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Demographic and Biological Determinants of Hypertension: Insights about ACE I/D Polymorphism in a population from northeastern Brazil

By Samuel dos Santos Oliveira, Mariane de Oliveira Barreto, Pedro Barros Cerqueira, Poliana Souza Santos Campos, Dariana Viegas Andrade, Márcio Galvão Oliveira, Mauro Fernandes Teles, Sandra Mara Bispo Sousa & Patrícia Santos Pereira Lima

UESB - Universidade Estadual do Sudoeste da Bahia

Abstract- Primary Hypertension is a multifactorial condition and a significant public health concern due to its link with cardiovascular disease. Factors such as obesity, smoking, and genetics contribute to its development. This study examined demographic and biological parameters in hypertensive and normotensive individuals, revealing that ethnicity and family history strongly influence Hypertension risk, particularly among Black participants and those with affected relatives. Hypertensive patients showed higher BMI and waist circumference, with age also contributing to onset. Other parameters, including hip circumference and lipid profiles, were similar between groups. Positive correlations were found among weight-related measures and between total and LDL cholesterol, while HDL cholesterol showed weak negative correlations.

Keywords: genetic polymorphism, cardiovascular disease, angiotensin converting enzyme, blood pressure & hypertension.

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Demographic and Biological Determinants of Hypertension: Insights about ACE I/D Polymorphism in a population from northeastern Brazil

Samuel dos Santos Oliveira [α], Mariane de Oliveira Barreto [σ], Pedro Barros Cerqueira [ρ], Poliana Souza Santos Campos [ω], Dariana Viegas Andrade [¥], Márcio Galvão Oliveira [§], Mauro Fernandes Teles [χ], Sandra Mara Bispo Sousa [ν] & Patrícia Santos Pereira Lima [Θ]

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Keywords: genetic polymorphism, cardiovascular disease, angiotensin converting enzyme, blood pressure & hypertension.

I. Introduction

Primary Hypertension is a highly heterogeneous disease of multifactorial etiology characterized by persistent elevation of blood pressure (BP) (1–3). Hypertension is defined as a systolic blood pressure (SBP) equal to or exceeding 140 mmHg or a diastolic blood pressure (DBP) equal to or exceeding 90 mmHg (4,5) This condition is a significant risk factor for the development of cardiovascular complications, which are the leading causes of death worldwide, surpassing cancer and infectious diseases, thereby constituting a significant public health issue (6). In Brazil, Hypertension affects 32.5% (36 million) of adult individuals, with over 60% of the elderly population, contributing directly or indirectly to 50% of deaths from cardiovascular disease (CVD) in the country (7).

A range of risk factors has been associated with the development of Hypertension (8), including obesity, smoking, imbalance in the renin-angiotensin-aldosterone system (RAAS), mental stress, ethnicity, among others. The genetic influence on the development of the disease is a consensus within the scientific community; however, its polygenic nature complicates the clear determination of the contribution of each genetic variant in individual patients, rendering each case unique (9,10).

A genome-wide association study (GWAS) comprising over 1,000,000 rigorously phenotyped individuals with measured blood pressure elucidated 901 loci related to blood pressure regulation. Notably, it identified genes encoding proteins of the renin-angiotensin-aldosterone system (RAAS), proteins involved in vascular remodelling, and proteins associated with immune response regulation, such as TGF-β and SMAD. This expands the diversity of therapeutic targets for the treatment of Hypertension(11).

The aberrant action of the renin-angiotensin-aldosterone system (RAAS) is central to the pathogenesis of Hypertension, as it promotes sodium retention, vasoconstriction, endothelial dysfunction, and vascular injury. Renin is released by the kidneys under low-pressure conditions; it cleaves angiotensinogen into angiotensin I, which serves as a substrate for angiotensin-converting enzyme (ACE), converting angiotensin I into its physiologically active form, angiotensin II. This component accelerates sodium reabsorption in the proximal tubule and exhibits pro-fibrotic and pro-inflammatory actions, mechanisms through which angiotensin II is pathological in Hypertension(3,12).

