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Global Journal

of Medical Research: F

Diseases

Cancer, Ophthalmology & Pediatric

Different Cardiovascular Risk

Latinoamerican Case and Review

Highlights

Residents of Kamwenge Town

Clinical Trial of Saccharomyces

Discovering Thoughts, Inventing Future

VOLUME 19 ISSUE 2 VERSION 1.0

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A Randomized Clinical Trial of Saccharomyces Cerevisiae versus Placebo in the Irritable Bowel Syndrome

By Dr. Amjad Atef Suliman Alhelo

Abstract- Background: We aimed to evaluate clinical symptoms in subjects with irritable bowel syndrome receiving Saccharomyces cerevisiae in a randomized double-blind placebo-controlled clinical trial.

Methods: 347 adults with irritable bowel syndrome (Rome III criteria) were randomized to receive twice daily 1000 mg of Saccharomyces cerevisiae, delivered by two tablets for four-week n=177 age: 35 ± 15, or placebo n=170 age: 35 ± 15 for 4 weeks.

Ibs symptoms (Abdominal pain/discomfort, bloating/ distension, bowel movement difficulty) and changes in stool frequency and consistency were recorded daily and assessed each week. A safety assessment was carried out throughout the study.

Result: The proportion of responders, defined by an improvement of I.b.s symptoms (abdominal pain/ discomfort, bloating/distension, bowel movement difficulty) and changes in stool, was significantly higher (p value < 0.001) in the treated group than the placebo group (130 vs 47), (73.4% vs 27.64%).

Keywords: abdominal pain, irritable bowel syndrome, probiotic saccharomyces cerevisiae, yeast.

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Conclusion: Saccharomyces cerevisiae is well tolerated and reduces irritable bowel syndrome symptoms with stool modification.

Keywords: abdominal pain, irritable bowel syndrome, probiotic saccharomyces cerevisiae, yeast.

I. Introduction

Irritable Bowel Syndrome (IBS) is the most common functional gastrointestinal disorder. IBS is characterized by chronic and/or recurrent abdominal pain or discomfort and altered bowel habits.

IBS has an estimated worldwide prevalence of 14% in women and 9% in men, and usually occurs before age 50 years.

IBS has been sub typed according to predominant bowel habit as:

- IBS with constipation.
- IBS with diarrhea.
- Mixed type.
- Unclassified.

a) *Rome III Criteria for IBS*

The criteria for a diagnosis of Irritable Bowel Syndrome (IBS) requires that a person be experiencing chronic abdominal pain or discomfort at least three days over the course of the last three months, with an onset of symptoms at least six months prior. These symptoms must also show:

- Pain symptoms are improved with a bowel movement.
- Symptom onset is related to a change in the frequency of stool.
- Symptom onset is related to a change in the appearance of stool.

Numerous pathophysiological mechanisms have been explained IBS, but the contribution of the gastrointestinal microbiota and variations in its composition and function have only recently begun to be evaluated as a significant component in the pathogenesis and pathophysiology of irritable bowel syndrome.

b) *Intestinal microflora*

Human intestine contains 1014 bacterial cells, which are 10 times higher than the number of cells in the human body. Seventy percent of our body normal microflora in the colon, which contains bacteria, fungi, viruses.

The number of bacteria increases from stomach (101 to 103 bacteria/g) to the colon (1011 to 1012 bacteria/g).

The small intestine contains mainly Gramm positive and aerobic bacteria, the large intestine contains predominantly Gram negative and anaerobic bacteria. 95% of intestinal bacteria are anaerobes, Bacteroidetes and Firmicutes.

c) *Benefits of intestinal flora*

Fermentation of undigested food, endogenous mucus producing short chain fatty acids, which are nutrients to the colonic epithelial cells and conservation of energy, absorption of NaCl and water, from the right colon, synthesis of vitamin K, control of epithelial cell proliferation, protection against pathogens by a barrier effect and training of the immune system.

Intrinsic and extrinsic factors that prevent overgrowth of bacteria in the small intestine, *intrinsic factors* include:

1. Gastric juice and bile.
2. Peristaltic movement which prevent adherence of bacteria.

Author: Family physician, Jordanian Board of Family Medicine.
e-mail: amjadrrrr@hotmail.com

3. Normal gut defense including humoral and cellular mechanisms.
4. Mucin production by intestinal mucosa.
5. Gut antibacterial peptide.
6. Ileocecal valve preventing retrograde translocation of bacteria from colon to the small intestine.

Extrinsic factors include diet and drugs modulating gut flora, such as antibiotics and ppis and h2 blockers.

d) Evidences of Bacterial Disturbance Causing IBS

i. *Post-infectious IBS*

After acute gastroenteritis infectious etiology, up to 30% of patients complain of gastrointestinal symptoms for a long time, which meet irritable bowel syndrome criteria.

Probiotics is effective in restoring the intestinal microbiota in patients with post infectious irritable bowel syndrome.

ii. *Small Intestinal Bacterial Overgrowth and IBS*

A study undertaken at Cedars-Sinai Medical Center used 448 subjects who were referred by their doctors for detection of SIBO. After completing a questionnaire, the researchers determined that 202 subjects could be considered as having irritable bowel syndrome according to standard symptom criteria (see sidebar). Of these, 157 (78%) were positive for bacteria overgrowth using the LHBT.

The subjects' doctors then prescribed a 10-day course of antibiotics (e.g. Neomycin, ciprofloxacin, flagyl, or doxycyline) to eradicate their bacterial overgrowth. Of the 157 initially qualifying subjects, 47 were referred back by their doctors for a follow-up LHBT and were given a second questionnaire without being given the results of their LHBT. Of these 47 subjects, 25 achieved complete eradication, and 22 incomplete eradication of their SIBO. Antibiotic treatment significantly reduced hydrogen production in all 47 subjects, with greater reduction in hydrogen production seen in those subjects whose SIBO was completely eradicated.

iii. *Antibiotics and IBS (Iatrogenic IBS)*

Antibiotics significantly alter gut microflora causing imbalance of the intestinal microflora, for example many antibiotics causes pseudomembranous colitis.

a. *Antibiotics*

A risk factor for irritable bowel syndrome in a population-based cohort Krogsgaard LR1, Engsbro AL2, Bytzer P1, 3.

An internet-based web panel representative of the Danish background population was invited to participate in a survey regarding the epidemiology of IBS in 2010, 2011 and 2013. A questionnaire based on the Rome III criteria for IBS were answered at all three occasions. In 2013, a question regarding use of antibiotics in the past year was included.

e) Results

In 2013, use of antibiotics was reported by 22.4% (624/2781) of the population. A higher proportion of individuals with IBS reported use of antibiotics compared with asymptomatic controls [29.0% (155/534) vs. 17.9% (212/1,184), $p < .01$]. For asymptomatic respondents in 2010 and 2011 ($n = 1004$), the relative risk of IBS in 2013 related with use of antibiotics was 1.9 [95% confidence interval (CI): 1.1-3.1]. Adjusting for sex by logistic regression, development of IBS was predicted by use of antibiotics with an odds ratio of 1.8 (95% CI: 1.0-3.2).

f) Conclusions

Antibiotics is a risk factor for IBS in asymptomatic individuals. Possible mechanisms should be investigated in future studies.

g) Probiotics

The World Health Organization define probiotics as "*live microorganisms, which when taken in adequate amounts, confer a health benefit on the host*", Probiotics can be bacteria, virus, parasites, or yeasts.

Probiotics benefit to the body by various mechanisms:

1. Pathogen suppression
2. Improvement of barrier function
3. Immunomodulation
4. Neurotransmitter production

Strain of *Saccharomyces cerevisiae* CNCM I-3856 secretes saccharolytic enzymes and assists intestinal flora by generating short-chain fatty acids that accelerate bowel movement. It also acts as a visceral analgesic, increasing resistance to pain by up to 40 percent. Additionally, it also acts as an anti-inflammatory to combat intestinal inflammation. To top it all off, the probiotic rebalances microbial composition in the gut as it has been shown to reduce harmful bacteria such as *Enterococcus spp.*, *Escherichia coli* and *Candida albicans*. The result is decreased inflammation, bloating, pain, discomfort, constipation all of which are symptoms of IBS.

II. Materials and Methods

a) Patients

Patients were selected in two investigative sites in Jordan, Jordanian Ministry of Health, and Saudi Arabia, Riyadh National Hospital from 1/09/2010 to 1/07/2015. Patients involved in the study were males and females between 18 and 75 years of age with a diagnosis of IBS according to the Rome III criteria.

A pain/ discomfort score strictly above 1 and strictly below 6, as determined on a pain/discomfort scale using arbitrary grading from 0 to 7.

Patients had normal blood counts, complete blood count, liver function test, renal function, thyroid function, before participating the study.

Subjects were excluded if they had organic intestinal diseases, underwent treatments that influence ibs, or taking any medication or herbals or probiotics.

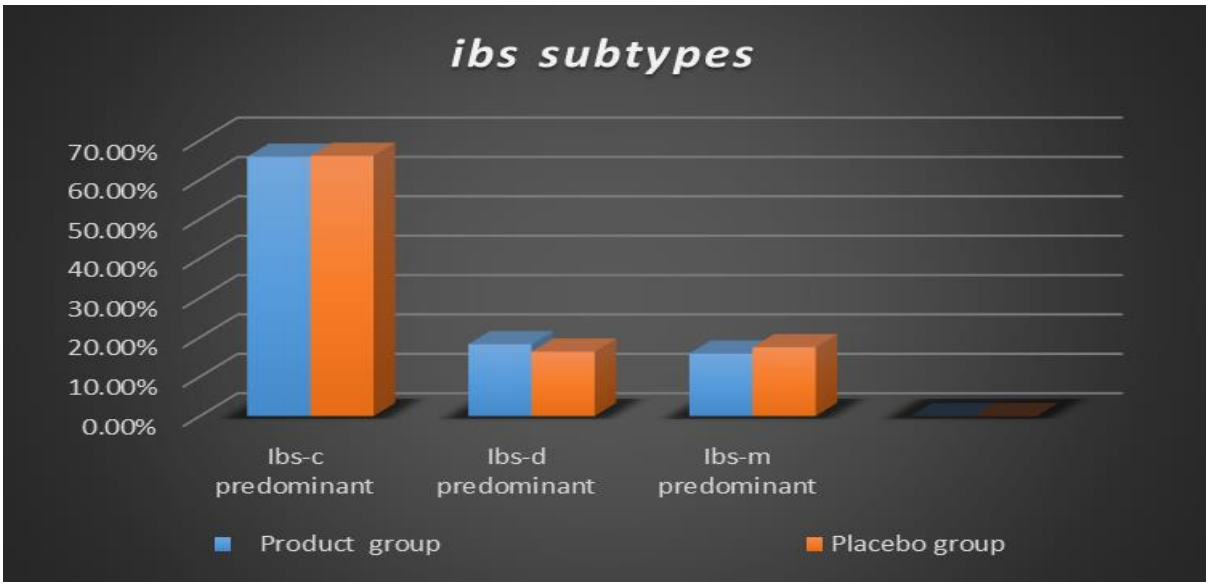

Figure 1

b) Study design

This is 4-week double-blind placebo- controlled clinical study randomizing two parallel group of IbS patients 177 experimental and 170 placebos, During a four week period, scores for abdominal pain/discomfort (defined as a non-comfortable sensation corresponding to a continuum between discomfort and pain), bloating And flatulence, difficulty with defecation, stool frequency, and consistency were recorded.

