cadavers showed 4 heads bilaterally. One right limb demonstrated 4 headed gastrocnemius. 3 heads of gastrocnemius (Figs. 1and 2) were seen in 4 (6.66%) limbs. One cadaver showed bilateral 3 heads of gastrocnemius. One right and one left side limb showed 3 heads of gastrocnemius. All the heads of gastrocnemius were supplied by tibial nerve. No variation in course of sural nerve was noted. The popliteal artery was seen passing between the accessory medial head and normal medial head of gastrocnemius in 2 cadavers (Fig. 3).

A single belly was formed by joining of all heads of gastrocnemius. This muscle belly joined with belly of soleus to form the Achilles tendon, which inserted on posterior surface of calcaneum. Length of the heads of gastrocnemius before forming common tendon and length of Achilles tendon is tabulated in Table 1. Maximum length seen for medial and lateral bellies are 7.5 cm and 9 cm respectively Length of Achilles tendon of limbs with additional heads of gastrocnemius ranged from 6.1 cm to 12.8 cm.

Fig. 1 showing three headed gastrocnemius: A – Medial head of gastrocnemius, B – Additional medial head of gastrocnemius, C – Lateral head of gastrocnemius, D – Additional lateral head of gastrocnemius, T – Achilles tendon, S – Soleus and N – Tibial nerve

Fig. 2. Showing three headed gastrocnemius: A – Medial head of gastrocnemius, B – Additional medial head of gastrocnemius, C – Lateral head of gastrocnemius, D – Additional lateral head of gastrocnemius and T – Achilles tendon

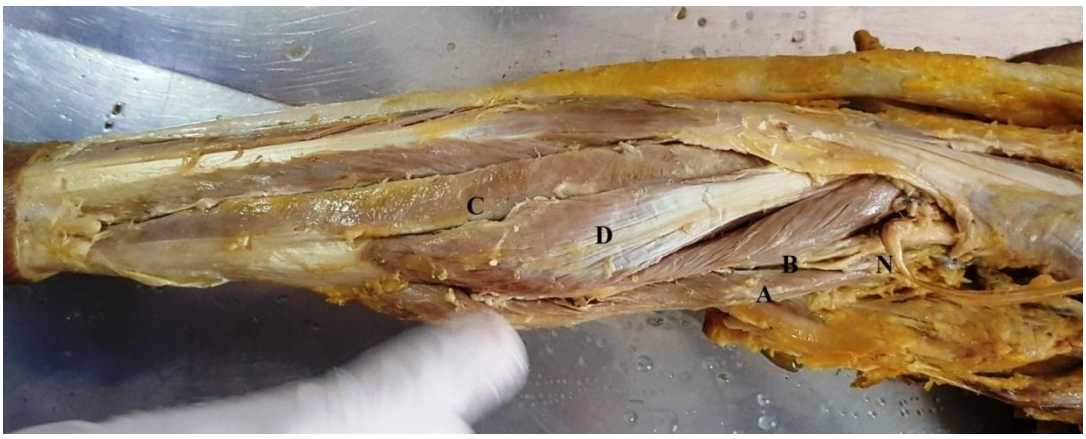

Fig. 3. Four headed gastrocnemius : A – Medial head of gastrocnemius, B – Additional medial head of gastrocnemius, C – Lateral head of gastrocnemius, D – Additional lateral head of gastrocnemius and N – Tibial nerve

Table 1. Showing lengths of bellies of gastrocnemius and Achilles tendon in limbs with accessory bellies of gastrocnemius

Sr number	Side	Number of bellies of Gastrocnemius	Muscle length (cm) medial to lateral	Tendon length (cm)
1	Right	4	5.2 5.9 7.4 8.6	6.1
2	Left	4	5.6 5.4 6.9 7.1	6.5
3	Right	4	7.5 7.1 8.3 9.0	12.6
4	Left	4	6.8 7.1 9.2 9.4	12.8
5	Right	4	6.7 6.9 8.8 9.3	12.5
6	Right	4	5.9 6.2 8.4	11.9
7	Right	3	5.6 6.1 7.5	7.2
8	Right	3	7.1 7.2 7.9	12.5
9	Left	3	7.3 7.7 8.3	12.8

3. DISCUSSION

Embryological reasoning: Gastrocnemius muscle originates from the calcaneum blastomere and migrates by ascending towards the inferior epiphysis of femur. Before termination on femur it splits into medial and lateral bellies. Any variant mode of embryological migration and termination may result as variation in number of bellies and attachment [3].

Gastrocnemius is the superficial calf muscle. It originates as medial and lateral head . The medial head is more common to be injured. Medial head of gastrocnemius sprain is also called 'Tennis leg' as it is commonly seen in tennis players. Risk of strain of gastrocnemius is more as it crosses two joints (knee and ankle) and has type two fast acting muscle fibers. Strain of gastrocnemius is called 'coup de fouet' or snap of a whip [4].

Sookur et al. [5] in a study of variations of muscles of lower limb state that variations of medial and latereral heads of gastrocnemius are due to anomalous origins and accessory slips. Medial and lateral heads of gastrocnemius take origin from posterior surface of femur, above the condyles and from the capsule of the knee joint.Additional medial head may arise from intercondylar notch instead of medial condyle. Additional slips may arise from intercondylar notch and pass between popliteal artery and vein to get inserted into medial head. Lateral head may have a more medial origin on posterior surface of femur, but lateral to politeal artery. Additional slips may pass anterolatral to popliteal vessels and insert in the lateral head.

In a study of gastrocnemius in Nigerian population, Ashaolu et al. [3] give a 51.7% incidence of four headed gastrocnemius and 13.3% incidence for 3 headed gastrocnemius. These figures are more than our study where we got incidence of four headed gastrocnemius as 8.33% and three headed gastrocnemius as 6.66%. Knowledge of such variations will help during popliteal nerve blocks and autografting of gastrocnemius muscle.

Shinde et al. [6] in a study of Soleus muscle report 8% cases of accessory head of soleus. One limb demonstrated three heads of gastrocnemius with accessory head of soleus. This condition is called gastrocnemius tertius. Coexistence of gastrocnemius tertius and accessory soleus is considered a rare variation.

Yildirim et al. [7] Report a case of bilateral gastrocnemius terius with unilateral accessory head of soleus. Accessory head of gastrocnemius can lead to entrapment syndromes in popliteal fossa. Decrease in pulse of distal arteries on passive dorsiflexion suggests popliteal artery entrapment,while pain and tenderness in popliteal fossa points towards nerve compression in the popliteal fossa.

Calf injuries are one of the most common muscle injuries of leg. Gastrocnemius acts to flex the leg at knee joint and plantar flex the foot at ankle joint .Gastrocnemius injury is considered high risk because it crosses two main joints, the knee and ankle. Calf injuries occur with strenuous exercise and also on innocuous movements in middle aged people with sedentary lifestyle. Differential diagnosis for calf injury are gastrocnemius tear, thrombophlebitis, soleus tear, Achilles tendon rupture and posterior compartment syndrome. Pain in the medial head of gastrocnemius just above the musculotendinous junction with palpable tenderness are clinical signs to diagnose gastrocnemius tear [8].

A case report of 4 headed gastrocnemius was given by Rodrigues et al. [9]. They mention the medial head of gastrocnemius splitting into 3 heads. The multiple heads join with the lateral head to form the Achilles tendon. The sural nerve was seen entrapped between the multiple heads of gastrocnemius. Sural nerve is primarily a sensory nerve but they state that considering some communication with tibial nerve in leg, motor loss is also reported along with sensory loss in case of entrapment. No variation in course of sural nerve was seen in our study.

Politeal entrapment syndrome was first reported by Anderson Stuart. Unilateral calf pain in a athletic young patient is a symptom seen in popliteal artery entrapment syndrome. Accessory medial head of gastrocnemius compressing the artery in popliteal fossa is the most common cause. Stenosis of the artery and early atherosclerotic changes may be seen in chronic cases. Surgical intervention to

relieve the compression is considered. The popliteal artery is surgically decompressed with myotomy. This is followed by embolectomy and prophylactic fasciotomies [10].

In a study of patients with entrapment syndromes, Rosset E et al. [11] found the popliteal artery was susceptible for compression in cases of accessory heads of gastrocnemius. CT and MRI were used to define the muscular origin of popliteal compression. Higher insertion of gastrocnemius, presence of a fibrous band linking the medial and lateral head of gastrocnemius and muscular hypertrophy were causes for arterial compression.

Popliteal artery entrapment syndrome has been reported due to aberrant course of popliteal artery or accessory heads of gastrocnemius or plantaris muscle compressing a normal popliteal artery. The range of prevalence of popliteal artery entrapment syndromes is 0.16 to 3.5%. The syndrome is classified as type I in case of an aberrant course of politeal artery, type II in case of an accessory medial head of gastrocnemius compressing the popliteal artery with a normal course. Type III anomaly has an accessory slip from the medial head of gastrocnemius, forming a sling around the popliteal artery and in Type IV a popliteal artery with normal course gets compressed by passing deep to the popliteus muscle. Any of type I to IV anomaly with popliteal vein involvement is called type V. Type VI is popliteal artery compression due to a hypertrophied gastrocnemius muscle [12]. Our study shows the popliteal artery passing between the accessory and normal medial heads of gastrocnemius in two cadavers (Fig 1). This condition is seen in type II popliteal artery entrapment syndrome.

Radonic et al. [13] Describe three cases of popliteal artery entrapment syndrome. Accessory head of gastrocnemius compressing the popliteal artery was reported. Intermittent claudication on physical activity is seen in young adults. Soldiers and athletes are more likely to succumb to popliteal artery entrapment syndromes as their muscles are well toned and enlarged. Any accessory muscle forming the boundary of popliteal fossa may lead to compression of the artery.

Popliteal artery entrapment syndrome [PAES] is a condition in running athletes who suffer from posterior leg pain and paraesthesia. Miller et al. [14] mention accessory head of gastrocnemius as the most common cause for PAES.