A polymorphism resulting from the insertion/deletion of 287 base pairs in the ACE gene, initially described as hypertensin I (13), has been widely studied in the context of Hypertension (14). The allele frequencies (AF) of the I and D alleles vary significantly

Author α: Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brasil; Departamento de Ciências Naturais, Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista, Brasil.
e-mail: oliveirass@usp.br
Author σ ρ ω ν Θ: Departamento de Ciências Naturais, Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista, Brasil.
Author ¥: Departamento de Ciências da Saúde, Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista, Brasil.
Author §: Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Vitória da Conquista, Brasil.
Author χ: Departamento de Saúde, Universidade Estadual do Sudoeste da Bahia, Jequié, Brasil.

among populations (15–21). In Brazil, different AF are observed in distinct populations across the country, underscoring the importance of investigating various populations, particularly considering the continental dimensions and the significant ethnic and cultural diversity, as well as the profound social inequality (15,16).

Regarding the physiological relevance of this polymorphism, the D allele appears to be associated with increased expression of ACE in the kidneys (22) and elevated serum levels of angiotensin II (23,24), a pathological component in Hypertension. Furthermore, pharmacological inhibition of ACE is a widely used therapeutic intervention for managing Hypertension (25,26). The convergence of these factors supports the notion that the D allele is a risk factor for Hypertension and a range of cardiovascular complications, highlighting the importance of investigating the allele frequencies of I/D and their association with Hypertension and related pathologies (24), which is the objective driving our research.

II. Methodology

a) Sample Collection

Blood samples were collected at the Municipal Laboratory of Vitória da Conquista (LACEMp), interior of Bahia, Brazil. Patients who attended for blood sampling to undergo laboratory tests were invited to participate in the research, having been initially informed about the study. After clarification and signing the Informed Consent Form (ICF) and self-reported Hypertension (or not), the patients completed a questionnaire, at the time of blood sampling for lab tests, an additional 8 ml blood tube containing EDTA was collected. The tubes were stored and maintained refrigerated for a minimum of 24 hours, after which plasma and cellular fractions were separated in the Genetics Laboratory of UESB. A total of 183 samples were collected, including 95 from hypertensive individuals and 88 from normotensive individuals.

b) DNA Extraction Genomic

DNA was extracted using the QIAGEN DNA extraction kit. Subsequently, the quality of the DNA was assessed by agarose gel electrophoresis at 2%.

i. *Polymerase Chain Reaction Agarose Gel Electrophoresis*

The I/D polymorphism of the ACE gene was identified using the Polymerase Chain Reaction (PCR) technique. To amplify the polymorphic region located in intron 16 of the ACE gene, primers with the following sequences were used: 5′-CTGGAGACCACTCCCATC CTTTCT-3′, 5′-TCGAGACCATCCCGGCTAAAAC-3′, and 5′-GATGTGGGCATCACATTCGTCA-3′(27) Amplifications were conducted in a total volume of 25 μl under the following conditions: 2.5 mM of 10x reaction buffer (Invitrogen), 1.5 mM MgCl2 (Invitrogen), 1.25 mM dNTPs (Invitrogen), 2.5 μM of each primer (Invitrogen), 1 U of Taq polymerase (Invitrogen), 2 μl of genomic DNA, and ultra-pure water. The PCR was initiated with 10 minutes of denaturation at 94°C, followed by 35 cycles as follows: 94°C for 1 minute, 67°C (primer annealing temperature) for 1 minute, and 72°C for 1 minute. The reaction concluded with an extension at 72°C for 5 minutes. The PCR product (fragments of 479 and 277 bp for the I allele and 199 bp for the D allele) was checked on a 3% agarose gel stained with ethidium bromide and visualised under ultraviolet light.