Dietary recommendations were explained to each patient, After verification of the inclusion/exclusion criteria, eligible IBS patients were randomized to consume daily, for 4 weeks, two tablets of s.cerevisiae CNCM I-3856 (1000 mg) with meal and placebo (calcium gluconate 500 mg). Patients were followed weekly and provided consent before inclusion in the study.

c) Study products and compliance evaluation

The products studied were presented in all tablets of active product and placebo was without flavour, and had the same size, colour. They were to be taken orally, two tablets a day with launch and dinner time with a glass of water. The probiotic preparation specifically 1000mg per tablet of S. cerevisiae CNCM I-3856, and the placebo consisted of calcium gluconate 500 mg.

d) Assessment of symptoms and study endpoints

Ibs symptoms evaluated daily and assessed each week during the 4-week study according to a 7-point Likert scale.

Abdominal pain/discomfort scores were first analyzed, where the score at week 0 (W0) to (w4).

Secondary outcome measures were the weekly scores of bloating/distension and bowel movement difficulty, recorded daily in the same condition using the 7-point Likert scales Changes in stool frequency and consistency were followed daily using the Bristol Stool Scale (ranging from 1, corresponding to separate hard lumps, to 7 for entirely liquid stools).

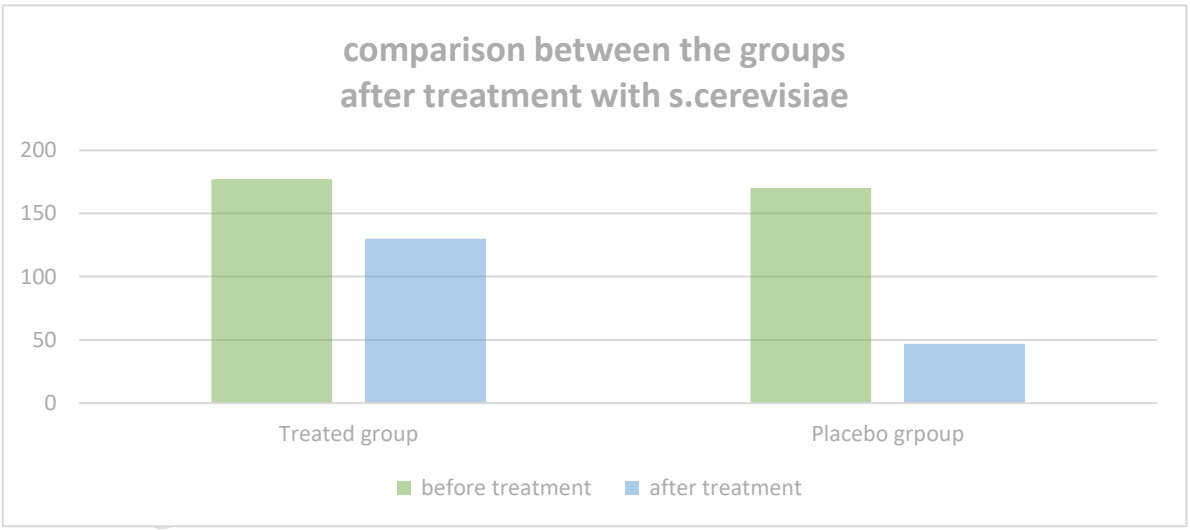

Figure 2

e) Safety variables

Adverse events were recorded by patients and immediately transmitted to the investigator to estimate their severity.

f) Randomization and statistical methods

Randomization and statistical analyses were conducted using *SPSS* software.

Each subject included at the visit (V1) received in a random manner one of the two products (placebo or active).

Block randomization was performed by type of subject (with predominant constipation) (IBS-C), with predominant diarrhea (IBS-D), or mixed symptoms (IBS-M)) with dynamic allocation software using the block permutation technique.

The AUCs (W1-W4) of the abdominal pain/discomfort scores, bloating/distension scores, and bowel movement difficulty scores was calculated and analyzed.

III. Results

a) Primary outcome measures

Abdominal pain/discomfort scores, expressed in AU on a scale from 0 (no symptoms) to 7 (severe symptoms), Intra group analysis revealed a significant reduction of the score in the probiotic groups throughout the 4 weeks of treatment period (W0–4); this led to a mean score reduction of (130 vs 47), (73.4% vs 27.64%) compared with baseline, respectively in the product group ($p < 0.001$) in both treated groups.

IV. Discussion

The present randomized double-blind placebo-controlled study demonstrates, in Jordanian population and Saudi population, that S. cerevisiae CNCM I- 3856 is safe and improves abdominal pain/discomfort. In IBS and other patients fulfilling the Rome III criteria, the 4-week clinical trial was performed according to the recommended designs of treatment trials for functional gastrointestinal disorders in order to demonstrate statistical superiority of a treatment with S. cerevisiae for IBS patients.

Based on these data and expecting a 45.76% therapeutic gain over placebo for the score assessing abdominal pain/discomfort, 347 IBS patients were randomized and treated for 4 weeks with either S. cerevisiae CNCM I-3856 at a daily dose of 2000 mg 1000mg bid, or placebo 500 mg calcium gluconate.

After the first week of the study abdominal pain in the treatment group significantly decreased, score of 1 was 40 percent at the first week, and at the second week was 54 percent, and at the third week was 63 percent, and at the fourth week score of 1 was 70 percent. As a result, (abdominal pain/discomfort, bloating/distension, bowel movement difficulty and changes in stool frequency and consistency) had improved, if we compare treated group and the placebo group (130 vs 47), (73.4% vs 27.64%).

Probiotic administration is considered safe and acceptable strategy in IBS. Most studies evaluating the effects of probiotics in IBS patients have been performed with bacterial strains of lactobacilli and/or bifidobacteria. Despite the numerous advantages offered by yeast compared to bacteria, including antibiotic and phage resistances, as well as higher natural resistance against gastric acid and bile salts, and stronger capacity to regulate the immune response, only two clinical trials assessed the effect of yeast in patients with IBS.

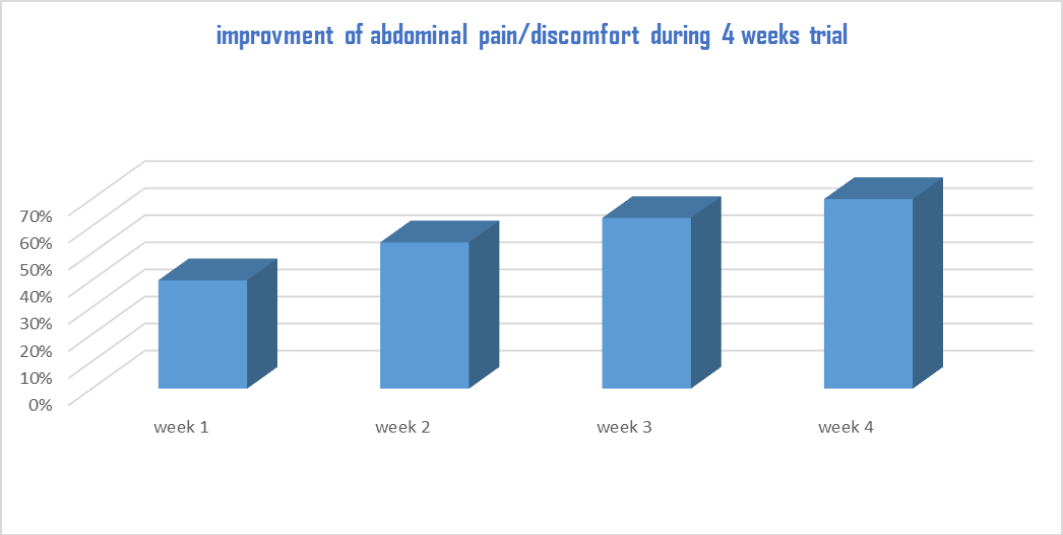

Figure 3

V. Conclusion

In conclusion, S. cerevisiae CNCM I-3856 at 2000 mg/day, conveniently delivered bid by two tablets 1000 mg, is well tolerated and reduces abdominal pain/discomfort scores with altering stool frequency and consistency. Further clinical studies are warranted to confirm that S. cerevisiae could be a new promising candidate to improve abdominal pain/digestive discomfort in subjects with IBS.

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Bohring-Opitz Syndrome: First Latinoamerican Case and Review of the Literature

By Daniela Alejandra Tolosa Quintero, Carolina Rivera Nieto & Juan Sebastián Leguizamón Melo
University of Rosario

Introduction- The Bohring-Opitz syndrome (BOPS) was first described in 1999 by Bohring et al (1). It is an extremely rare genetic condition, of unknown prevalence, which is caused by de novo or nonsense mutations in the ASXL1 gene. To date 46 people with BOPS have been described, of whom only 20 have a confirmed molecular diagnosis. The BOPS diagnosis is established by clinical suspicion and / or identification of a constitutional heterozygous pathogenic variant in the ASXL1 gene (2). This article shows the first case in Latin America of BOPS confirmed by molecular diagnosis.

Keywords: *Bohring Opitz Syndrome, ASXL1, HOX genes, BOPS.*

GJMR-F Classification: *NLMC Code: QU 450*

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Daniela Alejandra Tolosa Quintero [α], Carolina Rivera Nieto [σ] & Juan Sebastián Leguizamón Melo [ρ]

Keywords: Bohring Opitz Syndrome, ASXL1, HOX genes, BOPS

I. Introduction

The Bohring-Opitz syndrome (BOPS) was first described in 1999 by Bohring et al (1). It is an extremely rare genetic condition, of unknown prevalence, which is caused by de novo or nonsense mutations in the ASXL1 gene. To date 46 people with BOPS have been described, of whom only 20 have a confirmed molecular diagnosis. The BOPS diagnosis is established by clinical suspicion and / or identification of a constitutional heterozygous pathogenic variant in the ASXL1 gene (2). This article shows the first case in Latin America of BOPS confirmed by molecular diagnosis.