Gamas et al. [15] state that progressive compression of popliteal artery by muscular and tendinous structures will lead to fibrosis causing Popliteal artery entrapment syndrome. Calf pain on persistent walking for even a short distance is seen. Accessory heads of gastrocnemius are commonly the causative factor. Surgical removal of this accessory head has lead to relief in walking for patients.

4. CONCLUSION

Gastrocnemius injuries have been one of the leading causes of calf injuries. Medial head of gastrocnemius is more commonly involved. Accessory heads of gastrocnemius have given rise to entrapment syndromes in the popliteal fossa. Our study gives a 15% incidence of accessory heads of gastocnemius. Bilateral variation is seen in 10% cadavers. Three (6.66%) and four (8.33%) headed gastrocnemius muscles are reported. Knowledge of additional heads of gastrocnemius is important while autografting and using imaging techniques for entrapment syndromes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

Dr. Shinde Amol A.
Department of Anatomy, Dr. D. Y. Patil Medical College, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, Maharashtra, India.

He is an Associate Professor in Dept of Anatomy at the Dr. D. Y. Patil Medical College, Pimpri, Pune. He has 16 Research articles published in various National and International Journals. He has done various oral and poster presentations in conferences. He has worked as Judge for oral presentations in International conferences. He has done various research projects for Dr. D. Y. Patil Vidyapeeth. He has guided students for STS ICMR projects.

Dr. Patel Dinesh K.
Department of Anatomy, Dr. D. Y. Patil Medical College, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, Maharashtra, India.

He is an Associate Professor in Dept of Anatomy at the Dr. D. Y. Patil Vidyapeeth and Medical College, Pimpri, Pune, India. He has 9 Research articles published in various National and International Journals. He has done various oral and poster presentations in conferences. He has done various research projects for Dr. D. Y. Patil Vidyapeeth. He has guided students for STS ICMR projects.

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Chapter 19
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Association of Vitamin D Deficiency with Knee Osteoarthritis (KOA) in Population of Tamil Nadu, India

Regupathy Annamalai [a#*] and Amar Nagesh Kumar G. [bϖ]

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ABSTRACT

The objective of this study is to evaluate the serum 25–OH vitamin D status in patients with knee osteoarthritis (KOA) scheduled for joint replacement along with healthy individuals for comparison. This pilot study conducted in and around the Chennai city and rural places of Tamil Nadu population. Study subjects were selected during the medical camps conducted by our teaching hospital in and around the Chennai city and rural places of Tamil Nadu. . This study showed that about 5 patients out of 10 with knee osteoarthritis were vitamin D deficient with serum levels <10 ng/ml. The study also tried to correlate serum levels of vitamin D with related anthropometric predisposing factors such as age, gender, and BMI. A significant association with gender was observed, with female patients having lower serum levels of vitamin D. Identification of high risk subjects and correction of risk factors such as low level serum 25-OH vitamin D in KOA patients will give beneficial effects and even decreases fracture risk in elderly people.

Keywords: 25 – OH Vitamin D; osteoarthritis; body mass index; age; knee joint pain.

1. INTRODUCTION

The prevalence of vitamin D deficiency is the burning topic worldwide. Vitamin D deficiency is a under diagnosed medical condition since a significant proportion of the population in many countries and regions around the world have low serum 25 –OH vitamin D levels [1–4]. The serum level of 25-hydroxyvitamin D depends on various parameters such as ultraviolet irradiation (determined by the time of day) to skin, skin pigmentation, season [5–7], latitude, age, dietary habits, gender, obesity, and many others factors [6-9]. It is known that vitamin D has role in mineralization, demineralization, remodeling, and maintenance of bone. Therefore its deficiency may be implicated in the pathogenesis of osteoarthritis (OA) [10,11]. The pathogenesis of OA is still unclear. Recent studies on changes in sub-chondral bone remodeling phases of bone absorption and of bone sclerosis may be responsible for the cartilage damage [12-14]. The reason behind these changes in the cartilage and bone is that low levels of 25-hydroxyvitamin D.

Knowledge of the serum status of 25-OH vitamin D may provide additional information to recognize patients at risk for progression of OA knee. The objective of this study is to evaluate the serum 25–OH vitamin D status in patients with knee OA scheduled for joint replacement along with healthy individuals for comparison. And also to find out association between serum25 – OH vitamin D levels

[#] Professor;
[ϖ] Assistant Professor;
[a] Department of Orthopedics, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Madurantagam, Tamil Nadu, India.
[b] Department of Biochemistry, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Madurantagam, Tamil Nadu, India.
**Corresponding author: E-mail: amarnageshkumar@gmail.com;*

with age and body mass index (BMI) and with the knee osteoarthritis. Since, there are very few studies available on the association of 25 – OH vitamin D in knee osteoarthritis patients, the present study was undertaken.

2. MATERIALS AND METHODS

Study setting and Design: This is a pilot study conducted in and around the Chennai city and rural places of Tamil Nadu population. Subjects were selected during the medical camps conducted by our teaching hospital in and around the Chennai city and rural places of Tamil Nadu. The study was approved by the institutional ethics committee. An informed consent was obtained from all the participants after explaining the purpose of the study. The study consists of 120 ages matched controls (Group 1) and 120 patients with knee osteoarthritis as cases (Group2).Further study the subjects were divided into two sub groups based on age (<60 years and >60 years) in both group 1 and group 2. Based on body mass index (BMI) there are four subgroups i.e, BMI <20, 20 – 25, 26 – 30 and >30 in each group. Based on vitamin D status there are three subgroups in each group.

Inclusion criteria: Patients who presented with knee joint pain to the medical camps conducted by the department of Orthopaedics, Karpaga Vinayaga Institute of Medical Sciences and Research Centre (KIMS&RC), Madhuranthagam, Tamil Nadu were recruited for the study after explaining all the study objectives clearly. American College of Rheumatology classification was considered as standard for the diagnosis of KOA [13,14]. Subjects of the control group were selected over the same period patients without non-skeletal symptoms and had no clinical features of KOA based on history and clinical examination.

Exclusion criteria: Patients with history of rheumatic diseases other than osteoarthritis, chronic kidney diseases, gastrointestinal disorders, pulmonary systems, and persons on anticonvulsant drugs were excluded from the study. Stage 4 Kelegren-Lawrence stage, severe radiographic knee osteoarthritis, and those with knee joint instabilities were also excluded from the study. Patients with physical disabilities were excluded from the study.

2.1 METHODOLOGY

Vitamin D status was measured by the serum concentration of 25-OH vitamin D which has a reasonably long half life in the circulation. Measurement of serum 25-OH vitamin D was made by ELFA using fully automated Minividas (Biomeriux, Germany) hormone analyzer. Based on the results obtained vitamin D status was categorized into 3 groups as serum 25-OH vitamin D levels <10 ng/ml, between 10–29 ng/ml, and 30 - 100 ng/ml, indicating vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency respectively.

2.2 Statistical Analysis

In statistical analysis the mean serum 25-OH vitamin D levels and proportion of serum 25-OH vitamin D deficiency were determined in both the groups and compared with the age matched controls. Additional subgroup analyses were performed according to age groups of <60 years versus >60 years. Comparisons were also made with respect to gender and BMI with KOA as well. Chi square analysis was done to know the association of vitamin D deficiency and knee osteoarthritis. Odds ratio (OR) and its 95% confidence interval (95% CI) was also calculated. The independent effect of serum 25-OH vitamin D deficiency was determined after adjustment for sex and age using logistic regression analysis. SPSS software version 16.0 was used for statistical analysis.

3. RESULTS

A total of 240 patients were recruited for the study. All the patients were selected during the medical camps conducted at KIMS&RC various place in Tamil Nadu. Study population was divided into two major groups, Group 1 (Controls) and Group 2 (OA Cases).Group 1 includes 120 patients without the clinical symptoms of Knee OA and group 2 consists of 120 knee osteoarthritis (OA).Serum 25-OH

vitamin D deficiency was observed in 67(55.8%) patients in Group 2 versus 15(12.5%) of Group 1 (Table 1). In Group 1, 57(46.5%) were males and 63 (53.5%) females, while in Group 2, 57(45.5%) were males and 63 (54.5%) females. Age range of group 1 was 37 – 69 years with a mean age of 57.5 years while in Group 2,the age range of subjects was 38 – 68years with a mean age of 58.7 years (Table 1).Further study subjects were divided into two sub groups based on age in both group 1 and group 2 (<60 years and >60 years). Number of patients <60 years were 55 and 56 respectively in Group 1 and Group 2.Based on body mass index (BMI) there are four subgroups i.e, BMI <20, 20 – 25, 26 – 30 and >30 in each group. Based on vitamin D status there are three subgroups in each group (Table 1). In the entire study population serum 25-OH vitamin D deficiency was not homogenously distributed across age but was more frequent in subjects aged<60 years compared with >60 years particularly in the patient group (Tables 1-5). Further analysis of subgroups, showed that the mean serum 25-OH vitamin D level in Group 2 aged <60 years was significantly lower than Group 1 (8.2 ± 1.1 vs. 9.2 ± 0.8 ng/ml, $P < 0.05$). In this age group, knee OA was significantly associated with serum 25-OH vitamin D deficiency. In Table 2 comparisons of age, BMI, and 25-OH vitamin D status in controls and osteoarthritis patients was shown. There is no statistical difference with respect to age of the patients in the both the groups. But, statistical significant difference was observed with respect to BMI and serum 25 – OH vitamin D in both control group (Group 1) and OA group (Group 2) (Table 2). The association between knee OA and mean serum 25-OH vitamin D levels in patients aged >60 years did not reach a significant level among both groups (7.9 ± 1.9 vs. 8.1 ± 1.5). All in all percentage of number of patients with serum 25 – OH vitamin D deficiency was significantly higher in Group 2 than Group1(67 vs 15, p<0.001) (Table 3).