c) Statistical Analyses

Allelic and genotypic frequencies of the ACE I/D polymorphism were estimated by direct counting. Genetic and genotypic differentiation tests were performed using the Genepop software. All other analyses described below were conducted using JASP software (version 0.19.1). The chi-square test was used to assess whether the groups were in Hardy-Weinberg equilibrium (HWE) and to evaluate associations between Hypertension and sociodemographic variables (sex, education level, self-reported race/ethnicity, smoking, alcohol consumption, self-reported family history ofHypertension, self-rated health status, and self-reported kidney disease and/or diabetes), as well as with the ACE I/D polymorphism. For association analyses between Hypertension and anthropometric variables (BMI, waist circumference, and waist-to-height ratio), the t-test was applied. For parameters such as waist-to-hip ratio, age, triglyceride levels, blood glucose, cholesterol, and HDL, the Mann-Whitney U test was used. A p-value < 0.05 was considered statistically significant for all analyses.

III. Resultados

The sociodemographic data of the studied population are presented in *Table 1*. We found an association between hypertention andlower education level, no-whiteethnicity, self-reported family history Hypertension, a poor self-assessment of health and self-reported kidney disease and/or diabetes.

Table 1: Sociodemographic Characteristics of the Study Population

Characteristics	N	HT	NT	X^2	p
Gender (F/M)	183	68/27	56/32	1,319	0,251
Education (1/2/3)	182	66/21/07	37/42/09	15,234	**<0,001**
Smoking (N/Y)	183	65/30	57/31	0,272	0,601
Alcohol consumption (N/Y)	183	62/33	54/34	0,299	0,584
self-declared ethnicity (W/N-W)	182	15/79	30/58	8,030	**0,005**
Reports Family History of Hypertension (+ / -)	183	88/07	69/19	7,581	**0,006**
self-reported kidney disease and/or diabetes (+ / -)	183	73/22	82/06	9,411	**0,002**
Self-assessment of health (4/5)	183	27/68	51/37	16,293	**<0,001**

HT = hipertensive, NT = normotensive, F = female, M = male, 1 = up to elementar school, 2 = until high school, 3 = higher education, N = smokes or has smoked / no drink, Y = smokes / drink, W = white, N-W = no-white, + = yes, - = no, 4 = good to very good, 5 = regular to very bad.

Biological parameters were also evaluated. The analyses of the anthropometric data showed that, on average, hypertensive individuals exhibited higher BMI, WHtR, and WC values (among women) compared to normotensive individuals (Table 2). It was also observed that age, blood glucose levels, and WHR (among men) tended to be higher in the hypertensive group (Fig. 1).

Table 2: Analysis of Anthropometric Parameters of Study Groups

	Mean ± SD	t	*p*	*CI (95%)*
BMI				
Hipertensive (n=95)	28,751 ± 4,656	3,679	**< 0,001**	1,103 – 3,657
Normotensive (n=88)	26,371 ± 4,045			
WHtR				
Hipertensive (n=95)	0,598 ± 0,080	3,683	**< 0,001**	0,019 – 0,062
Normotensive (n=88)	0,558 ± 0,066			
WC - Women				
Hipertensive (n=68)	97,125 ± 10,212	3,229	**0,002**	2,695 – 11,233
Normotensive (n=56)	90,161 ± 13,208			
WC - Men				
Hipertensive (n=27)	98,852 ± 12,287	0,972	0,335	- 3,323 – 9,590
Normotensive (n=32)	95,719 ± 12,382			

Significant difference between both groups, p<0,05. BMI; Body Mass Index, WHtR; Waist-to-height Ratio, WC; waist circunference.

A total of 183 PCR reactions were conducted, including 95 cases and 88 controls (*Fig 2.*). The Hardy-Weinberg equilibrium was assessed, which indicated that the distribution of genotypes in the case and control groups did not differ from what was expected, suggesting that both populations are in equilibrium. As análises também indicaram que não há diferenciação gênica e genotípica entre os grupos, indicando que as os grupos são homogêneos.