II. Case Report

We present an 8-year-old male patient, born to term at 38 weeks, presented intrauterine growth restriction with birth weight of 2200 grams (P 0.4, Z -3.1) and a birth lenght of 48 centimeters (P 17, Z 0.95). He was hospitalized for 40 days in the neonatal intensive care unit (NICU) where he received mechanical ventilation for the first six days. During his hospital stay in NICU an echocardiogram was performed and a moderate mitral regurgitation, a moderate pulmonary hypertension and an ostium secundum interatrial communication were identified. No evidence of hemodynamic repercussion was found. At one year of age a ventricular dilatation are evidenced in brain MRI. Developmental delay was documented. Physical exam showed discrete synophris, ocular hypertelorism, myopia, bilateral sensorineural hearing loss, cleft palate, velo-palatal incoordination, cryptorchidism, left thora-columbar scoliosis, pre-axial polydactyly in the hands and hypotonia. Current athropometry was: weight 25 kg (P21, Z -0.8) and height 120 cm (P2, Z -2.1), IMC 17.4 (P75, Z 0.67)

In the first year of life, severe uptake-swallowing disorder managed with gastrostomy is documented; later, in the preschool age he presented multiple hospitalizations due to infectious diseases, pneumonias and recurrent otitis, because of this, inmmunodeficiency was ruled out. He developed symptomatic refractory epilepsy that was treated with carbamazepine and topiramate. Finally, Bohring-Opitz syndrome was suspected [25], a complete sequencing of the ASLX1 gene was performed, identifying the heterozygous variant C.2893C>T (p.Arg965*) that confirmed the diagnosis of BOPS.

III. Discussion

The Bohring-Opitz Syndrome (BOS, OMIM 605039) is caused by de novo or nonsense mutations in the ASXL1 gene (OMIM 612990) (3), that accounts for about 50% of the cases that meet the clinical criteria. The gene locus is located on chromosome 20q11.21, contains 13 exons and codes for the sex-combs-like 1 protein, which is a polypeptide of 1543 amino acids (2, 4). The sex-combs-like 1 protein is involved in the remodeling of chromatin in localized areas and helps to activate and silence the transcription of different genes involved in the regulation of the expression of HOX genes, involved in embryonic development for determination of the basic structure and orientation of the embryo (5), which proposes a mechanism of loss of function, that is, haploinsufficiency, as the fundamental cause of the BOPS clinical picture. (3)

To date, genotype-phenotype correlations have not been reported because of the few number of individuals in whom pathogenic variants in the ASXL1 gene have been identified (2). The described phenotype of the BOPS includes distinctive facial and postural features, delayed neurodevelopment, failure of thrive and other associated clinical conditions of variable presentation. Table 1 summarizes the most representative clinical characteristics.

Author α: Medical Resident of pediatrics, Master of Education. University of Rosario. Bogota Colombia.
e-mail: daleja2012@gmail.com
Author σ: Medical Geneticist. Chief of Medical Genetics. Cardioinfantil Foundation. Bogota Colombia. e-mail: carolinariveran@gmail.com
Author ρ: Resident doctor of pediatrics, University Foundation of Health Sciences. Bogota Colombia. e-mail: juancho1309@gmail.com

Table 1: Summary of BOPS clinical characteristics the adapted from Russell et al.

Skull	Microcephaly Or Trigonocephaly And Prominent Metopic Crest.
Face	*Nevus flammeus* that fades with age, telecanthus, hypoplastic supraorbital crests, ptosis, strabismus, glaucoma, hypertrichosis, anteverted nostrils, cleft lip, cleft palate, arched palate or prominent palatine ridges and micrognatia / retrognathia.
Central Nervous System	Ventriculomegaly, delayed myelination, delayed neurodevelopment, severe or profound intellectual disability, Dandy-Walker malformation and seizures.
Osteomuscular	congenital dislocations.
Gastrointestinal	Functional constipation, chronic emesis and gastroesophageal reflux.
Cardiovascular	Defects of the atrial septum, persistent ductus arteriosus, valvular anomalies and pulmonary stenosis.
Respiratory	Repeated broncho-obstructive episodes and obstructive sleep apnea
Hematological	Acute myeloid leukemia, sideroblastic anemia, Wilms tumor and myelodysplastic syndromes.
Inmunological	Upper respiratory tract repeated infections
Typical Posture	Elbow, wrists and flexed metacarpophalangeal joints, ulnar deviation, of the hands and hypertonic limbs with central hypotonia.

The facial features are distinctive and include pronounced microcephaly in the early years of childhood, trigonocephaly that is generated from the prominence of the metopic ridge and a narrowing at the bitemporal level (6), hypotonic facies, "nevus flammeus", telecanto, hypoplastic supraorbital crests, upward slanting palpebral fissures, depressed nasal bridge, anterverted nostrils, hairy and arched eyebrows, posteriorly rotated ears, hypertrichosis, micrognathia and narrow and high palate (see figure 1) (7). Facial manifestations tend to become less noticeable with age. (8)

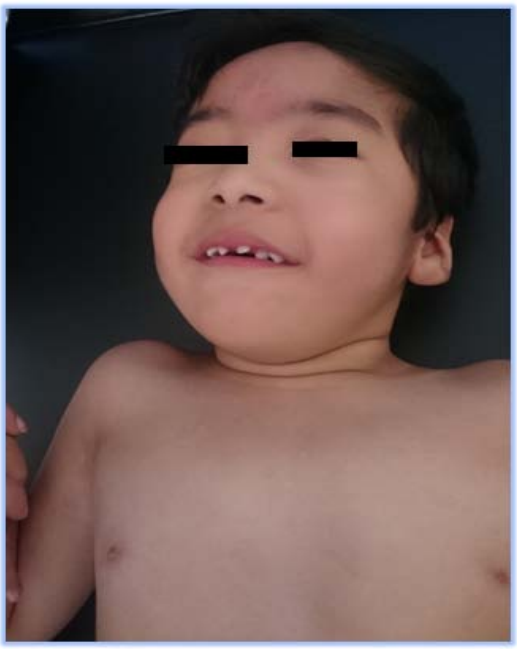

Figure 1: Facial lesion type "Nevus flammeus", telecanthus, thick arched eyebrows, hypoplastic supraorbital ridges, depressed nasal bridge, anteverted nostrils and hypertelorism are observed

At the ocular level, ptosis, strabismus that does not resolve, and glaucoma due to pressure increase at the level of the anterior chamber of the eye are evident. (7) Similarly, alterations of the retina and the optic nerve, which include colobomas, optic nerve atrophy, atrophy of the retina and / or abnormal pigmentation that explain visual impairment (2, 9) are common. Patients diagnosed with BOPS who have a mutation identified in the ASXL1 gene have a higher incidence of myopia (87% versus 40%) and hypertrichosis (89% versus 17%), compared to those without mutations. (5)

A typical BOPS posture that is commonly identified in early childhood and becomes less apparent with age has been described: it presents with shoulders directed towards the midline, extremities flexed distally (elbows, wrists and fingers) forming a fixed contracture position (see figure 2) (6). Despite the typical posture, no studies have been found that relate it to CNS alterations, muscle tone or joint dislocations (1).

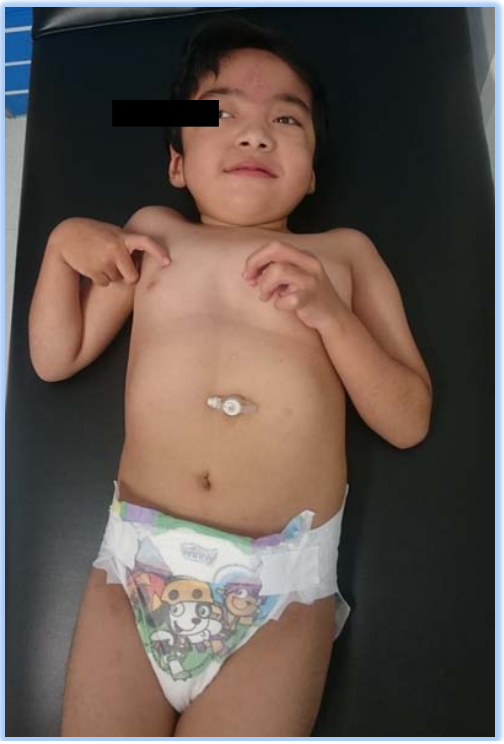

Figure 2: Typical posture, elbows, wrists and metacarpophalangeal joints in fixed forward contracture. At the abdominal level, a gastrostomy button is evident.

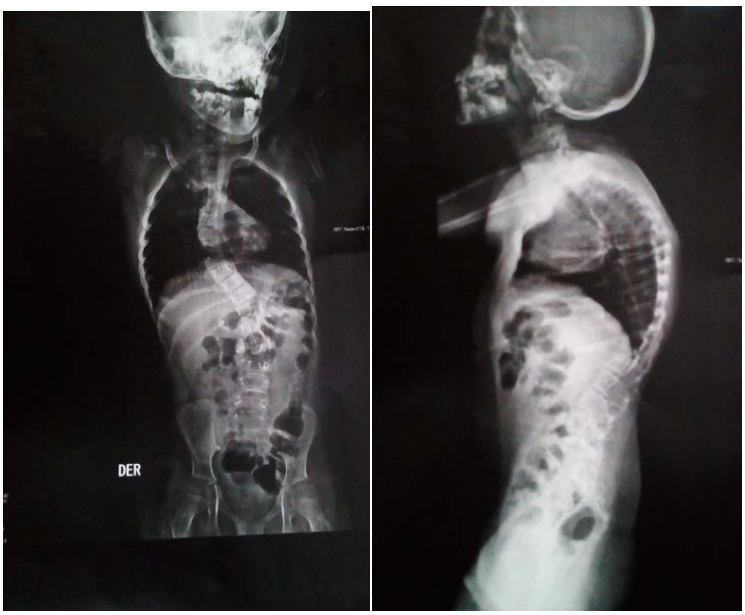

Figure 3: Ortho-radiography at 7 years of age, showing a scoliotic left lumbar curve with a scoliosis angle of 46 ° and a right dorsal compensatory curvature with a scoliosis angle of 34 °. On the lateral side there is an increase in the dorsal kyphosis at an angle of 63 °.

In addition, alterations in muscle tone ranging from flaccidity to hypotonia of the upper and lower extremities leading to alterations in the curvatures of the spine are described (Figure 3) (6). Similarly, congenital contractures, dislocations and pectus excavatum are observed (2); the congenital dislocation of the hip and the radial head are reported in up to 33% of the cases, the first was evidenced in the present patient (Figure 4).

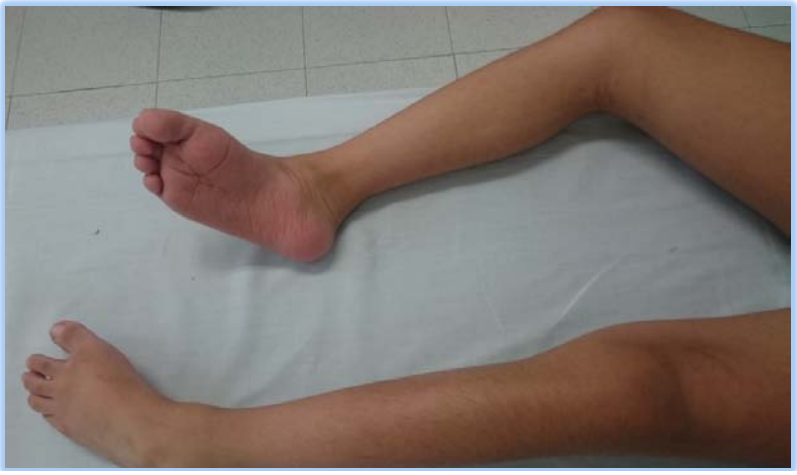

Figure 4: Congenital dislocation of ankles that hinders independent sitting and bipedestation.