Table 1. Baseline characteristics of study population

			Controls (Group 1) n (%)	Osteoarthritis (Group 2) n (%)
Gender	Male		57 (47.5)	41 (34.2)
	Female		63 (52.5)	79 (65.8)
Age (Years)	< 60 years	Male	29 (21.1)	26 (21.6)
		Female	30 (25)	30 (25)
	> 60 years	Male	28 (23.4)	31 (25.9)
		Female	33 (27.5)	33 (27.5)
BMI (Kg/m^2)	< 20		2 (1.7)	3 (2.5)
	20 – 25		23 (19.2)	16 (13.3)
	26 – 30		58 (48.3)	70 (58.4)
	> 30		37 (30.8)	31 (25.8)
Vitamin D status	Normal (30 – 100ng/ml)		70 (58.4)	21 (17.5)
	Insufficient (10 – 29ng/ml)		35 (29.1)	32 (26.7)
	Deficient (<10 ng/ml)		15 (12.5)	67 (55.8)

Table 2. Comparison of Age, BMI and 25 – OH vitamin D status in controls and osteoarthritis patients

Parameter	Group 1 All Controls (n=120)	Group 2 All Osteoarthritis (n=120)	P value
Age	57.5 ± 9.4	58.7 ± 9.7	0.33
BMI	25.4± 7.5	28.5 ± 6.3	0.006*
25 – OH Vitamin D	38.2 ± 15.6	34.4 ± 11.9	0.03*

**statistically significant p value*

Table 3. Status of vitamin D in control and cases who are less than 60 year of age

Vitamin D status	Controls <60 years (n=55)	Osteoarthritis <60 years (n=56)
Normal	35.9 ± 12.3 (n=39)	33.4 ± 2.9 (n=12)
Insufficient	28.6 ± 2.5 (n=12)	24.5 ± 3.5 (n=19)
Deficient	9.2 ± 0.8 (n=04)	8.2 ± 1.1 (n=25)

In Table 3 and Table 4 distribution of patients who are under the age of 60 years according to the status of 25 – OH vitamin D in both the groups was shown. Table 5 shows the gender wise distribution of patients according to their vitamin D status. Though there is vitamin D deficiency in knee osteoarthritis patients who are >60years of age but percentage of number of patients with 25 – OH vitamin D deficiency was not significant (Table 4). Thus, additional analysis in respect to mean serum 25-OH vitamin D and proportion of serum 25-OH vitamin D deficiency demonstrated a significant difference in age of less than 60 years in Group 2 vs Group 1, but was not significant when compared with 60 years and older (Table 2). Table 6 shows the distribution of patients according to their vitamin D status and body mass index. In table 7, Odds ratio, 95% confidence interval (95% CI) by logistic regression analysis showing association of serum 25-hydroxy vitamin D deficiency, age, gender, and, BMI with knee osteoarthritis (OA) was shown.

Table 4. Status of vitamin D in control and cases who are greater than 60 year of age

Vitamin D status	Controls >60 years (n=65)	Osteoarthritis >60 years (n=64)
Normal	35.9 ± 7.8 (n=31)	32.5 ± 8.8 (n=09)
Insufficient	26.7± 3.4 (n=23)	22.5 ± 2.3 (n=13)
Deficient	8.1 ± 1.5 (n=11)	7.9 ± 1.9 (n=42)

Table 5. Showing the gender wise distribution of study subjects and their vitamin D status

Vitamin D status	Controls (n=120)		Osteoarthritis (n=120)	
	Male (n=57)	Female (n=63)	Male (n=57)	Female (n=63)
Normal	38.9 ± 6.8	34.8 ± 5.5	31.5 ± 0.7	31.8 ± 0.5
Insufficient	26.8 ± 3.7	25.7 ± 6.1	24.6 ± 3.5	21.7 ± 1.3
Deficient	8.8 ± 0.6	8.5 ± 0.4	7.4 ± 1.7	7.6 ± 1.2

Table 6. Showing the 25 – OH vitamin D status according to body mass index

Vitamin D status	BMI (Body Mass Index)			
	< 20 (n= 03)	20 – 25 (n=16)	26 - 30 (n=70)	> 30 (n=31)
Normal	Nil	33.4 ± 1.6	31.8 ± 0.8	Nil
Insufficient	21.6 ± 6.2	26.8 ± 4.2	22.8 ± 5.9	21.5 ± 2.7
Deficient	Nil	8.9 ± 1.4	8.3 ± 1.6	7.9 ± 2.5

Table 7. Odds ratio, 95% confidence interval (95% CI) by logistic regression analysis showing association of serum 25-hydroxy vitamin D deficiency, age, gender, and, BMI with knee osteoarthritis (OA)

Patients	Variables	Odds ratio (95% CI)	P value
Total OA patients	25 – OH vitamin D (< 10 vs>10ng/ml)	0.16 (0.09 – 1.42)	0.07*
	Gender (Female vs Male)	4.7 (1.3 – 7.8)	< 0.001*
	BMI	4.3 (0.9 – 7.3)	< 0.001*
OA patients < 60 year	25 – OH vitamin D (< 10 vs>10ng/ml)	0.32 (0.11–1.65)	0.04*
	Gender (Female vs Male)	4.5 (1.34 – 8.69)	0.015*
	BMI	3.5 (1.01 – 6.38)	0.005*
OA patients >60 year	25 – OH vitamin D (< 10 vs>10ng/ml)	1.01 (0.49 – 5.7)	0.89
	Gender (Female vs Male)	3.21 (1.28–8.04)	0.01
	BMI	4.1 (1.2 – 8.3)	0.003*

**p <0.05 is statistically significant*

On comparison of data among the same group also, there was a significant difference in 25–OHvitamin D levels of patients <60 and>60 years in Group 2 (Table 7), while it was not significant in control group. There was an association between serum 25-OH vitamin D deficiency and knee OA which was not statistically significant after adjustment for gender (Table 7).

4. DISCUSSION

Vitamin D deficiency and knee OA occur in elderly people, seen all over the world [12]. The changes in sub-chondral bone play a vital role in the development of cartilage lesions in OA patients [4,9,8,11]. At this stage, bone resorption markers are higher and bone formation markers are lower [6,8,15,16]. Low serum 25-OH vitamin D increases osteoblastic activity and bone turnover [1,9,14,17]. Many mechanisms were put forwarded to explain role of vitamin D and alterations in properties of bones in the progression of the disease osteoarthritis. Increasing bone resorption by the elevated levels of parathyroid hormone (PTH) increasing bone turn over, or effect of vitamin D metabolites directly on articular chondrocytes were some of the mechanisms put forwarded to explain the role of vitamin D deficiency in osteoarthritis [3,6,9,15].

Early structural changes in the joints such as defects in cartilage, decrease in volume of cartilage, expansion of sub-chondral bone, and lesions in bone marrow will appear before the onset of clinical symptoms of OA. Reports and observations from earlier studies provided a rationale for the measurement of serum 25-OH vitamin D levels with appearance of knee OA in elderly people and encourage supplementation to raise the serum vitamin D concentration to adequate levels [14-16]. Epidemiological studies showed an association between dietary intake and serum levels of 25-hydroxyvitamin D and the progression of hip [14,15] and KOA [12, 16]. In a recent study, decreased serum level of 25-hydroxyvitamin D was reported in a significant proportion of patients with OA of hip and knee joints [14,16–19]. All these epidemiological suggests that by achieving normal serum vitamin D level may prevent or delay loss of cartilage loss and reduce the pain and other symptoms of OA [15-18].

Observation from studies on knee osteoarthritis evaluated by Cao *et al.,* suggested that serum 25-OH vitamin D level play an important role in structural changes of knee osteoarthritis [17]. In another study done by Bergink *et al* also, reported three times increased risk of KOA and disease progress was associated with serum 25-OH vitamin D <20 ng/ml in the Rotterdam Study [18]. In patients with hip OA who underwent total hip replacement, 25-hydroxyvitamin D levels were found to have positive correlation with both pre and post operative Harris hip scores [4,6,16-19]. Therefore, it is obvious that in patients undergoing total hip replacement vitamin D deficiency was a risk factor for a suboptimal outcome [5, 7, 19]. However, few studies failed to show significant association between the low level of serum 25-OH vitamin D and the development of OA [20–22]. It may be because of low sample size, inclusion of different ethnicity, seasonal variation, their time of exposure to sunlight, usage of sun cream lotions, different dietary habits etc. In the present study these problems were eliminated since all the studied population belongs to South India, Tamil Nadu territory. Therefore, earlier studies did not able to establish the association of serum 25 – OH vitamin D levels with hip or knee osteoarthritis.
The most important finding of this study is the high prevalence (55.8% deficiency and 26.7% insufficiency; around 78%) of low serum levels of 25-hydroxyvitamin D in a population with OA, in a sunny region like South India of Asian country. This study showed that about5 patients out of 10 with knee osteoarthritis were vitamin D deficient with serum levels <10 ng/ml. Several studies have shown a high incidence of vitamin D deficiency in patients with OA of hip or knee [17–19, 23]. We also tried to correlate serum levels of vitamin D with related anthropometric predisposing factors such as age, gender, and BMI. A significant association with gender was observed, with female patients having lower serum levels of vitamin D.

The findings of the present study are on par with some previous studies (4, 8-10, 14, 15, 19-21) showing a significant positive association between serum 25-OH vitamin D deficiency and OA knee in persons less than 60 years of age and in contrary to some other studies as well [5,7,16 -18,17,23].

In the present study these problems were eliminated since all the studied population belongs to South India, Tamil Nadu territory. Therefore, earlier studies did not able to establish the association of serum 25 – OH vitamin D levels with hip or knee osteoarthritis. Keeping the limitations of earlier studies in view present study was planned and executed effective to obtain results which are not affected by seasonal variation, diet, and exposure to sunlight or less physical activity due to pain and other confounders because patients and control population recruited for this study were selected from the

same geographic territory who had unique racial and cultural backgrounds, with similar diet and sunlight exposure.