Finally, we found no association between the ACE I/D polymorphism and Hypertensionin our study population, nor was there a significant difference in the distribution of genotypes based on the presence or absence of Hypertension. Resultados semelhantes também foram observados considerando os modelos de análise dominante e recessivo. The D allele was the most frequent in our study population, both in normotensives and hypertensives, with the ID genotype also being the most prevalent in both groups, while the I allele and II genotype were the least frequent within the population (*Table 3*).

Table 3: Allelic and Genotypic Frequencies of ACE I/D Polymorphism

ACE I/D	HT (n=95)	NT (n=88)	X^2	p
Genotypes				
II	16	17	0,496	0,708
ID	50	48		
DD	29	23		
Alleles				
I	82	82	1,221	0,543
D	108	94		
Dominant Model				
II	16	17	0,189	0,663
ID + DD	79	71		
Recessive Model				
II + ID	66	65	0,433	0,511
DD	29	23		

Significant difference between both groups, p<0,05

IV. Discussion

The development of cardiovascular complications driven by Hypertensionis responsible for a significant number of deaths in Brazil and worldwide (3,4). As a multifactorial disease, both genetic and environmental factors interact in promoting or protecting against the condition (28). Early identification of genetic factors that predispose an individual to Hypertension may be a key strategy in medical counseling and in promoting lifestyle modifications, such as dietary and behavioral changes, aimed at counterbalancing the altered genetic factor.

In our study, we identified several risk factors that differentially affected normotensive and hypertensive individuals. We found that Hypertension was more prevalent among self-identified Black individuals in our study population. Our findings align with existing literature regarding ethnicity and its relationship with Hypertension (29). It is well established that mortality from cardiovascular diseases is significantly higher in African Americans (30), who, by age 45, exhibit average blood pressure levels comparable to those of 55-year-olds living in the same region, supporting the notion that early primary Hypertension predominantly affects this population (29). This group also shows an increase in blood pressure that positively correlates with salt sensitivity, which may help explain the relationship between ethnicity and blood pressure (31). Other aspects related to Hypertension, such as body weight, appear to be elevated among African American patients (32).

Age is also an extremely relevant factor when discussing Hypertension, as there is a linear relationship between increasing age and elevated blood pressure after the age of 40, which tends to reach a plateau in the later stages of life (5,33,34). This information supports our observations that the hypertensive patient group is older than the normotensive group.

Indicators of obesity, such as BMI and waist-to-hip ratio, are included in the list of classic factors contributing to increased blood pressure (4). It is well established that obesity is associated with the activation of the RAAS through the exaggerated stimulation of renin production by the kidneys in response to signals from the sympathetic nervous system (SNS) observed in overweight patients (35). Abdominal adipocytes also exhibit an aberrant capacity to produce angiotensinogen in these patients (36). Renin and angiotensinogen are precursors and intermediates, respectively, of angiotens in II, a pro-inflammatory component that induces increased blood pressure (37,38). Our data align with the literature and support the concept that age, weight, and ethnicity are key risk factors for blood pressure. We conducted an association analysis between the polymorphism and various anthropometric characteristics in our population; however, no differences were observed (data not shown).

It is estimated that genetics accounts for up to 30% of the variation in blood pressure; thus, the influence of a family history of Hypertensionon the development of the disease is clear (39). In our study, we identified a higher prevalence of Hypertensionin individuals with a positive family history ofHypertension, which is associated with the sharing of a series of genetic variants that may influence blood pressure regulation. Interestingly, it has been demonstrated that the genetic contribution to blood pressure similarity in twins can be as high as 65%. Furthermore, genetic factors are associated with stress, a component that also affects blood pressure (2).