In the central nervous system, ventriculomegaly, delayed myelination, Dandy-Walker malformation and generalized atrophy with abnormalities in neuronal migration that favors the development of seizures have been identified (4). These findings may explain the variable intellectual disability, the deficit of language and the difficulties for independent bipedestation. Very few achieve an independent walking.(6) It is described that children with BOPS can recognize caregivers and have a social and interactive nature, so they are seen with a happy and pleasant attitude.

Patients with BOPS have swallowing difficulties since early childhood, functional constipation, gastroesophageal reflux (GER), cyclical vomiting and poor suction; which are related to poor weight gain and are thought to be have a neurogenic origin (10). Gastrointestinal disorders are observed from the first months of life and tend to improve with age. Nevertheless, 53% of the patients require parenteral nutrition or gastrostomy as a definitive form of nutrition (Figure 2), and have a risk of aspiration and dehydration.

Half of patients may have minor cardiac anomalies, transient bradycardia and apnea (2). Similarly, septal and cardiac hypertrophy, persistent ductus arteriosus and valvular anomalies, pulmonary stenosis being the most representative (6). Other findings at the pulmonary level are recurrent broncho-obstructive symptoms and obstructive sleep apnea syndrome (8); the latter improves with the use of CPAP or mandibular traction. People affected with micrognathia may also present airway obstruction based on glossoptosis of the tongue (10).

Patients with BOPS have an inadequate innate and adaptive immune response and are more predisposed to upper respiratory tract infections, pneumonia and acute otitis media. (1) The literature also reports cases of recurrent urinary infections, urinary retention and increased risk of kidney stones (2).

Somatic mutations related to the ASXL1 gene have been associated with acute myeloid leukemia and appear to affect approximately 7% of people with myelodysplastic syndrome; they also present in sideroblastic anemia and Wilms tumor (2, 10). A systematic review demonstrated the relationship between a somatic mutation of the ASXL1 gene and the risk of developing hematological cancers, including chronic myelomonocytic leukemia (up to 43% of cases), myelodysplastic syndrome (20%), myelo- proliferative neoplasms (10%) and acute myeloid leukemia (20%) (11).

If a diagnosis of BOPS is suspected, a sequencing of exons 12 and 13 of the ASXL1 gene, must be performed. (5). In the absence of a mutation identified in these exons of the ASXL1 gene, a somatic mosaicism or genetic heterogeneity is possible (other causal genes not identified to date) (2, 12).

The BOPS prognosis is poor, with a high incidence of childhood mortality (2). Respiratory infections and recurrent wheezing episodes are common and represent 42% of deaths in the first two years of life (10). Death due to cardiovascular causes is associated with bradycardia and apnea, which represent four (33%) of the 12 deaths published in the literature (although none of these individuals had a molecular confirmation of BOPS) (2).

The treatment is symptomatic and focuses on clinical manifestations, cyclic vomiting can be controlled by identifying and avoiding the triggering factors (2). An earlyan enteral route such a gastrostomy has been proposed to reduce bronchoaspiration, improve nutrition and avoid unnecessary hospitalizations (10). Tracheostomy is recommended for patients with recurrent bronchoaspiration who develop secondary lung disease and those with severe sleep apnea who do not improve with non-invasive treatment (CPAP, BiPAP) or with surgery (for example, mandibular distraction) (2).

If a sleep disturbance is present, polysomnography is indicated in order to identify early obstructive apnea (10). The referral to a craniofacial team should be considered for children with palatal anomalies, with micrognathia or with obstructive sleep apnea. Similarly, renal ultrasonography should be performed every three months from birth to eight years to detect the development of a Wilms tumor (2).

Given the rarity of BOPS and the diversity of the phenotype, the clinical suspicion and diagnosis is a real challenge. The awareness of BOPS is needed to describe its natural history and develop tools to establish an early diagnosis and clinical management that could improve the quality of life of these patients.

Ethical Responsibilities

The authors declare that they have followed the protocols of their work center on the publication of patient data and the patient family has given informed consent to it.

Conflict of Interests

The authors declare no conflict of interests.

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Factors Associated with Utilization of Insecticide Treated Nets among Residents of Kamwenge Town Council-Kamwenge District-Uganda

By Ikiriza Antony, Maureen Andinda, Prof. Rubaihayo John & Prof. A R Semana
Mountains of the Moon University

Abstract- *Background:* Malaria continues to be a leading cause of morbidity and mortality in sub-Saharan Africa. In Uganda, malaria remains the leading cause of morbidity, accounting for 30 to 50 percent of outpatient visits, 15 to 20 percent of admissions, and 9 to 14 percent of inpatient deaths. The first national-wide Insecticide Treated Nets (ITNs) coverage campaign was launched in 2010 targeted at households with pregnant women and children below 5yrs of age. The study assessed factors associated with utilization of Insecticide Treated Nets (ITNs) among residents of Kamwenge Town Council, Kamwenge District-Western Uganda.

Methodology: The study design was cross-sectional in which data were collected using an interviewer administered structured questionnaire and analyzed using Stata version 13.

Keywords: *residents Insecticide treated nets (ITNs), utilization.*

GJMR-F Classification: *NLMC Code: QW 160*

Strictly as per the compliance and regulations of:

Factors Associated with Utilization of Insecticide Treated Nets among Residents of Kamwenge Town Council-Kamwenge District-Uganda

Ikiriza Antony [α], Maureen Andinda [σ], Prof. Rubaihayo John [ρ] & Prof. A R Semana [ω]

Abstract- Background: Malaria continues to be a leading cause of morbidity and mortality in sub-Saharan Africa. In Uganda, malaria remains the leading cause of morbidity, accounting for 30 to 50 percent of outpatient visits, 15 to 20 percent of admissions, and 9 to 14 percent of inpatient deaths. The first national-wide Insecticide Treated Nets (ITNs) coverage campaign was launched in 2010 targeted at households with pregnant women and children below 5yrs of age. The study assessed factors associated with utilization of Insecticide Treated Nets (ITNs) among residents of Kamwenge Town Council, Kamwenge District-Western Uganda.

Methodology: The study design was cross-sectional in which data were collected using an interviewer administered structured questionnaire and analyzed using Stata version 13.

Results: A total of 285 respondents were interviewed of which 39.7% were male. Ownership of ITNs was 60% whereas utilization in the night prior to the study was 54.4%. The factors associated with the ownership of the ITNs included: marital status: married respondents *(p=0.05),* level of education *(p=0.001)*, knowledge of malaria cause *(p=0.033),* presence of children under 5 years *(p=0.025).* The factors associated with the utilization of ITNs included: marital status of the respondents: married respondents *(p=0.018)*, education level *(p=0.009)*, presence of children under five years *(p=0.048),* knowledge of cause of malaria *(p=0.019)*, having faced challenges in using ITNs *(p=0.001),* and malaria episode in the last one month *(p=0.011).*

Conclusion: The study concludes that the ownership and utilization of ITNs were low since they were all below the national target of universal coverage. The factors statistically associated with the ownership of the ITNs included: marital status, level of education, knowledge of malaria cause and the number of children under 5 years in a household. The factors statistically associated with the utilization of ITNs were marital status, education level of the respondents, and presence of children under five years of age in the household, having knowledge about malaria, having faced challenges in using ITNs in the past and malaria episode in the last one month.

Keywords: residents Insecticide treated nets (ITNs), utilization.

Author α σ: Assistant Lecturer, Department of Public Health Mountains of the Moon University. e-mail: iantony2011@gmail.com
Author ρ: Professor, Department of Public Health Mountains of the Moon University.
Author ω: Professor, School of Agriculture and Environmental Science, Mountains of the Moon University.

I. Background

Malaria is the leading cause of morbidity and mortality in the tropical religion accounting for 3.2 billion deaths with its peak of 1.2 million in 2013 and Malaria mortality has been steadily decreasing since 1990 but 90% of the deaths occurred in Africa with Uganda having the third highest number of malaria cases after Democratic Republic of Congo and Nigeria [1], [2], [4].

The World Health Organization's Global Malaria Programme recommends use of Insecticide-Treated Nets (ITNs) as one of the three major means of malaria vector control to reduce malaria transmission but in 2013, only 49% of the people at risk in Sub Saharan Africa had access to ITNs yet ITNs efficacy against malaria episodes of approximately 50% [2], [3], [4], [5].

Uganda adopted a policy of universal LLIN coverage of one net per two persons to protect all people from malaria and conducted a community mass campaigns and through public health facilities that target pregnant women and children under 5 years on a routine basis and several community mass distribution of nets since 2010 [6].

However the utilization of ITNs in Kamwenge Town Council is still not known and the study aimed at determining the factors that influence utilization of ITNs among residents of Kamwenge Town Council.

II. Methodology

a) Study Design and setting

This was a household based survey that was conducted in Kamwenge Town Council that the main town of the district and a centre for most of the activities like Administrative services, business and other social amnesties located in Western Uganda a distance of 400km from the capital city Kampala by road.

b) Sample Size estimation, sampling procedure and data collection

The sample 299 was determined using a standard formula by Kish Leslie (8) assuming a standard error of 5% and 77.1% of households with at least one insecticide- treated net in Tooro region and 10% non-response was included [8], [9]. Kamwenge Town council was selected purposively because it in the

center of the district and has a mix of people from different backgrounds. A household list for each ward was obtained with the aid of the ward agents and they were used to randomly assign households to different research assistants during the data collection process. Household heads were interviewed and incase they were absent, the oldest household member 18 years and above was selected for the interview using a structured questionnaire that contained both close and open ended questions.

c) Ethical Considerations

Ethical approval was obtained from the Mountains of the Moon University, Directorate of Postgraduate Studies & Research seeking permission to conduct the study which was presented to the officials of the Kamwenge Town and written informed consent was obtained from the study participants.

d) Data Analysis

Data was analysed using STATA version 13 for data analysis, Chi square test was used to identify the factors that influence the utilization of ITNs. To identify the factors that are associated with utilization of ITNs, logistic regression analysis was used in which bivariate logistic regression was first used to identify the factors that could be associated with utilization of ITNs at a p-value <0.05 and the factors that were turned out to be associated with utilization of ITNs in the bivariate analysis were included into a multivariate logistic regression model to cater for the effect of confounders and effect modifiers and identify the independent predictors of utilization of ITNs.

III. Results

a) Demographic Characteristics

A total of 285 respondents were surveyed in July 2017. Majority of the respondents were aged 30-49 years 151(53%) with a mean age of 39.7 years, females 172 (60.4%) and married 221(77.8%) more demographic characteristics shown in table 1.