5. CONCLUSION

All in all 57% of vitamin D deficiency was observed in this study. From the observations of this study, it can be concluded that there is a significant association between knee osteoarthritis and vitamin D deficiency when compared to control population with respect to age, gender and BMI. This association also corresponds with the development of knee cartilage damage in radiographic study. Therefore, identification of high risk subjects and correction of risk factors such as low level serum 25-OH vitamin D in KOA patients will give beneficial effects and even decreases fracture risk in elderly people.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

Prof. Dr. Regupathy Annamalai
Department of Orthopedics, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Madurantagam, Tamil Nadu, India.

Research and Academic Experience: He has 15 Years of Academic Experience, and 15 Years of Clinical Research Experience.

Research Area: His Research Area includes Orthopaedics Surgery and Joint replacement surgery.

Number of Published papers: He has 7 research articles.

Special Award: Medical Academic Entrepreneur Award by IMA, Tamil Nadu State in 2018, Edupreneurs 2015 Award by The Times of India, given by the then Hon'ble Tamil Nadu state Governor.

Dr. Amar Nagesh Kumar G.
Department of Biochemistry, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Madurantagam, Tamil Nadu, India.

Research and Academic Experience:
He has 5 Years of Academic Experience, and 4 Years of Research Experience.
He has Experience in the Role of Vitamin D, its mechanism of Action, Prevalence

Number of Published papers: He has 20 research articles in the national and international journals.

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Sentinel Lymph Node (SLN) Metastases in Breast Carcinoma Whole Slide Image (WSI) through Densenet Deep Learning Network: An Approach towards Clinical Management and Treatment

Rajasekaran Subramanian [a*], R. Devika Rubi [a], Abhay Krishna Kasavaraju [a], Samayk Jain [a], Swathi Guptha [a] and Suraj Raghavendra Pingali [a]

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ABSTRACT

This paper envisions a new and faster sentinel lymph metastases classification model which will help the pathology experts to perform fast and accurate diagnosis. This paper discussed a CNN based image classification model, to classify breast lymph node metastasis from WSI images, called DenseNet-161. Breast cancer intends to spread throughout the body. Cancer cells spread locally by infecting nearby healthy tissue. And it can spread throughout a region by infecting adjacent lymph nodes, tissues, or organs. CNN model initially learns the features from the training data. Subsequently after fitting the training data well it tries to generalize and make accurate predictions for the incoming new data which it has not seen earlier. Overfitting refers to a model that models the training data too well. The noise persists even after using the thresholding pre-processing strategy, necessitating extra pre-processing before training the model. Furthermore, increasing the dataset size by data-augmentation will significantly increase the accuracy.

Keywords: Sentinel lymph node; axillary lymph node; metastasis; metastasized breast cancer; dense net; CNN; diagnostic pathology; digital pathology; WSI images.

1. INTRODUCTION

Breast cancer intends to spread throughout the body. Cancer cells spread locally by infecting nearby healthy tissue. And it can spread throughout a region by infecting adjacent lymph nodes, tissues, or organs. Cancer can spread to other parts of the body at an advanced stage, which is known as metastatic cancer.

Under microscopic examination and diagnostic testing, metastatic cancer cells contain characteristics that are comparable to those of the primary cancer (from which it originated) and are generally distinct from the cells in the location where it is discovered. This makes it easier for doctors to determine if the cancer is metastatic or local. Despite the fact that metastatic breast cancer (MBC) is unlikely to be cured, significant increases in survival have been documented in recent years, coinciding with the advent of novel systemic therapies [1-3]. Median overall survival now is slightly over three years, with a range from a few months to many years [4].

Breast cancer staging is a method of determining severity of the disease and may include a physical examination of the skin, mammary glands, and lymph nodes, with the axillary, supraclavicular, and cervical nodes as the primary nodes of evaluation [5-7]. Different methods exist for classifying clinical and pathological findings into stages, though the most commonly used guidelines in the world are from the American Joint Committee on Cancer (AJCC) [8].

[a] *Keshav Memorial Institute of Technology, Hyderabad, Telangana, India.*
**Corresponding author: E-mail: rajasekarans@kmit.in;*

When cancer cells break away from the primary cancer, they can travel to other areas of the body either through the bloodstream or through the lymph system. The lymph nodes, under arm, inside the breast and the nearest collarbone are among the first places where breast cancer spreads. Tumor cells migrating from a primary tumor, metastasize to one or a few lymph nodes before involving others. The entire breast drains, the tumor cells to the same few lymph nodes, regardless of injection site [9]. These few lymph nodes are called "sentinel" lymph nodes. The status of sentinel lymph nodes would accurately predict the status of the remaining lymph nodes.

Lymph node assessment is considered to be one of the most independent prognostic factors in the breast cancer. It is also an important component of the TNM breast cancer staging system. In TNM staging system, patients with a sentinel lymph node positive will receive a higher pathologic TNM stage for sentinel lymph node metastasis than the patients with negative. This higher pathologic TNM staging frequently results in more aggressive clinical management including axillary lymph node dissection [10-12].

Assessing lymph node metastasis through histologic examination is the most accurate method. Each WSI image is gigapixels in size and analyzing the entire image manually is extremely difficult which consumes lot of pathologist's time. Due to the time-consuming and tedious nature of the diagnosis of breast cancer, there is a growing need for it to be automated.

Convolutional Neural Networks (CNNs) are shown excellent performance and gaining extensive attention in digital image processing tasks such as image classification, segmentation, object detection tasks etc [13]. CNNs are the most well-known type of deep learning architecture which perform well in the medical image analysis field. These CNN models which can automatically learn complex medical images characteristics and provide quantitative measures which facilitates increased accuracy in disease diagnosis with higher efficiency.

This paper envisions a new and faster sentinel lymph metastases classification model which will help the pathology experts to perform fast and accurate diagnosis. This paper uses a CNN model called Densenet-161, a novel version of DenseNet model with 161 layers to classify breast cancer metastases in the sentinel lymph node WSI [14]. The experimental PCam dataset contains 327,680 patches extracted from Cameylon16 Challenge dataset at a size of 96x96 pixels @10x magnification [15]. The experimental dataset is derived from 400 H&E stained breast cancer SLN WSIs of size 97792 x 220672 pixels.

2. MATERIALS AND METHODS

2.1 Technical Information

CNNs contain stack of various layers like input layer, a hidden layer and an output layer. The hidden layer of CNNs generally contains one or more convolutional layers, pooling layers, and fully connected layers.

Recently large-scale image analysis tasks especially complex medical image analysis tasks have shown that CNNs which contain shorter connections between layers close to the input and output, can be significantly deeper, more accurate and more efficient to train. The Dense Convolutional Network (DenseNet) leverages this observation and connects each layer with each of its previous layers in a feed-forward fashion. Traditional L-layers CNNs have L-connections, one connection between each layer and its subsequent layer. The number of connections in DenseNet is L*(L+1)/2. DenseNet keeps the feature-maps of all preceding layers as inputs to the current layer. All layers are connected so that the flow of information between layers in the network is maximum. There are multiple versions of Densenet in an L-layered network. This paper proposed Densenet-161 which consists 161 layers.

2.2 Data

Lymph nodes are oval-shaped organs found in numerous parts of the body, including the armpits, neck and groin. Sentinel lymph nodes spread cancer cells to other parts of the body as illustrated in Fig. 1. The experimental dataset contains.

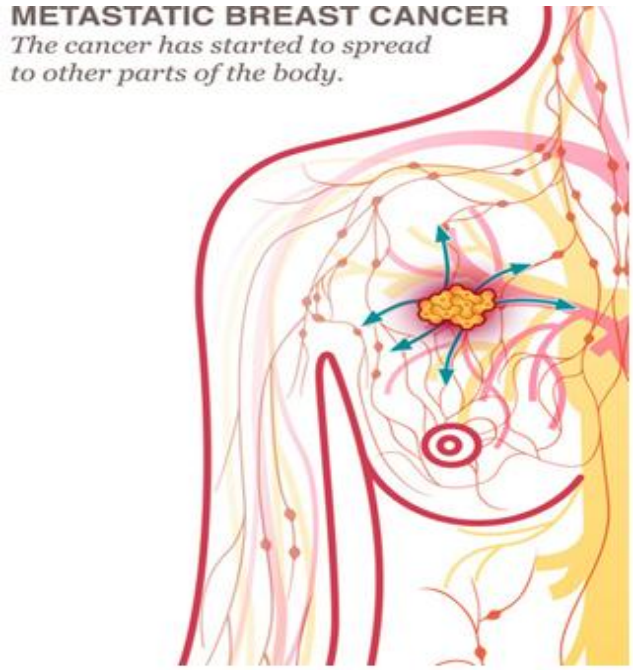

Fig. 1. Sentinel lymph node metastasize

2.3 CNN Training & Pre and Post processing Methods

The pre-processing consists of two steps. The first step consists of converting the three-channel RGB (colour) images into single-channel grayscale images. The second step is the application of truncation, which is a binary thresholding technique. Binary thresholding is done to highlight the image features i.e to make darker components darker and lighter components lighter. These single-channel images are stacked up three times to mimic three-channel RGB images.

Transfer learning technique is a machine learning technique where a model trained on one task is re-purposed on a second related task. This paper leverages the modern deep learning library Fastai for the implementation of deep learning model [16].

Training images in the experimental dataset has been shuffled and get_transforms function of fastai has been applied to augment the dataset. The concerned get_transforms function applies data augmentation techniques like image resizing, random cropping etc. Data is normalized with mean and deviation values range in [0.485, 0.456, 0.406] and [0.229, 0.224, 0.225] respectively in line with ImageNet model, a precursor to DenseNet model.

The pre-trained model is loaded using a fastai library function cnn_learner, which takes data, the model name, metric type and callback function as input parameters. Another fastai library function lr. find() which performs a mock training on data and plots the learning rate vs loss relationship has been used to reduce the amount of guestimates to pick initial learning rate for the learner. The experimental data set uses a learning rate of 1e-0.2. The well-known method fit_one_cycle method has been used to fit the model with the chosen learning rate and the number of epochs as input parameters.

Same pre-processing techniques such as three-channel conversion and binary thresholding are applied to test images. A prediction function is applied to compare the annotated value with the predicted value.