In our study, the D allele and the ID genotype of the ACE gene were found at high frequencies in both groups, consistent with observations in most Western populations where such studies have been conducted (40,41). A strong association was observed between Hypertensionand the combination of the *ACE I/D* polymorphisms and the G8790A polymorphism of the

ACE2 gene, which was not detected when the polymorphisms were assessed individually (40). This finding reinforces the importance of combined analysis of multiple polymorphisms, given that Hypertensionis a multifactorial disease with a polygenic nature (11).

Few studies have aimed to assess the allelic and genotypic frequencies of the *ACE I/D* polymorphism in Brazilian populations (41), and this number is even smaller when considering populations from the state of Bahia (42), which are highly admixed and therefore exhibit a broad genetic diversity that remains largely underexplored in the context of Hypertensiongenetics. This underscores the importance of conducting further studies like this one to fully understand the role of genetic polymorphisms in the development or predisposition to Hypertensionand other diseases, while taking into account the genetic diversity of the Brazilian population (43). It is essential to emphasise that different populations are exposed to distinct environmental conditions and possess unique genetic backgrounds, which helps to explain the varying allele frequencies and the presence or absence of an association between the ACE I/D polymorphism and Hypertension. Similar to our findings and those of other researchers in Brazilian populations, no such association has been observed, mirroring results in specific populations from other continents (21,44), whereas studies in European (45) and some Asian (24,46) populations have reported a significant association. A study conducted with obese children in São Paulo State also found an association between the D allele and Hypertension (47).

V. Conclusion

The absence of an association between the polymorphism and Hypertensiondoes not refute the hypothesis that it may exert an influence on the development ofHypertension. Here, we evaluated only one polymorphism, and it is essential to emphasise that the combination of different polymorphisms, as well as the interplay between genotype, phenotype, and behaviour, may reveal that the cumulative effect of minor influences from various factors, often overlooked when considered in isolation, can robustly contribute to the development of the pathology. Therefore, a comprehensive evaluation of the ACE I/D polymorphism in conjunction with other polymorphisms and non-genetic risk factors is warranted.

Abbreviations

BMI - Body mass index
HDL - High-density lipoprotein
LDL - Low-density lipoprotein
ACE2 - Angiotensin converting enzyme – 2
I/D - Insercion/delection
BP – Blood pressure
SBP - Systolic blood pressure
DBP - Diastolic blood pressure
CVD - Cardiovascular disease
RAAS - Renin-angiotensin-aldosterone system
GWAS - Genome-wide association study
TGF-β - Transforming growth factor beta
SMAD - Suppressor of mothers against decapentaplegic
AF - Allele frequencies
LACEM - Municipal Laboratory of Vitória da Conquista
ICF - Informed Consent Form
EDTA - Ethylenediamine tetraacetic acid
UESB -State University of Southwestern Bahia
DNA - Deoxyribonucleic acid
dNTP - Deoxynucleotide triphosphates
HWE - Hardy-Weinberg equilibrium
WHtR - Waist-to-height Ratio
WC - Waist circunference
SNS - Sympathetic nervous system

Ethics Committee Approval

We declare that this study was approved by the Ethics Committee of UESB (Opinion No. 2,627,076) and the Public Health Foundation Isaú Matos, where LACEM is located (Opinion No. 2,792,660)

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

All patients and healthy donors included in the study agreed to participate and signed the informed consent form.

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Author Contributions

SO: Conceptualization, Investigation, Methodology, Writing – original draft.
MB: Investigation.
PBC: Investigation.
PSZC: Investigation.
DA: Funding acquisition.