Table 1: Demographic Characteristics of the respondents (n=285)

Variable	Category	Frequency, n (%)
Age in years	10-29	72 (25.3)
	30-49	151 (53)
	50-69	56 (19.7)
	70 and above	6 (2)
Sex	Female	172 (60.4)
	Male	113 (39.6)
Marital status	Single	19(6.7)
	Married	222(77.8)
	Divorced	15(5.3)
	Widow	29(10.2)
Level of Education	No Education	136 (47.7)
	Primary	96 (33.7)
	Secondary	37 (13)
	Tertiary	16 (5.6)
Occupation	Business	45 (15.8)
	Civil Servant	16 (5.6)
	Farmer	210(73.7)
	Housewife	14 (4.9)
Religion	Bornagain	28(9.8)
	Catholic	150(52.6)
	Islam	12(4.2)
	Protestant	95(33.3)
No children under 5years	0	126(44.2)
	1	77(27.0)
	2	59(20.7)
	3	17(6.0)
	4	6(2.1)
the household	1-5	186 (62.3)
	6-10	90 (31.6)
	11-16	8 (2.8)
	17-22	1(0.35)
Ownership of ITNs	No	114(40)
	Yes	171(60)

Utilization of ITNs by adults	No	130(45.6)
	Yes	155(54.4)
Utilization of ITNs by children <5years	No	54(34)
	Yes	105(66)
Number ITNs observed per household	1	74(43.3)
	2	66(38.6)
	3	22(12.9)
	4	7(4.1)
	6	1(0.6)
	7	1(0.6)
Type of the ITN owned	Polyester nets	42(24.6)
	Nylon nets	129(75.4)

Source: Field data, 2017

b) TN Ownership by Demographic Characteristics

The study found out a significant difference in the ownership of ITNs a cross the different marital statuses *(X^2 =12.50, p=0.006)*, levels of education whereby those with secondary and above level of education were more likely to own the nets *(X^2 =33.55, p=0.001)* as well as occupation of the respondents, number of children <5years in the household and number of people in household shown in table 2 below:

Table 2: ITN Ownership by demographic Characteristics

Variable	ITN ownership		X^2	P-value
	No n (%)	Yes n (%)		
Age				
10-29	30(41.7)	42(58.3)	3.17	0.366
30-49	55(36.4)	96(63.6)		
50-69	25(44.6)	31(55.4)		
70 and above	4(66.7)	2(33.3)		
Sex				
Female	67(38.9)	105(61.1)	0.20	0.656
Male	47(41.6)	66(58.4)		
Marital status				
Single	10(52.6)	9(47.4)	12.50	0.006*
Married	82(36.9)	140(63.1)		
Divorced	12(80.0)	3(20.0)		
Widow	10(34.5)	19(65.5)		
Education level				
No Education	68(50.8)	66(49.2)	33.55	0.001*
Primary	43(43.9)	55(56.1)		
Secondary	1(2.7)	36(97.3)		
Tertiary	2(12.5)	14(87.5)		
Occupation				
Business	14(31.1)	31(68.9)	17.51	0.001*
Civil Servant	0(0.0)	16(100.0)		
Farmer	97(46.2)	113(53.8)		
Housewife	3(21.4)	11(78.6)		
Religion				
Bornagain	15(53.6)	13(46.4)	4.10	0.252
Catholic	62(41.3)	88(58.7)		
Islam	3(25.0)	9(75.0)		
Protestant	34(35.8)	61(64.2)		
Number of children <5 years				
0	62(49.2)	64(50.8)	10.90	0.028*
1	25(32.5)	52(67.5)		
2	21(35.6)	38(64.4)		
3	6(35.3)	11(64.7)		
4	0(0.0)	6(100.0)		

Number of people in household				
1-5	71(38.2)	115(61.8)	8.53	0.036*
6-10	43(47.8)	47(52.2)		
11-16	0(0.0)	8(100.0)		
17-22	0(0.0)	1(100.0)		

Source: Field data, 2017

* *Significantly associated at p<0.05*

c) *ITN utilization by demographic Characteristics*

There was a significant difference in ITN utilization across the different marital statuses of the respondents, with married respondents utilizing nets more than any other *(X^2 =11.49, p=0.009),* education level where by those educated to secondary level and beyond utilizing the nets than those who are not *(X^2 =35.25, p=0.001)* and other characteristics as shown in table 3 below:

Table 3: ITN utilization by demographic characteristics

Variable	ITN utilization		X^2	P-value
	No n (%)	Yes n (%)		
Age				
10-29	32(44.4)	40(55.6)	1.13	0.769
30-49	69(45.7)	82(54.3)		
50-69	25(44.6)	31(55.4)		
70 and above	4(66.7)	2(33.3)		
Sex				
Female	79(45.9)	93(54.1)	0.018	0.895
Male	51(45.1)	62(54.9)		
Marital status				
Single	10(52.6)	9(47.4)	11.49	0.009*
Married	95(42.8)	127(57.2)		
Divorced	13(86.7)	2(13.3)		
Widow	12(41.4)	17(58.6)		
Education level				
No Education	79(59.0)	55(41.0)	35.25	0.001*
Primary	45(45.9)	53(54.1)		
Secondary	3(8.1)	34(91.9)		
Tertiary	3(18.8)	13(81.2)		
Occupation				
Business	17(37.8)	28(62.2)	17.78	0.001*
Civil Servant	1(6.2)	15(93.8)		
Farmer	109(51.9)	101(48.1)		
Housewife	3(21.4)	11(78.6)		
Religion				
Bornagain	17(60.7)	11(39.3)	5.60	0.133
Catholic	71(47.3)	79(52.7)		
Islam	3(25.0)	9(75.0)		
Protestant	39(41.0)	56(59.0)		
Number of children <5 years				
0	68(54.0)	58(46.0)	10.34	0.035*
1	30(39.0)	47(61.0)		
2	25(42.4)	34(57.6)		
3	7(41.2)	10(58.8)		
4	0(0.0)	6(100.0)		
Number of people in household				
1-5	82(44.1)	104(55.9)	6.13	0.105
6-10	47(52.2)	43(47.8)		
11-16	1(12.5)	7(87.5)		
17-22	0(0.0)	1(100.0)		

Source: Field data, 2017

* *Significantly associated at p<0.05*

d) *Factors associated with utilization of ITNs among residents of Kamwenge Town Council*

The factors that were identified to have a statistically significant relationship with the utilization of ITNs in the bivariate analysis at a p-value <0.05 included: marital status, level of education, occupation, religion, knowledge of malaria cause, number of children under 5 years in a household, malaria experience in the last one month and challenge in using ITNs. These were included in a multivariate logistic regression model to identify the predictors of utilization of ITNs as shown in table 5 below:

Table 4: Factors associated with utilization of ITNs among residents of Kamwenge Town council

	Bivariate analysis		Multivariate analysis	
Variable	cOR (95% CI)	P-value	aOR (95% CI)	P-value
Age				
18-29	1			
30-49	0.95 (0.54-1.67)	0.861		
50-69	0.99(0.49-2.00)	0.982		
70 and Above	0 .4(0.07-2.32)	0.308		
Sex				
Female	1			
Male	1.03(0.64-1.66)	0.895		
Marital status				
Divorced	1		1	
Married	8.70(1.92-39.42)	0.005*	8.4(1.44-49.40)	0.018*
Single	5.85(1.03-33.33)	0.047*	4(0.40-31.26)	0.257
Widow	9.21(1.75-48.53)	0.009*	12(1.70-84.05)	0.013*
Education level				
No education	1		1	
Primary	1.69(1.00-2.86)	0.050*	1.1(0.58-2.23)	0.713
Secondary	16.28(4.76- 55.67)	0.001*	6.3(1.57-25.65)	0.009*
Tertiary	6.22(1.69-22.88)	0.006*	2.0(0.30-13.64)	0.486
Occupation				
Business	1		1	
Civil servant	9.11(1.10- 75.27)	0.040*	3.7(0.27-50.20)	0.328
Farmer	0.56(0.29-0.09)	0.088	0.5(0.16-1.24)	0.121
Housewife	2.23(0.54-9.13)	0.267	2.5(0.43-14.71)	0.308
Religion				
Bornagain	1		1	
Catholic	1.72(0.75-3.92)	0.197	0.9(0.29-3.10)	0.926
Islam	4.64(1.02-21.00)	0.047*	1.3(0.18-9.23)	0.791
Protestant	2.22(1.00-5.25)	0.070	1.1(0.32-3.62)	0.907
Number of children < 5years				
0	1		1	
1	1.84(1.03-3.27)	0.039*	2.1(1.01-4.47)	0.048*
2	1.59(0.85-2.98)	0.143	3.0(1.34-6.64)	0.007*
3	1.67(0.60-4.68)	0.325	3.3(0.90-12.14)	0.071
Knowledge of malaria cause				
Knowledgeable	1		1	
Not Knowledgeable	1.53(0.33-0.85)	0.009*	0.5(0.26-0.90)	0.019*
Knowledge of use of ITNs				
Knowledgeable	1			
Not knowledgeable	0.44(0.15-1.26)	0.125		
Quality of the nets				
Intact	1			
Torn	1.29(0.46-3.62)	0.628		
Challenge in using ITNs				
Had challenge	1		1	
Had no challenge	4.27(2.50-7.27)	0.001*	6.1(3.12-12.03)	0.001*
Malaria experience in the last one month				
No	1		1	
Yes	0.42(0.25-0.69)	0.001*	0.4(0.20-0.81)	0.011*

Source: Field data, 2017,

* Significantly associated at p<0.05,
cOR-Crude odds ratio,
aOR-Adjusted odds ratio,
CI-Confidence interval.

IV. Discussions

More than a half of the respondents 54.4% had slept under ITNs the night prior to the study. This could be because some people do not have the bed nets and others prefer not to use them. This agrees with Uganda Malaria Indicator Survey [6] findings that reported that 69% of the de facto household population slept under an ITN the night before the survey.

Of the 159 households that had a child below 5 years, 54(34%) reported that no child had slept under an ITN [10] also found out that despite the fact that ownership has been stable, available evidence shows that utilization of ITNs among children under five years of age has been low which is a gap between ownership and utilization is large.

Marital was found to statistically association with the utilization of the ITNs whereby married respondents slept under ITNs 8 times more than the divorced *(aOR=8.4, 95 CI: 1.44-49.40, p=0.018),* widows also slept under ITNs 12 times more than the divorced *(aOR=12, 95% CI: 1.70-84.05, p=0.013)* and the single respondents also slept under the ITNs 4 times more than the divorced ones. Given the fact they are in stable relationships, sleeping patterns could also be quite stable hence a high level of using ITNs than the divorced whose relationships are not stable.

The study found a statistically significant association between education level of the respondents and the use of ITNs whereby respondents who had attained secondary level of education slept under ITNs 6 times more than the un educated ones *(aOR=6, 95% CI: 1.57-25.65, p=0.009)* and the ones who had attained a tertiary level of education also slept under ITNs 2 times more than the un educated ones. This implies that such respondents are informed about the use of the ITNs and end up using the more than the uneducated who may not know. This finding is in line with findings of the study by Binka and others [11] who found out that demographic characteristics like age, education, size of household and ethnicity also influence use of bed nets.