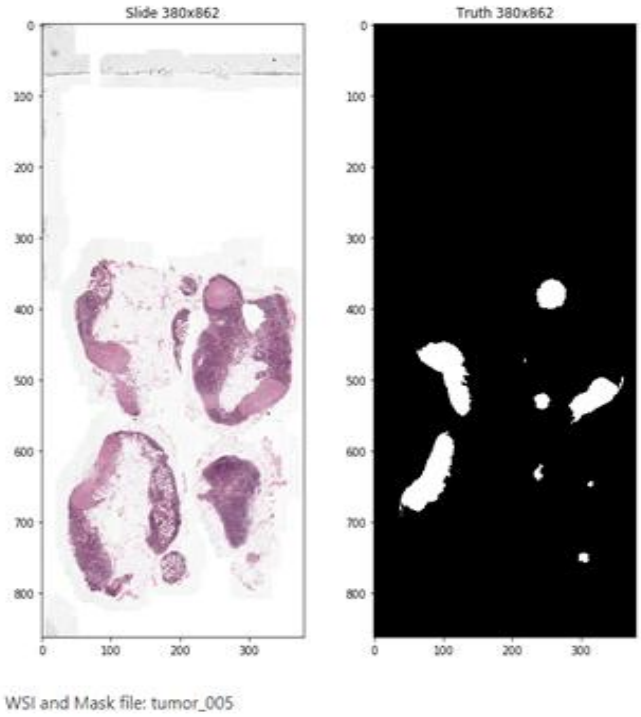

Fig. 2. Examples of camelyon-16 image – metastasized mask pair

3. RESULTS

Generally, the CNN models have been evaluated by using the metrics, dice coefficient, Jaccard coefficient, accuracy and F1-score [17-18]. Vikas Thada et al has done detailed comparison of the metrics for the researcher's reference [19].

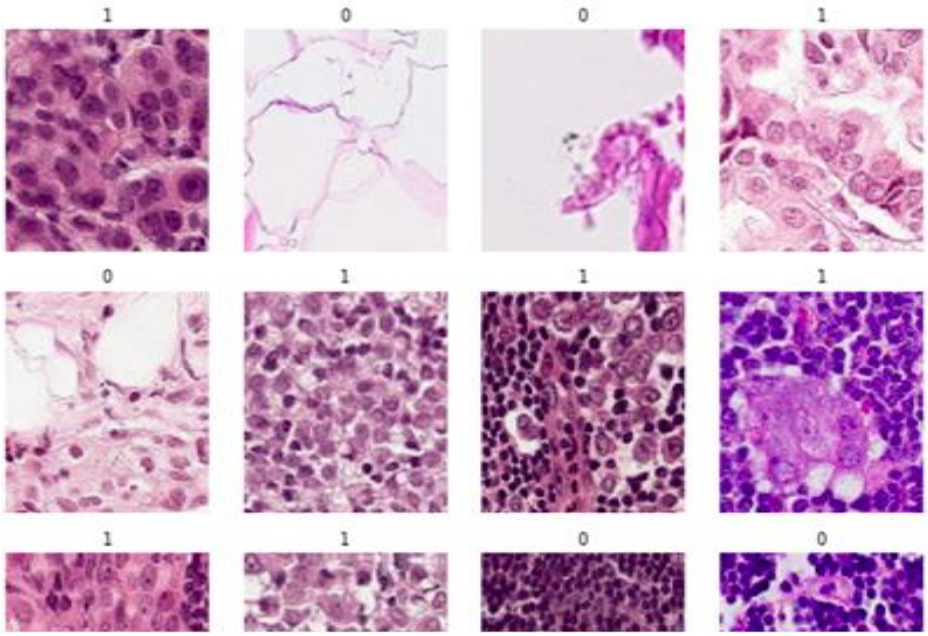

Fig. 3. PCAM patched image, 0-normal, 1-metastasize

Accuracy is one of the most common measure to evaluate models which measures all the correctly identified cases. In few cases, model evaluated by accuracy may fail to identify small, positive, and critical imaging regions. In imbalanced datasets, a model may have good accuracy, but may fail to identify such critical regions. In such scenario, to properly access the model, dice, Jaccard coefficients and F1-score are used.

CNN model initially learns the features from the training data. Subsequently after fitting the training data well it tries to generalize and make accurate predictions for the incoming new data which it has not seen earlier. Overfitting refers to a model that models the training data too well. It implies that model learns the features and noise in the training data to the extent that it negatively impacts the performance of the model on new data. Overfitting should be avoided. Data scientists do cross-validation to find out if their model is overfitting, wherein they split their data into two parts - the training set and the validation set. The training set is used to train the model whereas the validation set is used to evaluate the model's prediction performance while tuning model hyperparameters. The testing set is data applied on the final model to provide an unbiased evaluation of a final model fit on the training dataset.

Training accuracy measures the progress of model in terms of its training. Validation accuracy measures the quality of model measured on its ability of new predictions based on data it hasn't seen before. With an accuracy of ~82% on the validation set means that model can predict with ~82% accuracy on new data. Testing accuracy provides the actual (testing) performance numbers of the final model.

The proposed system has generated a training accuracy of 0.9477 and validation accuracy of 0.944. The proposed system has correctly classified 28127 images out of 32768 images belonging to the testing dataset, with an accuracy of 85.84%, as shown in Table 1.

Table 1. Actual testing accuracy

Actual	Predicted	True	False
True		12241	4136
False		505	15886
Correct Predictions (12241+15886)		28127	

Precision is measure of the correctly identified positive cases from all the predicted positive cases. Recall measures correctly identified positive cases from all the actual positive cases. F1-score is a harmonic mean of Precision and Recall and provides a better measure than Accuracy metric with incorrectly classified cases. In most and highly complex real-life classification problems like medical imaging analysis, imbalanced class distribution exists and thus F1-score is a better metric to evaluate the model. The proposed system has achieved a F1-score of 0.8406 as shown in Table 2.

Table 2. F1 Score Metrics

F1- Score	Precision	Recall
0.84	0.96	0,74

4. DISCUSSION

Initially, approaches to pathological image analysis in digital pathology have primarily focused on low level image analysis tasks like color normalization, nuclear segmentation, and feature extraction. Subsequently, classification models construction using machine learning methods like regression, support vector machines, and random forests are evolved over a period. Later on Deep Learning models, CNNs are gaining attention in complex medical image analysis tasks like image classification, image segmentation, object detection etc, which are part of disease diagnostic pipelines.

This paper discusses few of the well-researched, established and implemented CNN models on digital pathology imaging techniques.

Anant Madabhushi et al explains out performing various computational tumoral markers identification tasks like Carcinoma Localization, Nuclei Segmentation, Epithelium Segmentation, Tubule Segmentation, Lymphocyte detection, Mitosis Detection and Lymphoma classification on WSI pathology images by implementing various Deep Learning Techniques [20]. Authors explained-out epithelium segmentation task as an use case. Authors have used 34 digital pathology 1000x1000 pixels training images and 8 validation images and performed epithelium segmentation from WSI images. Multiple patches of size 32x32 are extracted from each WSI image and each patch containing the edges of epithelium regions are taken so that the network can learn crisp boundaries. The popular CNN algorithm, AlexNet is applied to classify the images on the generated patches. White regions are removed by applying user-defined thresholding and positive regions which aren't clinically relevant, of size less than 300 pixels are removed to generate the output. An average F1 score of 0.84 has generated by this method.

Muhammed Tao has introduced DenseNet-161 and ResNet-50 pre-trained CNN models and classified into multiple classes of WSI images, based on textural patterns of images by performing transfer learning on those images [21]. The author has used 23,916 images of size 1000x1000 pixels, which are extracted from 24 WSI images of various body parts with different texture patterns. Author has tried to prove that automated detection and classification of diseases using computational scanned microscope images with much higher accuracy and faster time. By performing the transfer learning of the pre-trained models DenseNet-161 and ResNet-50 on digitized histopathology images has achieved a classification accuracy of 97.89% using grayscale images and the ResNet-50 model obtained the accuracy of 98.87% for color images. Author has shown that the pre-trained models used in this experiment outperform state-of-the-art methods in all image classification performance metrics with 24 image categories.

Wang et al have evaluated classification performance of four different existing deep learning networks of the time for the classification of SLN with MBC. Proposed a GoogleNet based deep learning network for this classification task [22]. The researchers have implemented four well-known deep learning networks of the time namely, GoogleNet, AlexNet, VGG16 and a face oriented deep network to classify SLN with MBC. Subsequently, they used GoogleNet in their deep learning framework for their patch-based classification task. And also established that combining deep learning networks with pathologist's prediction have reduced the pathologist error rate from over 3 percent to less than 1 percent.

Based on observation of digital pathology images which are inherently symmetric under rotation and reflection B.S.Veeling et al proposed new deep learning model for pathology images segmentation [23]. B.S.Veeling model leverages inherent symmetries of pathology images in a principled manner and shown that model has improved stability on predictions. Also demonstrated that exploiting rotation equivariance improves the performance of tumor detection on lymph node metastases dataset significantly. The proposed model, a patch-classification model is derived from the densely connected convolutional network (DenseNet). The DenseNet is primarily consisting dense blocks with layers which uses stack of all previous layers as input, alternating with 1 x 1 convolutional layer as transition blocks and 2 x 2 strided average pooling. Presented a novel large-scale pathology images derived dataset for precise machine learning model evaluation. Patients with a sentinel lymph node positive will receive a higher pathologic TNM metastasis breast cancer stage than patients negative for sentinel lymph node metastasis which frequently results in more aggressive clinical management and treatment including axillary lymph node dissection.

This paper discussed a CNN based image classification model, to classify breast lymph node metastasis from WSI images, called DenseNet-161. The DenseNets have substantial advantages over traditional CNNs, that they reduce the vanishing-gradient problem, feature reusage, strengthen feature propagation, significant reduction in number of parameters and less computation time. DenseNet with its novel architecture of connecting each of its layer to each of their previous layers in a feed-forward fashion has substantial advantage over traditional CNNs. Traditional data augmentation techniques like image resizing, random cropping etc. are used to artificially increase the dataset size to obtain a better fit on the model. This paper also discussed about pre-processing

techniques like binary thresholding to avoid the information loss. This paper also discussed about the modern library fastai which is used to perform transfer learning on the experimental dataset.