WA: Funding acquisition.
MO: Funding acquisition and Writing – original draft.
MT: Methodology and Funding acquisition.
SS: Conceptualization and Funding acquisition.
PL: Conceptualization, Funding acquisition, Supervision, and Writing – original draft

Figure Legends:

Generative AI Statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

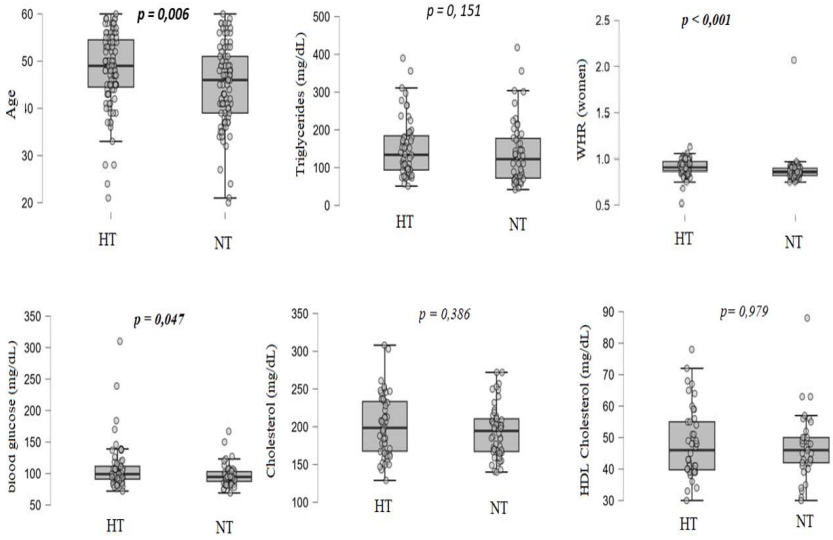

Figure 1: Analysis of anthropometric and clinical of study groups. Significant difference between both groups, p<0,05. BMI; Body Mass Index, WHR; Waist-to-hip Ratio

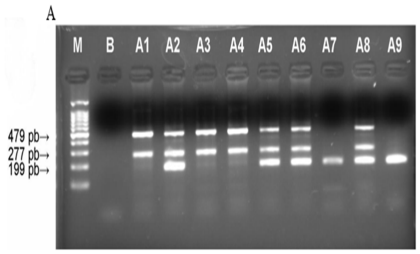

Figure 2: Allelic and genotypic frequencies of I/D ACE in the population: (A) Photograph of the agarose gel after the separation, by electrophoresis, of the fragments generated from the PCR reaction; (B-E) frequency of alleles I and D in normotensive and hypertensive individuals; (F-I) frequency of genotypes DD, ID, and II in normotensive and hypertensive individuals

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Psychometric Evaluation and Validation of the Questionnaire on Stress in Diabetic Patients in Brazilian Portuguese

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Objective: To analyse the psychometric properties of the Brazilian version of the Questionnaire on Stress in Diabetic Patients– Revised (QSD-R).

Methods: A cross-sectional methodological study was conducted at both public and private healthcare services involving 315 patients diagnosed with type 2 diabetes. Construct validity was evaluated through exploratory and confirmatory factor analysis. Reliability was estimated using Cronbach's alpha coefficient and the intraclass correlation coefficient (ICC). Convergent validity was tested with the Diabetes-21 Quality of Life Scale and the Hospital Anxiety and Depression Scale (HADS).

Keywords: *diabetes mellitus, quality of life, surveys and questionnaires, psychological stress.*

GJMR-F Classification: *NLM Code: WT 135*

Strictly as per the compliance and regulations of:

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Results: Exploratory factor analysis revealed a structure consisting of four factors, with satisfactory factor loadings and communalities ranging from 0.26 to 0.79. Confirmatory factor analysis indicated good fit for the model (CFI = 0.91; TLI = 0.87; RMSEA = 0.06; SRMR = 0.067). Cronbach's alpha was 0.87, and the overall ICC was 0.91. Convergent validity was demonstrated by significant correlations between the QSD-R domains and both the Diabetes-21Scale and HADS.

Conclusion: The Brazilian Portuguese version of the Questionnaire on Stress in Diabetic Patients includes 34 items across five domains, showing reliable results and a good fit to the proposed factor model. These findings confirm the robustness of the cross-cultural adaptation, validating the instrument for use within Brazilian contexts.