Households in which there were children under five years of age used ITNs more than those in which there were no children and the association between the numbers of children in the household was statistically associated with the use of ITNs with households with one child using ITNs 2 times more than those in which there are no children and household with two children 3 times more than those without any children. This could be due to the fact that the Government of Uganda promotes use of mosquito nets by pregnant women and children under five years by providing mosquito nets to such women when they were pregnant which increases the ownership as well as use. This could also be due to the fact that the parents want to protect their children from malaria. This is in line with the findings by Biadgilign et al, [12] on determinants of ownership and utilization of insecticide treated nets for malaria control in Eastern Ethiopia that showed that households which had at least one under- five child the odds of owning any net was about 60% higher than those with no under-five children.

Having knowledge about malaria cause was statistically associated with the utilization of ITNs. The knowledgeable respondents slept under ITNs 0.5 times more than those who were not knowledgeable *(aOR=0.5; 95% CI: 0.26-0.90, p=0.019)*. This implies that knowing how to prevent increases possibility for such people to sleep under ITNs and this is in agreement with findings of studies by Berhane, and Worku, 2012 [13] that documented that the key factors which explain the use of ITNs for malaria prevention relate to perceptions, attitude and retention level of ITNs and that use of ITNs was found to be low among people with little knowledge on malaria prevention methods.

Having faced challenges in using ITNs in the past was also statistically associated with the use of ITNs where by the respondents who had no challenges slept under ITNs 6 times more than those that had challenges. This is due to the fact that such challenges like heat and allergies inconvenience the people's sleep and discourage use of the ITNs. This is in line with the findings of the study by [14] that found that the main reasons for not using LLINs were inconveniences due to heat, no LLIN and LLIN is washed or torn, [16] also reported similar findings such as forgetfulness, unavailability of nets, net washed, fatigue, illness, and heat.

Respondents who had suffered from malaria in the last one month slept under ITNs 0.4 times less than the ones who had not suffered from malaria and the relationship between malaria experience and ITNs use was statistically significant *(aOR= 0.4, 95%CI: 0.20-0.81, p=0.011).* This implies that suffering from malaria does not influence the use of ITNs but Onwujekwe *et al, in 2013* reported a different findings that perceived risk of malaria and benefits of the nets by the population also drive demand and that households with a recent attack of malaria were more likely to purchase net than their counterparts and that such communities have a perceived need for utilizing ITNs [15].

V. Conclusions

Utilization of ITNs was found to be 54.4% and almost a half of the respondents surveyed did not sleep under the net a night prior to the study. The factors that were found to be significantly associated with the use of ITNs are marital, education level of the respondents, presence of children under five years of age in the household, having knowledge about malaria, having faced challenges in using ITNs in the past and malaria experience in the last one month. To maximize the

benefits of use of mosquito nets, strategies to deal with unwanted or damaged ITNs and other bed nets should be put in place since people are still using torn and old ITNs.

List of abbreviations

ITNs	Insecticide Treated Nets
LLINs	Long Lasting Insecticide Nets
MOH	Ministry of Health
NMCP	National Malaria Control Programme
UMIS	Uganda Malaria Indicator Survey
WHO	World Health Organization

Ethics and Consent to participate

Ethical approval was sought from the Directorate of Graduate studies and research Mountains of the Moon University Research and Ethics Committee and all participants consented in writing at the time of interview in their homes.

Competing interests

Authors declare that there is no conflict of interest in this study.

Authors' contributions

All authors conceived and designed the study; I A, RJ and AM collected, analysed, interpreted the data and drafted the manuscript; ARS critically revised the manuscript. All authors read and approved the final manuscript.

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Vascular Aging Factors in Individuals with Different Cardiovascular Risk

By G. D. Fadeienko, O. V. Kolesnikova, O. V. Vysotska & A. O. Radchenko
L. T. Malaya Therapy Academy of Medical Sciences of Ukraine

Abstract- *Objective:* Study of vascular aging markers in conjunction with metabolic parameters and the degree of cardiovascular risk (CVR).

Materials and methods: The study included 298 patients aged 40 to 69 years old. Parameters of lipid metabolism, immune inflammation, endothelial dysfunction, telomere length, insulin resistance, biological age (BA), and CVR were determined. Differences were deemed statistically significant at $p < 0.05$.

Results: Significant changes occur in lipid and carbohydrate metabolism parameters, as well as markers of immune and endothelial inflammation, the degree of intensity of which depends on the CVR degree. During this process, significant changes occur in the length of telomeres, which have a relation with hyperinsulinemia. Telomeres are shorter in more than 50% of patients suffering from even moderate CVR.

Keywords: *vascular aging, calendar age, biological age, cardiovascular risk, lipid profile, carbohydrate profile, immune inflammation, endothelial dysfunction, telomere length, insulin resistance.*

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Conclusions: Patients with moderate CVR develop vascular aging processes. Knowledge of vascular aging parameters in people with high CVR dictates the need for early medical intervention.

Keywords: vascular aging, calendar age, biological age, cardiovascular risk, lipid profile, carbohydrate profile, immune inflammation, endothelial dysfunction, telomere length, insulin resistance.

I. Introduction

Every year, cardiovascular diseases (CVD) are becoming more prevalent among people of both working age and old age, which leads to a significant increase in the cost of treatment of these diseases and complications, and also generally reduces the quality of life of the population. In some high-income ESC member countries, the decline in mortality from CVD has led to cancer becoming the more common cause of death, but in the middle- and low-income countries, CVDs remain the predominant cause of death (European Society of Cardiology: cardiovascular disease statistics 2017) [1]. Vascular aging has a major impact not only on the morbidity and mortality rates in people of the older age group as a whole but is also a primary risk factor for CVD. At the same time, an increase in blood pressure itself contributes to the accelerated aging of blood vessels, which predisposes to complications from the target organs through a variety of mechanisms [2]. The combination of epigenetic and genetic factors, as well as activation of the renin-angiotensin-aldosterone system, inflammation, oxidative stress, lifestyle, leads to structural and functional changes that are characterized by endothelial dysfunction, thickening, excessive fibrosis of the arterial wall, reduced elongation and arterial stiffness.

According to research data, activation of proinflammatory cytokines in the arterial wall increases with age. Also, the presence of arterial hypertension increases the synthesis of fibronectin, collagen and plasminogen-1 activator inhibitor (PAI-1), with a decrease in collagenase production, stimulation of tissue inhibitors of metalloproteinases (TIMPs), which affects the process of vascular fibrosis [3].

The leading factor in age-related diseases is oxidative stress, which aggravates vascular inflammation, supported by cardiovascular risk factors, including obesity, type 2 diabetes mellitus, metabolic disorders, etc. Increased oxidative DNA damage and increased expression of multiple biomarkers of double-stranded DNA breaks are present in atherosclerotic plaques. A violation of the mechanisms responsible for maintaining the appropriate length and functionality of telomeres plays a role in the aging of vessels and arterial hypertension, causing cellular aging [4]. Critically short telomeres can lead to cellular aging and apoptosis, which contribute to the development of atherosclerosis and predispose people to plaque instability. But telomere length is a consequence of the action of not only genetic but also environmental factors, which requires studying them in complex with other CVD risk factors and aging [5, 6, 7]. According to other studies, the measurement of telomeres length and telomerase activity reflects their useful rather than harmful effect, and, thus, can serve as a surrogate marker of the vascular system [8].

Rankinen, Tuomo, et al. provide evidence of genomic sequence variants and positional genes that have a pleiotropic effect on CVD risk factors, especially the lipid profile [9].

Eaton et al. have established that the concentration of vascular endothelial growth factor A (VEGF-A) had a moderate relationship with C-reactive protein (CRP), age, lipid profile parameters, systolic blood pressure, BMI, and physical activity. In the course of a large number of studies, an association exists between VEGF and glycemic profile in both healthy

Author α σ: State Institution "L. T. Malaya Therapy National Institute of the National Academy of Medical Sciences of Ukraine", 2-a, Avenue L. Malaya, Kharkiv, Ukraine, 61039. e-mail: kolesnikova1973@gmail.com
Author ρ: National Aerospace University H. E. Zhukovsky "Kharkiv Aviation Institute", 17, Chkalov street, Kharkiv, Ukraine, 61070.
Author ω: Kharkiv National Medical University, 4, Nauky Avenue, Kharkiv, Ukraine, 61022.

individuals and patients with diabetes mellitus [10]. Hautero O. et al. show that the level of circulating VEGF is significantly higher, the severer ischemia manifestations are [11].

There is an assumption that biological age (BA), in contrast to the calendar age (CA), can serve as an indicator of vascular aging. Establishment of BA by anthropometric parameters according to the method by A. H. Horelkin and B. B. Pinkhasov is convenient to use since it does not require any specialized laboratory equipment [12].

When developing new and effective therapeutic strategies for improvement and prevention of "vascular aging" processes in cardiovascular disorders, it is essential to understand the cellular and functional changes that occur in the bloodstream during aging. The proposed factors that take into account the formation of cardiovascular risk (CVR) do not always allow classifying patients into risk groups of CVD development, which is why the search for cardiometabolic predictors that could influence the more reliable identification of patients at risk continues. In this connection, the objective of our study was an investigation of vascular aging markers in conjunction with metabolic parameters and CVR degree.

II. Materials and Methods

This study included 298 patients. The age of the subjects was 40-69 years, median 44.9 years. Medical documentation (outpatient and inpatient patient histories) was analyzed to assess the presence of risk factors and calculate the total CVR on the SCORE scale.This became the basis for the distribution of patients into CVR groups: low and moderate (0-4% on the SCORE scale), high (5-9% on the SCORE scale) and very high (≧10% on the SCORE scale) without clinical manifestations of CVD. According to the study protocol, patients were divided into groups according to the level of total cardiovascular risk according to SCORE: group I included patients (n = 101) with low CVR - 33.9%; group (II) included patients (n = 125) with moderate risk - 41.9%; group (III) included patients (n = 72) with high/very high risk - 24.2%. Table 1 shows the distribution of patients according to the calculation of the total CVR on the SCORE scale.

Table 1: Distribution of patients according to the calculation of the total CVR on the SCORE scale

Age, years	CVR on the SCORE scale					
	Low/moderate, n=101		High, n=125		Very high, n=72	
	Abs.	%	Abs.	%	Abs.	%
40-49	38	38.4±3.5%	44	35.2±4.3%	14	19.8±1.7%
50-59	51	49.5±5.0%	38	30.2±4.1%	17	23.5±5.0%
60-69	12	12.1±3.2%	43	34.6±4.2%	41	56.7±5.8%

a) The inclusion criteria for the study were as follows

1) Age of men and women - 40 to 69 years;
2) The presence of one or more of the following risk factors: essential hypertension of 1-2 degree, smoking, dyslipidemia, dysglycaemia, overweight or obesity;
3) The presence of signed informed patient consent to participate in the study.

b) The non-inclusion criteria for the study were

1) The presence of heart disease (clinically pronounced coronary artery disease, history of MI, coronary revascularization, chronic cardiac failure of blood circulation above functional class 2 according to NYHA), cerebral circulation disorders, atherosclerotic lesion of peripheral arteries;
2) Decompensated liver and kidney diseases with impaired function;
3) Oncological diseases;
4) Rheumatic diseases;
5) Allergic and autoimmune diseases;
6) Diabetes mellitus;
7) Pregnancy;
8) Use of lipid-lowering drugs;
9) Use of medicinal products affecting the state of the hemostas is system and blood rheology within 6 months prior to the inclusion in the study;
10) Essential hypertension of the third degree according to the criteria recommended by the European Society of Hypertension (ESH, 2016) [13].