Each breast WSI image is in gigapixels size and analyzing gigapixels image manually is extremely difficult and time consuming to pathologists. As diagnosing the breast cancer is time-consuming and tedious, there is a growing need to automate this process. This paper envisions an image classification model to classify breast lymph node metastasis on WSI image which facilitate pathology experts to perform fast and accurate diagnosis task.

This model involves extraction of complex information from the medical images dataset, which requires the removal of noise. Even after applying thresholding pre-processing method the noise persists, which requires additional pre-processing before training the model. And by increasing the dataset size through data-augmentation will also improve the accuracy considerably.

5. CONCLUSION

This model entails extracting complex information from a dataset of medical images, which necessitates noise removal. The noise persists even after using the thresholding pre-processing strategy, necessitating extra pre-processing before training the model. Furthermore, increasing the dataset size by data-augmentation will significantly increase the accuracy.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

Rajasekaran Subramanian
Keshav Memorial Institute of Technology, Hyderabad, Telangana, India.

Research and Academic Experience: He is PMP certified having 24 years of Software Industry experience, in managing multiple, multi-million-dollar Software Engineering- Delivery-Programs-Projects across various geographies, in the capacity of Country Head, Program/Projects Management Head and Microsoft Practice Head. Also, he has Biomedical Informatics Research experience, especially with Pathomics (Pathology Data Science and Onco-Genomics) and worked as a Research scientist at the Dept. of Neurology, School of Medicine, Emory University, Atlanta, USA

Research Area: Digital Pathology, Cancer Imaging Analysis, Onco-Genomics.

Number of Published papers: He has 12 research articles in the National and International Journals.

R. Devika Rubi
Keshav Memorial Institute of Technology, Hyderabad, Telangana, India.

Research and Academic Experience: Bioinformatics Researcher having 15+ years of Genomics/Bioinformatics, Biomedical Informatics Research experience in Data analysis algorithms development, Mathematical modeling of Data. She was the research lead for Genome International Corporation, Madison, Wisconsin, USA and also she worked as Researcher at Teknuance, VA, USA. She worked as a Post-doc Research associate for Cancer Imaging and Genomics studies at the Dept. of Neurology, School of Medicine, Emory University, Atlanta, USA.

Research Area: Digital Pathology, Cancer Imaging Analysis, Onco-Genomics.

Number of Published papers: 11 research articles in the National and International Journals.

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Study on Association of Systemic Diseases on Tooth Loss and Oral Health

Farzeen Tanwir [a*] and Chander Kumar [b]

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ABSTRACT

Aim: To evaluate the level of oral hygiene in patients of various systemic illnesses.
Materials and Methods: A total of 450 patients from Pakistan Institute of Medical Sciences, Capital Development Authority, Islamic International Dental College, Islamabad and Jinnah Postgraduate Medical Center, Karachi, suffering from diseases such as diabetes mellitus, cardiovascular disorders (with and without diabetes mellitus), respiratory disorders, gastrointestinal disorders, hepatic disorders, renal disorders, bone/joint disorders and hypertension were selected. Dental examination was performed to calculate the number of missing teeth, decayed, missing and filled teeth (D.M.F.T.) and decayed, missing and filled surfaces (D.M.F.S.) scores in order to compare oral health between different disease groups.
Results: Age, gender, number of missing teeth, D.M.F.T. and D.M.F.S. scores were significantly associated with the systemic diseases ($p < 0.05$). The diseases causing poor oral hygiene in descending order of severity were as follows: cardiovascular disorders, diabetes mellitus with cardiovascular disorders, diabetes mellitus, bone/ joint disorders, hypertension, renal diseases, hepatic disorders, gastrointestinal disorders and respiratory diseases.
Conclusion: Patients with systemic diseases have greater risk of compromised oral hygiene, tooth loss, high D.M.F.T. and D.M.F.S. scores showing the association between systemic diseases and oral health. There is a need for collaboration between dentists and medical physicians to improve the health of the patient.

Keywords: DMFT; DMFS; systemic diseases; oral hygiene; tooth oral health; cardiovascular disorders; diabetes mellitus; renal diseases; respiratory diseases and gastrointestinal diseases.

1. INTRODUCTION

Pakistan ranks as sixth most populous country of the world yet only 2.6% of total gross domestic product is allocated for public health. Approximately 70% of the population is resident in rural areas making the access to healthcare facilities difficult as most of tertiary care hospitals are in the urban cities. The doctor to patient ratio is 1: 1254 while dentist to patient ratio is 1: 6557 reflecting a dearth of health care facilities in the country.

There is high prevalence of systemic diseases in the population. One in every four middle aged adult carries risk of coronary artery disease [1]. Pakistan is ranked as fifth and sixth country world over to be affected by pulmonary infections such as tuberculosis and diabetes mellitus (10% diabetes prevalence) [2,3]. Hepatitis B and C infections affect 9-10 Million people [4,5]. At 45 years, every third person is reported to be hypertensive (33% prevalence) while more than 50% of hypertensive often remains undiagnosed [6]. Likewise, the rate of cerebral stroke is 4.8 % which is said to be one of the highest in the world [7]. Similarly, 37% of the newborn are low weight [8].

[a] *Department of Dentistry, Dow Dental College / Bahria Dental College, Bahria University, Pakistan.*
[b] *Department of Periodontology, Dow Dental College, Dow University / Bahria University, Pakistan.*
**Corresponding author: E-mail: farzeen_tanwir@yahoo.com;*

Many of the above-mentioned systemic disorders are either caused or aggravated by poor oral hygiene and vice versa. It is now believed that compromised oral health can increase the complications of diabetes mellitus, cardiovascular diseases, respiratory infections, gastrointestinal pathologies, renal ailments, liver problems, bone and joint disease as well as complicate the outcome of pregnancy by increasing the risk of pre-term delivery of low weight babies [9]. These systemic disorders may deteriorate oral health leading to the development of infections such as gingivitis, periodontitis and if left untreated, may cause tooth loss.

Therefore, the objective of this study, which is a part of a larger research project was to evaluate the impact of systemic illnesses on oral hygiene, and to observe and compare the level of oral hygiene in patients with systemic disorders. To the authors' knowledge, there has been less data available comparing oral health in patients of various systemic diseases.

2. MATERIALS AND METHODS

This observational study was approved by the Ethics committee at Ziauddin University, Karachi, Pakistan. The sample size was calculated using online sample size calculator by Raosoft. The recommended minimum sample size was 377, which was increased to 520 for accuracy (non-probability sampling). Data was collected from November 2012 to February 2013 from two public sectors; tertiary care hospitals in Islamabad namely Pakistan Institute of Medical Sciences (P.I.M.S.), Capital Development Authority (C.D.A.) hospital, outpatient department at Islamic International Dental College and Hospital, Islamabad and Jinnah Postgraduate Medical Center (J.P.M.C.) Karachi which is also a public tertiary care hospital. Patients from nine systemic illnesses were chosen for this study, which are listed in Table 1.

Conscious patients with permanent dentition suffering from one of the diseases listed in Table 1 were selected after obtaining informed consent. Patients with acute infections, pain, pregnancy, loss of teeth due to trauma and total absence of teeth were excluded which reduced sample size to 450 patients. For every disease, 50 patients were chosen in the consecutive order with the recruitment stopping when 50 patients were assigned to every disease group. The patients were inquired about their disease and the reason for visiting hospital. Their medical reports were assessed to rule out co-morbidities.

Dental examination was performed using a sterilized dental mirror, periodontal probe, wooden tongue depressor and a torch light. Data for age, gender, number of remaining teeth, decayed, missing and filled teeth (D.M.F.T.) and decayed, missing and filled surfaces (D.M.F.S.) was recorded for every patient (WHO). The normal number of teeth was taken to be 28 excluding 3rd molars for this study.

For statistical analysis, Statistical Package for Social Sciences SPSS 16.0 was used to calculate the mean for each variable along with the standard error. One way ANOVA test was used to find significant differences between study variables. Moreover, Post-hoc Duncan test was applied to categorize diseases that are similar to each other with respect to number of teeth, age, D.M.F.T. and D.M.F.S. The level of significance (α) was 0.005.

3. RESULTS

There were equal number of males and females in the selected sample of 450 patients. The mean age of study population was 43.28 years (± 14.45) with an age range of 13-84 years. The mean number of teeth, D.M.F.T. and D.M.F.S. scores were 23.13 (±5.76), 7.93 (± 5.78) and 22.94 (± 19.27), respectively.

Table 2 Results from one way ANOVA analysis for systemic diseases and dependent variables such as age, gender, number of remaining teeth, D.M.F.T. and D.M.F.S scores. The mean value along with standard error in parenthesis is represented.

By using post-hoc Duncan test, the values for number of remaining teeth, age, D.M.F.T. and D.M.F.S. are represented in Tables 3 and 4. Diseases showing similar patterns were categorized vertically in subset of groups for number of teeth, age, D.M.F.T. and D.M.F.S.

Table 3 The vertical groups represent diseases showing similar pattern. Some of the diseases in a group share common features with diseases in other group which is shown by repetition of the value of these diseases in the next group. Each subset is significantly different to other subset but non-significant within a subset. Similarly for age groups, but here the order is different than number of teeth.

Table 4 Diseases with similar patterns are represented in vertical groups with diseases sharing common features with other diseases are shown by repetition of the value of these diseases in the next group. Each subset is significantly different to other subset but non-significant within a subset. Similarly for age groups, but here the order is different than number of teeth.