Study protocol and materials pertinent to the study were reviewed and approved by the ethics committee of the Sociological Association of Ukraine and after obtaining informed verbal consent from the patients according to the Helsinki Declaration II.

Determination of lipid and carbohydrate metabolism parameters was carried out according to the generally accepted procedure, CRP concentration was measured using a test system (Best Diagnostics, Ukraine).Tumor necrosis factor-α (TNF-α) was measured using a test system (Vector-Best JSC, Russia), serum insulin was measured using a test system (DRG Instruments GmbH, Germany) under fasting conditions via enzyme-linked immunoassay (ELISA) on a semi-automatic micro plate analyzer Immuno Chem - 2100 (High Technology, Inc., USA). Biochemical marker of endothelial dysfunction, VEGF-A, was tested by enzyme-linked immunoassay on photometer-analyzer Huma Reader using a set of reagents from IBL International GmbH, Germany.

DNA for measurement of the relative length of telomeres was isolated from buccal epithelium and peripheral blood leukocytes using DNA-sorb-AM and DNA-sorb-B reagents (Amplisense, Russia), respectively. A fluorometric method was applied to measure DNA concentration in the samples using via Qubit 3.0 fluorometer (Life Technologies, USA) and the Qubit dsDNA HS Assay Kits (Life Technologies, USA). DNA samples were diluted at a concentration of 2-4 ng/μl and stored until amplification at -20 ° C.

PCR with real-time detection of fluorescence was used to measure the relative length of telomeres according to the protocol described by Cawthon R. M., 2002 [14].The following primers produced by Invitrogen (Thermo Fisher Scientific) were used to amplify telomeric sequences:

– Tel1 GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT;
– Tel2 TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA.

The following primers produced by Invitrogen (Thermo Fisher Scientific) served for amplification of the reference single-copy gene 36B4 (ribosomal phosphor protein):

– 36B4u CAGCAAGTGGGAAGGTGTAATCC;
– 36B4d CCCATTCTATCATCAACGGGTACAA.

For each of the primer systems, we prepared two reaction mixtures per the required number of samples using the iQ SYBR Green Supermix master mix (BioRad Laboratories, USA). We made the reaction mixture immediately before use. 11μl of the reaction mixture and four μl of DNA were added to strip PCR tubes. Series of dilutions of the reference DNA sample (dilution range from 0.28 to 7.5 ng/μl) were performed separately for telomeric sequences and a single-copy gene to plot the calibration curves for estimation of the average telomere length. We examined each sample in 3 technical replicates.

Amplifications were performed using the CFX96 Touch detection system (BioRad Laboratories, USA) according to separate protocols for the target and reference gene. PCR protocol for telomeric sequences: DNA pre-denaturation - 95 ° C, 5 minutes, followed by 35 cycles at 95 ° C, 20 s., 54 ° C, 2 minutes; for single-copy gene 36B: DNA pre-denaturation - 95 ° C, 5 min., and then 35 cycles at 95 ° C, 20 s., 58 ° C, 1 min.

The obtained results were processed using CFX96 Touch Software V.3 (BioRad Laboratories, USA) to generate telomeric signal curves (T) or a single-copy reference gene signal (S), evaluate the amplification reaction efficacy and determine Ct (the number of cycles required to achieve thethreshold level of fluorescence). To estimate the relative length of telomeres (T/S), the difference of threshold cycles for telomeric (Ct_{tel}) and reference (Ct_{ref}) sequences was calculated using the formula $\Delta Ct_x = Ct_{tel} - Ct_{ref}$. Besides, we calculated the average ΔCt_k for all reference and blank samples. We carried out normalization of the T/S value for each of the analyzed samples relative to the average ΔCt_k value using the formula:

$$\frac{T}{S} = \left[2^{-(Ct_x - \Delta Ct_k)}\right] = [\,2^{-\Delta\Delta Ct}].$$

Biological age was determined according to the procedure by A. H. Horelkin and B. B. Pinkhasov [12]. First, we calculated the aging rate factor, and then the biological age was calculated based on it. The formula for estimating the aging rate factor (ARF):

$$ARF_m = \frac{WC \times BW}{HC \times H^2 \times (17{,}2 + 0{,}31 \times AD_m + 0{,}001 \times AD_m^2)};$$

$$ARF_f = \frac{WC \times BW}{HC \times H^2 \times (14{,}7 + 0{,}26 \times AD_f + 0{,}001 \times AD_f^2)};$$

where ARF_m and ARF_f are aging rate factors for men and women, respectively, WC – waist circumference, BW – body weight, HC– hip circumference, H– body height, AD_m and AD_f– the difference between the calendar age and the age according to ontogenetic standard for men and women, respectively.

The ontogenetic standard is the age by which development and formation of the structure and functions of all systems of the human body are completed in the process of ontogenesis (individual human development). This age is 21 years old for men and 18 years old for women.

When ARF is 0.95 inclusive to 1.05 inclusive, the rate of aging is deemed compliant with the standard; when ARF is less than 0.95, the aging is delayed, when ARF is more than 1.05, the aging is accelerated.
Formulas for determining biological age:

$$BA_m = ARF_m \times (chronologicalage - 21) + 21;$$

$$BA_f = ARF_f \times (chronologicalage - 18) + 18;$$

where BA_m and BA_f is biological age for males and females, respectively.

All statistical analyses were performed using SPSS software (statistical package for social science), version 19.0. Analysis of the parameters studied by the normality of distribution was carried out using the Shapiro-Wilk test. We presented quantitative variables in the form of M ± m (M is the average value, m is its standard error), and described qualitative characters as the frequency of events (% of the normal number of observations). We used Student's t-test to determine the differences between dependent and independent samples. The rate of characters in the groups was compared using the χ^2 test. We carried out a correlation analysis using the Pearson test (r) and the Chad dock scale to determine the presence and nature of the relations between various manifestations and

pathogenetic factors of different processes. Analysis of variance was used to establish the role of individual factors, and a logistic regression method was used to determine the likelihood of development of a cardiovascular event. Differences were deemed to be statistically significant at $p < 0.05$.

III. Results and Discussion

Comparison of the calendar and biological age showed that BA of the subjects was lower than the calendar age (CA) by 3.02 ± 0.01 years in the low CVR group, by 1.14 ± 0.02 years in the moderate CVR group, and was higher by 2.23 ± 0.01 years in the high/very high CVR group, which is evidence of the increasing rate of aging with increasing CVR.

When analyzing the comparative characteristics of parameters in the group of patients with low and moderate CVR, statistically significant differences were found in carbohydrate metabolism: glucose level 4.20 ± 0.01 mmol/l vs 6.23 ± 0.17 mmol/l ($p = 0.049$), insulin 14.23 ± 0.65 mU/l vs 16.42 ± 1.16 mU/l ($p = 0.018$), immune inflammation parameters: CRP 6.71 ± 1.02 mg/l vs 9.46 ± 0.41 mg/l ($p = 0.026$), TNF-α 6.90 ± 0.36 pg/ml vs 8.9 ± 0.47 pg/ml ($p = 0.048$) (Table 2).

Table 2: Comparative characteristics of metabolic parameters in the group of patients with low and moderate CVR

Parameter	Low CVR group	Moderate CVR group	p-criterion
Lipid metabolism parameters			
Total cholesterol,mmol/l	5.68±0.10	5.86±0.13	0.931
Triglycerides,mmol/l	2.10±0.04	2.25±0.08	0.834
LDL cholesterol,mmol/l	3.19±0.013	3.62±0.17	0.854
VLDLcholesterol,mmol/l	0.76±0.02	0.87±0.01	0.784
HDL cholesterol,mmol/l	1.03±0.035	0.92±0.04	0.831
Carbohydrate metabolism parameters			
Glucose,mmol/l	4.20±0.013	6.23±0.17	0.049
Insulin,mU/l	14.23±0.65	16.42±1.16	0.018
Immune inflammation parameters			
CRP,mg/l	6.71±1.02	9.46±0.41	0.026
TNF-α,pg/ml	6.90±0.36	8.9±0.47	0.048
Endothelial dysfunction parameter			
VEGF-A1,pg/ml	319.94±66.47	422.82±10.01	0.461
Telomere length			
Blood	1.14±0.08	0.94±0.03	0.326
Buccal epithelium	1.30±0.02	1.21±0.05	0.235

Significant differences between moderate and high/very high CVR groups in lipid metabolism parameters are noted: total cholesterol (TC) is 5.86 ± 0.13 mmol/l vs 7.24 ± 0.22 mmol/l ($p = 0.000$), triglycerides (TG) 2.25 ± 0.08 mmol/l vs 2.75 ± 0.11 mmol/l ($p = 0.000$), low-density lipoprotein cholesterol (LDL cholesterol) 3.62 ± 0.17 mmol/l vs 4.31 ± 0.27 mmol/l ($p = 0.040$) and very low density cholesterol (VLDL cholesterol) 0.87 ± 0.01 mmol/l vs 1.03 ± 0.05 mmol/l ($p = 0.008$); carbohydrate metabolism: blood glucose 6.25 ± 0.17 mmol/l vs 7.09 ± 0.27 mmol/l ($p = 0.012$), insulin 16.42 ± 1.16 mU/l vs 23.59 ± 2.62 mU/l ($p = 0.018$); immune inflammation: CRP 9.46 ± 0.41 mg/l vs 11.43 ± 0.59 mg/l ($p = 0.027$), TNF-α 8.90 ± 0.37 pg/ml vs 11.96 ± 0.95 pg/ml ($p = 0.001$) and endothelial dysfunction: VEGF-A1 422.82 ± 10.01 pg/ml vs 646.44 ± 58.11 pg/ml ($p = 0.001$) (Table 3). Significant differences depending on CVR degree were found in telomere length among the groups of patients with moderate and high/very high CVR: 0.94 ± 0.03 vs 0.76 ± 0.05 ($p = 0.027$) in blood cells; 1.21 ± 0.05 vs 0.83 ± 0.07 ($p = 0.045$) in buccal epithelium (Table 3).

Table 3: Comparative characteristics of metabolic parameters in the group of patients with moderate and high/very high CVR

Parameter	Moderate CVR group	High/very high CVR group	p-value
Lipid metabolism parameters			
Total cholesterol, mmol/l	5.86±0.13	7.24±0.22	0.000
Triglycerides, mmol/l	2.25±0.08	2.75±0.11	0.000
LDL cholesterol, mmol/l	3.62±0.17	4.31±0.27	0.040
VLDLcholesterol, mmol/l	0.87±0.01	1.03±0.05	0.008
HDL cholesterol, mmol/l	0.92±0.04	0.90±0.05	0.764
Carbohydrate metabolism parameters			
Glucose, mmol/l	6.25±0.17	7.09±0.27	0.012
Insulin, mU/l	16.42±1.16	23.59±2.62	0.018
Immune inflammation parameters			
CRP, mg/l	9.46±0.41	11.43±0.59	0.027
TNF-α, pg/ml	8.90±0.37	11.96±0.95	0.001
Endothelial dysfunction parameter			
VEGF-A1, pg/ml	422.82±10.01	646.44±58.11	0.001
Telomere length			
Blood	0.94±0.03	0.76±0.05	0.027
Buccal epithelium	1.21±0.05	0.83±0.07	0.045

During the study, we found a strong inverse correlation between HDL cholesterol and the length of telomeres in blood in the low CVR group (r = -0.90; p = 0.014), which indicates the effect of this parameter on the rate of biological aging. This correlation corresponds to the data by Mazidi, Mohsen, et al., where the mean HDL cholesterol concentrations increased significantly with increasing telomere length (p = 0.013), and the level of C-reactive protein significantly decreased with increasing telomere length (p <001) [15]. Strong inverse correlation in the low CWR group was observed between HDL cholesterol and insulin (r = -0.87; p = 0.024), HDL cholesterol and TG (r = -0.95; p = 0.004) (Table 4). This is reflected in the studies by Sneha, S. et al., where HOMA-IR was higher among individuals with low HDL level (compared to normal HDL level), and the positive correlation of HOMA-IR and TG/HDL suggested that the TG/HDL ratio can be used as a marker of insulin resistance, as was also confirmed by Young, Kendra A., et al. [16, 17].

Table 4: The presence of a relationship between the vascular aging markers and metabolic parameters in the group of low CVR patients

	TG	p-value
Insulin	0.84	0.035
	HDL cholesterol	
CRP	-0.97	0.002
Telomere length (blood)	-0.90	0.014
Telomere length(buccal epithelium)	-0.79	0.065
TG	-0.95	0.004
Insulin	-0.87	0.024

The correlation analysis showed a strong inverse correlation between HDL cholesterol and CRP in group I (r = -0.97; p = 0.002) and a moderate inverse correlation in group II (r = -0.33; p = 0.029) (Tables 4, 5). Zangana S.N. reported similar results, where CRP concentration positively correlated with cholesterol, TG and LDL levels, but inversely correlated with HDL level, and CRP level showed increase in individuals with arterial hypertension versus the healthy population [18].

Table 5: The presence of a relationship between the vascular aging markers and metabolic parameters in the group of moderate CVR patients

	Insulin	p-value
Telomere length (buccal epithelium)	-0.42	0.005
	Total cholesterol	
Telomere length (buccal epithelium)	-0.29	0.059
	HDL cholesterol	
CRP	-0.33	0.029
TG	-0.28	0.074

A reliable moderate direct correlation was found in group III between CRP and total cholesterol (r = 0.49; p = 0.022), VLDL cholesterol (r = 0.43; p = 0.048) (Table 6). Rathore, Vedika, et al. also found significant changes in the lipid profile levels and inflammatory markers in patients with acute myocardial infarction; they have established a strong positive correlation between CRP and total cholesterol, TG, LDL cholesterol and VLDL cholesterol, and significant negative correlation with HDL cholesterol, which can be a confirmation of preceding development of immune inflammation and lipid profile disorders [19]. The data of studies by McGarrah R. W., et al., also emphasize the interrelation between systemic inflammation and HDL cholesterol with clinical outcomes, consideration of which allows to improve the accuracy of clinical risk assessment [20]. VEGF-A1 as an indicator of immune inflammation is a factor associated with a subsequent increase in the CVR degree, as evidenced by a significant relationship between VEGF-A1 and VLDL cholesterol (r = 0.59; p = 0.004), as well as VEGF-A1 and shortening of telomere lengths (buccal epithelium) (r = 0.43; p = 0.044) in the high/very high CVR group (Table 6).

Table 6: The presence of a relationship between the vascular aging markers and metabolic parameters in the group of high/very high CVR patients

	Total cholesterol	p-value
CRP	0.49	0.022
VLDL cholesterol	0.47	0.028
	VLDL cholesterol	
CRP	0.51	0.015
VEGF-A1	0.59	0.004
CRP	*Telomere length (buccal epithelium)*	
CRP	0.51	0.016
VEGF-A1	0.43	0.044

Considering that 68% (n = 203) of the patients included in the study were immune resistant, we evaluated the telomere length depending on the serum insulin concentration. In patients with hyperinsulinemia> 30 mU/l, their length in the blood was 0.82 ± 0.13 vs 0.95 ± 0.03 at insulin levels <30 mU/l (p = 0.016). Similar changes occurred in the buccal epithelium: 0.80 ± 0.03 vs 1.10 ± 0.04 (p = 0.004) (Table 7).

Table 7: Telomere length depending on the hyperinsulinemia level

Parameter	Insulin<30 mU/l, n=119	Insulin> 30 mU/l, n=179	p-value
Telomere length (blood)	0.95±0.03	0.82±0.13	p=0.016
Telomere length (buccal epithelium)	1.10±0.04	0.80±0.03	p=0.004

We determined the lower and upper margins of the confidence interval (CI) for interval estimates of the median. The sequence numbers of the sample values, which represented the lower (L) and the upper (U) margins, were determined using the formulas:

$$L = \frac{n}{2} - \left(z_{1-\alpha} \times \frac{\sqrt{n}}{2}\right),$$

$$U = 1 + \frac{n}{2} + \left(z_{1-\alpha} \times \frac{\sqrt{n}}{2}\right),$$

where n is the sample size, $z_{1-\alpha}$ is the value of the normal distribution for the selected confidence probability.

After calculating the sequence numbers of the lower and the upper CI margins, we determined their value in the sample. We used the L-th value of the formed variational series as the lower CI margin, and the U-th value as the upper CI margin.

Since the control group included 20 subjects, L = 6 is obtained for a confidence probability 95% z (1 - α) = 1.96. That is why the 95% CI for the parameter "telomere length of blood cells" was [1.38; 2.09]. By the obtained CI, an analysis of the frequency of occurrence of the normal and shortened telomeres of blood cells depending on CVR was carried out (χ^2 = 3.076, p = 0.215) (Table 8).

Table 8: The frequency of occurrence of normal and shortened telomeres of blood cells depending on CVR

		Length of blood telomeres		In total
		Shortened	Normal	
CVR	Low	17 (13.2±3.0%)	5(3.9±1.7%)	22 (17.1±3.3%)
	Moderate	62 (48.4±4.4%)	12 (9.3±2.6%)	74 (57.8±4.4%)
	High/very high	22 (17.1±3.3%)	10 (7.8±3.7%)	32 (25.0±3.8%)
In total		101(78,9±3,6%)	27 (21.0±3.6%)	100.0 %
χ^2=3.076, p= 0.215				

95% CI for the parameter "telomere length of buccal epithelium cells" was [1.45; 2.18]. Analysis of the frequency of occurrence of normal and shortened telomeres of the buccal epithelium cells depending on the CVR was carried out (χ^2 = 0.547, p = 0.761) (Table 9). According to the results of frequency analysis, we have revealed that the vast majority of the study patients who had shortened telomeres were the patients with moderate CVR (48.4 ± 4.4% in blood and 50.0 ± 4.4% in buccal epithelium) (Table 8, 9). Probably, already in the presence of moderate CVR in this patient category, timely diagnosis of the onset of vascular aging is necessary to prevent the development of CVR of higher degrees.

Table 9: The frequency of occurrence of normal and shortened telomeres of buccal epithelium cells depending on CVR

		Length of buccal epithelium telomeres		In total
		Shortened	Normal	
CVR	Low	20 (15.6±3.2%)	2 (1.5±1.1%)	22(17.1±3.3%)
	Moderate	64 (50.0±4.4%)	10 (7.8±2.4%)	74 (57.8±4.4%)
	High/very high	29 (22.6±3.7%)	3 (2.3±1.3%)	32(25.0±3.8%)
In total		113 (88,2±2.8%)	15 (11.7±2.8%)	100.0%
χ^2=0.547, p= 0.761				

Due to the increase in CRP and insulin levels and the degree of CVR, according to our study, patients experience a significant shortening of telomere length. Shortening can be associated with the destruction of the structure of telomere T-loop, which leads to cellular aging, increased oxidative stress and inflammation in the tissues (Morgan, R. G. et al.) [21].

Considering the results obtained, it can be assumed that the quality of control of the lipid spectrum and carbohydrate spectrum decreases in the high/very high CVR group, which leads to acceleration of immune inflammation and increase in the rate of vascular aging, which in turn leads to an increase in the number of cardiovascular complications, increased vascular aging rate.

IV. Conclusion

1. Patients with cardiovascular risk (CVR) of high degrees compared with low and moderate CVR show a more pronounced impairment in the lipid and carbohydrate profile. This can be the cause of acceleration of vascular aging processes and require more stringent control of the lipid profile and glucose parameters to improve secondary prevention.
2. The relationship between CRP and shortening of telomere length in the buccal epithelium in the high/very high CVR group, as well as between CRP and lipid profile parameters in all CVR groups indicates the development of premature aging processes. For timely secondary prevention, it is advisable to measure CRP and TNF-α in individuals with high CVR degrees.
3. For reduction of the activity of vascular aging and primary prevention of cardiovascular diseases (CVDs), it is essential to consider markers of systemic inflammation (CRP, TNF-α) and to ensure good glycemic control not only via screening of fasting glucose but also using HOMA index as a more reliable indicator.
4. To identify groups of patients at increased risk of complications and accelerated biological aging, it is advisable to determine the biological age of individuals with high/very high CVR at a stage even preceding laboratory examinations.
5. Patients from a risk group in the presence of even moderate CVR show a significant decrease in telomere length, which can serve as an essential factor that indicates the onset of premature vascular aging in this patient category and requires early preventive interventions.

Abbreviations

ARF	aging rate factor
BA	biological age
CA	calendar age
CRP	C-reactive protein
CVD	cardiovascular disease
CVR	cardiovascular risk
ELISA	enzyme-linked immunoassay
HDL cholesterol	high-density lipoprotein cholesterol
LDL cholesterol	low-density lipoprotein cholesterol
PAI-1	plasminogen-1 activator inhibitor
TC	total cholesterol
TG	triglycerides
TIMPs	tissue inhibitors of metalloproteinases
TNF-α	tumor necrosis factor-α
VEGF-A	vascular endothelial growth factor A
VLDL cholesterol	very low density cholesterol

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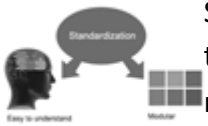

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Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

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Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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