3.1 Group 1 Diseases

Relatively more males (54%) compared to females (46%) had cardiovascular problems with the mean age of forty-nine years which did not differ from mean ages of D.M. sample with or without C.V.S. problems. C.V.S. patients on average had eight missing teeth and the highest D.M.F.T. score. There was greater percentage of females (54%) affected with D.M. compared to males (46%) with the diabetics having an average of seven missing teeth. When D.M. was present as co-morbidity in C.V.S. patients, the tooth loss and D.M.F.T. score increased with the D.M.F.S. score reaching maximum amongst all systemic diseases (Graphs 3 and 4). The last disease in the Group 1 type diseases (Table 3) was bone/joint disorder. 66% females with a mean age of 38.3 years suffered from these disorders making it the most common disease in females with the age group as young as 16-29 years. The patients in our study had an average seven missing teeth and third highest D.M.F.T. scores (Graph 3 and Table 4).

Table 1. Systemic disorders along with the most frequent forms of the disorders present in the study population

Systemic diseases	Conditions present
Diabetes Mellitus (D.M.)	Type 1 and Type 2
Cardiovascular disorders (C.V.S.)	Myocardial infarction, Angina Pectoris
Respiratory disorders	Tuberculosis, Asthma, Chronic obstructive pulmonary disease, Pneumonia
Gastrointestinal disorders (G.I.)	Gastric ulcer, Gastritis, Inflammatory bowel disease, Typhoid.
Hepatic disorders	Hepatitis B & C, Hepatocellular carcinoma.
Renal disorders	Chronic kidney failure, proteinuria.
Bone/joint disorders	Rheumatoid arthritis, osteoarthritis, osteoporosis.
Hypertension (H.T.N.)	Primary hypertension
Diabetes Mellitus + Cardiovascular patients (D.M.+ C.V.S.)	Diabetic patients with co-morbid heart disease.

3.2 Group 2 Diseases

Hypertension was the second most prevalent disease amongst females (62%) after Rheumatoid arthritis. The hypertensive patients in the study had on average five missing teeth. The D.M.F.T. and D.M.F.S. score ranked hypertension as fifth systemic disease to affect the oral health (Graphs 3, 4 and Table 4). The mean age of renal patients was lowest amongst all groups suggesting an earlier onset (35 years). Equal number of males and females were affected by renal disorders (Graphs 1 and 2). Dentally, the disease showed similar pattern to hypertension with five missing teeth.

Table 2. Mean (S.D) with ANOVA test for systemic diseases with respect to age, number of teeth, D.M.F.T. and D.M.F.S. is observed

Disease	D.M	C.V.S	Respiratory	G.I	Hepatic	Renal	Bone/ Joint	H.T.N	D.M+ CVS	P-value
Age	49 (16.1)	49.1 (12.1)	47.6 (10.1)	39.2 (16)	37 (13.3)	35 (12)	38.3 (12)	43.6 (16)	50.8 (11.7)	0.00
No. of teeth	21.8 (5.6)	20.4 (7)	25.7 (3.9)	25.6 (3.8)	25 (3.8)	23.6 (5)	21.9 (6.7)	23.5 (5.3)	20.7 (7.3)	0.00
DMFT	8.7 (5.4)	10.5 (6)	6.1 (4.5)	5.4 (4.8)	5.7 (3.9)	7.2 (5.2)	9.5 (6.5)	7.9 (6.1)	10.4 (6.9)	0.00
DMFS	26.9 (18.6)	32.2 (21.5)	13.9 (12.5)	14 (14.4)	16.6 (16)	20.7 (19)	26.4 (20.2)	23 (19.2)	32.6 (23.8)	0.00

Table 3. Homogeneous groups for number of teeth and age of patients with systemic illnesses.

Systemic Disease	Subset of number of teeth			Systemic Disease	Subset of age			
	1	2	3		1	2	3	4
C.V.S	20.4			Renal	35			
D.M + CVS	20.7			Hepatic	37			
D.M	21.8	21.8		Bone/ Joint	38.3	38.3		
Bone/Joint	21.9	21.9		G.I	39.2	39.2		
H.T.N		23.5	23.5	H.T.N		43.6	43.6	
Renal		23.6	23.6	Respiratory			47.6	47.6
Hepatic			24.9	D.M			49.0	49.0
G.I			25.5	C.V.S			49.1	49.1
Respiratory			25.7	D.M+CVS				50.8
P-value	0.20	0.14	0.07	P-value	0.15	0.06	0.06	0.28

3.3 Group 3 Diseases

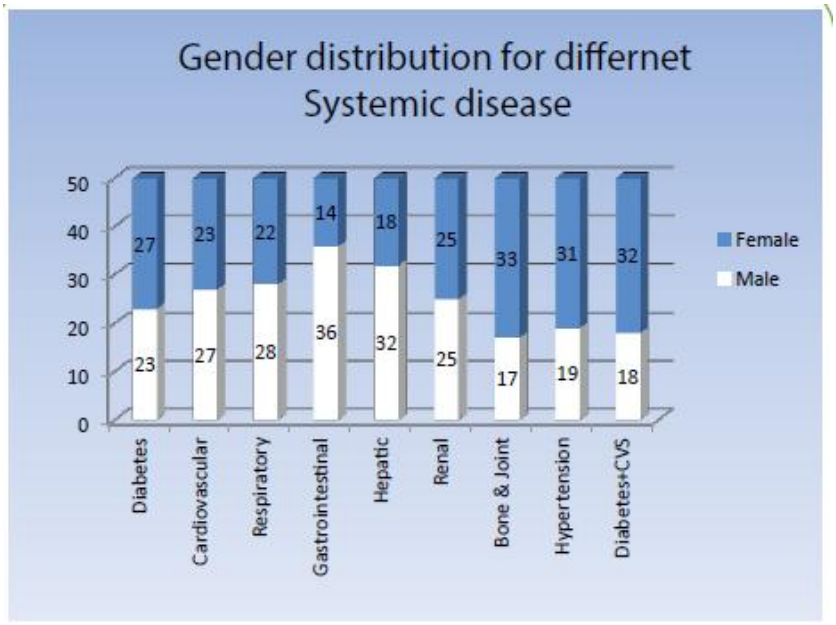
All the patients with systemic diseases in Group 3 had on average three missing teeth (Table 3). In terms of hepatic pathologies, more males (64%) compared to females (36%) suffered with a mean age of thirty-seven years with D.M.F.S. score higher than the D.M.F.T. In the current study, 72% of men suffered from G.I. disorders compared to 28% females with the mean age being thirty-nine years (Graphs 1 and 2). Finally respiratory ailments caused least number of tooth loss. As was true for all the Group 3, men had more pulmonary problems (56%) compared to females (44%) with a mean age of forty-seven years. These patients had the lowest D.M.F.T. score.

4. DISCUSSION

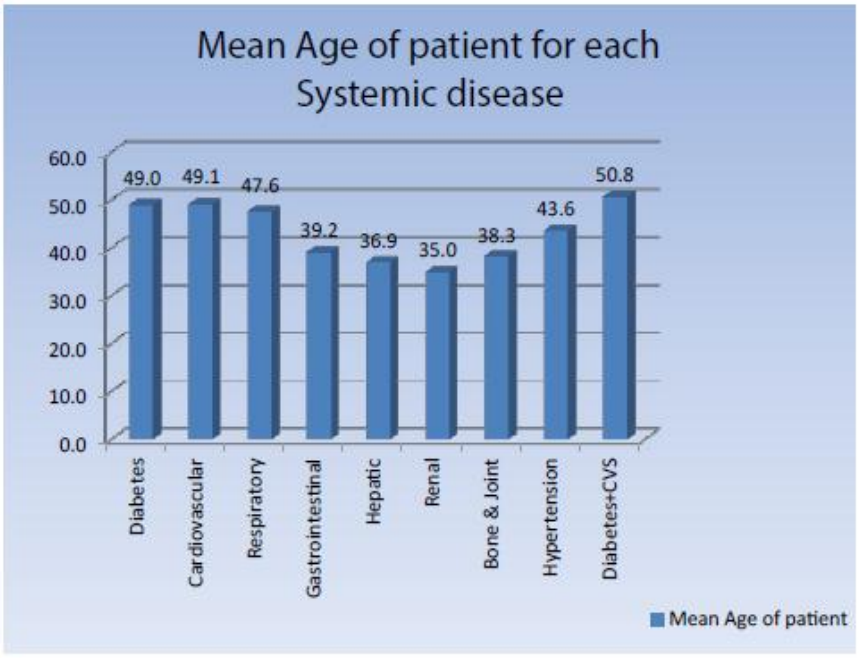
Age, gender, number of remaining teeth, D.M.F.T. and D.M.F.S. scores were found to be highly significant with the systemic diseases ($p < 0.05$). This is in accordance with previous findings which list age and gender as risk factors for certain diseases e.g., D.M., Rheumatoid arthritis and C.V.S. disorders [10-12]. Higher prevalence of C.V.S. disorders in men might be attributed to higher cholesterol levels and increased habit of smoking in men [13]. Maximum numbers of missing teeth in C.V.S. patients reflect the poorest oral hygiene amongst all other disease groups which is in accordance with a previous report [14]. Myocardial infarction deteriorates oral health and reports have linked level of periodontal detachment and loss of teeth in cardiac patients to earlier mortality [15]. People with less than ten teeth remaining had seven times higher mortality risk than those having greater than twenty-five remaining teeth [16]. Total loss of teeth (edentulism) also increases risk of cerebrovascular disorders such as stroke [17]. Not only C.V.S. problems can cause dental problems, but a compromised oral hygiene can also aggravate C.V.S. problems and it has been noted that improving oral hygiene in cardiac patients decrease the chances of thrombus formation and associated complications such as stroke [18]. This might be due to high levels of bacterial endotoxin, proinflammatory cytokines, lipids, fibrinogen and C-reactive protein and platelets, which can cause thromboembolic events such as ischemic stroke [19].

Table 4. Homogeneous groups for D.M.F.T. and D.M.F.S of patients with systemic diseases

Systemic Disease	Subset D.M.F.T. score				Systemic Disease	Subset D.M.F.S. score			
	1	2	3	4		1	2	3	4
G.I	5.4				Respiratory	13.9			
Hepatic	5.7	5.7			G.I	14.0			
Respiratory	6.1	6.1			Hepatic	16.6	16.6		
Renal	7.2	7.2	7.20		Renal	20.8	20.8	20.8	
H.T.N		7.9	7.86		H.T.N		23.0	23.0	
D.M			8.72	8.7	Bone/ Joint			26.4	26.4
Bone/ Joint			9.46	9.4	D.M			26.8	26.8
D.M+CVS				10.3	C.V.S				32.2
C.V.S				10.5	D.M+CVS				32.6
P-value	0.14	0.08	0.06	0.14	P-value	0.10	0.11	0.14	0.13

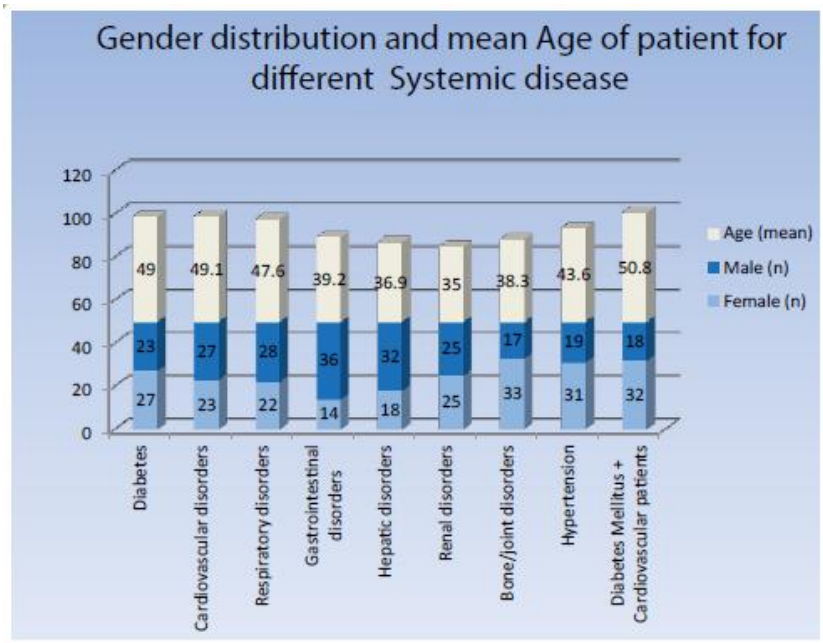


Graph 1. Representation of systemic diseases with respect to gender of patients

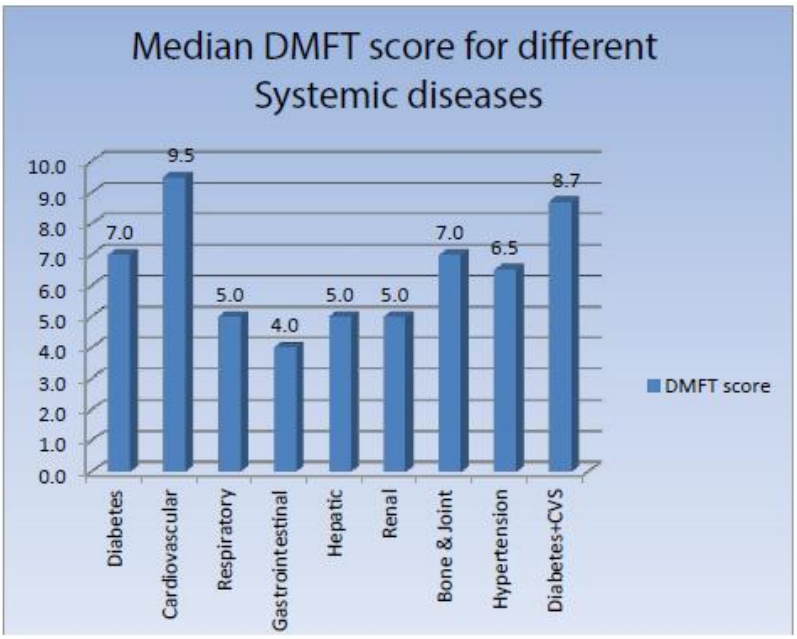


Graph 2. Mean age of patients with systemic diseases in the sample

D.M. and compromised oral health affect each other. People with neglected oral hygiene have poor glycemic control and higher Insulin resistance [20]. Periodontitis is listed as sixth complication of D.M. and both types of D.M. promote loss of teeth [21,22]. In terms of global prevalence, South Asians have a higher prevalence of C.V.S., D.M. (Type 2) and Insulin resistance which necessitates the maintenance of good oral hygiene particularly in this population [23].



Graph 3. D.M.F.T. scores amongst patients in various systemic diseases



Graph 4. D.M.F.S. scores amongst patients in various systemic diseases

The current study supports the higher ratio of females compared to males being affected with bone/joint problegms with mean age of 38.5 years in Pakistan [11]. It has been reported previously that 43.4% females above twenty years suffer from Osteopenia and about 25% have established Osteoporosis in Pakistan [24]. Periodontitis is also linked to the presence of bone/joint disorders, possibly due to Estrogen deficiency developing earlier in females compared to males [9]. A recent survey showed no strong association between oral health and bone/joint disease, however, the current study places bone/joint disorders in Group 1 diseases that cause greatest number of tooth loss similar to D.M. and C.V.S. disorders [25]. This finding is supported by other studies which relate higher frequency and severity of Periodontitis in Rheumatoid arthritis and Osteoporosis patients; however, few have noted a smaller number of decayed teeth in Rheumatoid arthritis patients

compared to controls [26-29]. Poor oral hygiene and presence of *Porphyromonas gingivalis*, a periodontal pathogen, promotes the development of autoimmune antibodies which are responsible for Rheumatoid arthritis [30].

H.T.N. has a high prevalence in Pakistan affecting an estimated 19% of population over the age of 15 years with female population being at a higher risk compared to men [31]. Elevated blood pressure causes alveolar bone loss and is associated with tooth loss in a bidirectional relationship as well [32,33]. People with lesser number of teeth and edentulous patients tend to have higher blood pressure, however one study found no relation between hypertension and oral health [34-36].

Earlier studies on Pakistani population found thirty years as the mean age whereby 25% have been diagnosed with reduction in kidney function [37]. A slightly greater predilection was observed in males in reference to the risk of renal disease by an earlier study which is not in line with our findings [38]. There is reportedly high D.M.F.T. score, poor oral hygiene and alveolar bone loss in patients with kidney disease particularly in patients undergoing hemodialysis [39,40]. Renal patients have more tooth loss and edentulous patients carry higher chances of developing chronic renal failure suggesting bidirectional relation between the two [41,42]. Two studies, however, found no association between kidney problems and oral health [43,44].

A previous study showed 68% of Pakistani men to be affected with hepatic problems compared to 31% females with the most cases occurring in the age range of 20-39 years which is similar to our findings [45,46]. Decreased oral hygiene has been linked to the presence of hepatic disease and in turn poor oral health can cause liver abscess formation [47,48]. It has also been observed that patients presenting with Hepatitis C have higher chances of developing Periodontitis [47].

Gastrointestinal disorders are also frequent with 60% reported prevalence of gastritis [49]. Furthermore *H.pylori*; bacteria linked to gastritis, gastric ulcers and increased insulin resistance has been found in the drinking water in many cities of the country [50,51]. G.I. disorders are highly prevalent in men (Graph 1) and a previous study also yielded same results [52]. People having neglected oral hygiene have greater number of Helicobacter pylori in the mouth which can cause gastric disorders as well as recurrence of these disorders [53]. This bacterium produces volatile Sulfur compounds responsible for halitosis and glossitis [54]. A study has linked the production of these compounds to the concomitant levels of gastric mucosal injury, thereby halitosis may be reflective of gastric injury [55]. Moreover, it is also linked to increased caries risk and higher D.M.F.T. score compared to controls [56]. Conditions such as inflammatory bowel disease, Crohn's disease and ulcerative colitis also affect oral hygiene negatively [57,58].

A higher incidence of respiratory problems in men compared to females with the mean age of 46.3 years has been already reported [56]. Tuberculosis, Chronic obstructive pulmonary disease, asthma and other respiratory diseases impact oral health negatively and less than optimum oral hygiene can cause decrease in lung function as well as increase the chance of pneumonia [59-65]. The less number of missing teeth along with lowest D.M.F.T. score reflects the fewest dental problems amongst in the sample.

The strength and weakness of the study deserve mentioning. This study is to compare oral health in patients with multiple systemic illnesses. Sample size was increased to have better evaluation of risk factors responsible for tooth loss. The weaknesses of the study include no adjustment for known cofounders like socioeconomic factors, failure to record the severity of the underlying systemic diseases owing to the lack of funds and lab facilities available and also not having control group of health subjects to compare the findings.

5. CONCLUSION

This study highlights association between age, gender, tooth loss, D.M.F.T., D.M.F.S. score and systemic diseases. Diseases such as cardiovascular disorders, diabetes mellitus with and without cardiovascular pathologies and bone/joint disorders negatively impact oral hygiene. Greatest loss of teeth and higher indices were observed in these patients. Hypertensive and renal patients had

comparable oral hygiene to diabetes mellitus and bone/ joint patients. The earlier onset of renal diseases is alarming as observed in this study. Patients suffering from pathologies of liver, gastrointestinal tract and respiratory system had compromised oral hygiene though relatively less compared to other diseases studied in this group. Therefore, it is the need of the hour to educate the dentists, general physicians and specialists about the association of systemic diseases with oral health and vice versa as many of the systemic diseases may be prevented and improved by improving oral hygiene.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Registered offices
India: Guest House Road, Street no - 1/6, Hooghly, West Bengal, PIN-712410, India, Corp. Firm Registration Number: L77527, Tel: +91 7439016438 | +91 9748770553, Email: director@bookpi.org, (Headquarters)
UK: 27 Old Gloucester Street London WC1N 3AX, UK
Fax: +44 20-3031-1429 Email: director@bookpi.org, (Branch office)