Keywords: diabetes mellitus, quality of life, surveys and questionnaires, psychological stress.

I. Introduction

Diabetes mellitus (DM) is the most prevalent chronic metabolic disease worldwide and a significant public health concern(1), with rapid increases and a considerable impact on individual well-being and healthcare costs (2–4). According to the International Diabetes Federation (IDF), one in 10 people globally (537 million) has diabetes (5,6) and approximately four million die from the disease each year. The projection for 2045 is that 783 million adults will be living with the condition (5–8).

DM affects people of different ages and genders and is a complex disease involving both genetic and environmental factors. The most common types are type 1 (DM1) and type 2 (DM2), with the latter accounting for 90% of cases, and there is a 15% higher risk of death from complications compared to healthy individuals (9).

Besides generating severe consequences due to the emergence of problems and complications, DM exerts an enormous impact on human physiology as well as cognitive, psychological, and social functioning (10–12). This disease incurs a significant social and economic burden, encompassing medical expenses, productivity loss, premature death, and intangible costs, such as diminished quality of life in many populations (11,13,14).

Capillary blood glucose measurements are essential for managing diabetes. However, the limitations of these assessments include poor patient adherence, physicians often encountering incomplete data, with few values measured throughout the day and scattered across irregular records, and patients and/or family members frequently forgetting to bring the blood glucose log to appointments (15,16) Additionally, difficulties in making lifestyle changes can lead to adverse effects in daily life, such as low self-esteem, anxiety, and depression, which directly affect quality of life (17).

The quality of life (QoL) is recognised as a vital aspect of health, associated with psychological well-being, mental health, stress, and personal experiences (18). However, perceptions of illness tend to become more negative when individuals have more comorbidities and depend more on others, which, in turn, impacts their QoL (19). Complications of DM increase morbidity and mortality, functioning as a stressor for the body (20). Over the years, many studies have demonstrated the detrimental effects on QoL in people with diabetes mellitus and severe or irreversible complications and comorbidities (4,6,12,17,19,21).

Psychological stress has increasingly been recognised as a risk factor for developing type 2 diabetes. Living with this diagnosis can also cause stress, as treatment requires constant discipline and can trigger fears related to hypoglycaemia and uncertainty about the future. These factors contribute to a cycle of emotional insecurity that can further worsen a person's psychological state (20). Evidence shows that external stressors, unsafe environments, and a poor

Author α: e-mail: amanda.segalla@unesp.br

understanding of the disease can disrupt glucose metabolism. If a stressor is perceived as negative, it is less about how often it occurs and more about how one interprets it and how it impacts or interferes with one's life (20, 22)

To measure stress in individuals with type 1 and type 2 diabetes, German researchers developed the Questionnaire on Stress in Diabetic Patients - Revised (QSD-R) in 1996. The final English version includes 45 items across eight domains. Given the limited availability of instruments in Brazil for assessing stress in people with diabetes, translating and culturally adapting the QSD-R in Brazil could provide a reliable and reproducible scale for collecting and analysing data on the stress experienced by these individuals, thereby contributing to the reorganisation of care practices. Therefore, the present study aimed to examine the psychometric properties of the Brazilian version of the Questionnaire on Stress in Diabetic Patients - Revised (QSD-R).

II. Methods

a) Study Design

A methodological study with a quantitative approach was conducted at two public primary health care services and a private endocrinology clinic in the state of São Paulo, Brazil, from May 2023 to October 2024, following authorisation from the creator of the instrument.

b) Population of Study

The sample was selected by convenience and included individuals of both sexes aged 18 years and above with a diagnosis of diabetes mellitus who agreed to participate in the study. Illiterate individuals were excluded. Although there is no gold standard for validating a new instrument, it is recommended that the sample size be at least four to ten times the number of items, with a minimum of 180 individuals, to ensure adequate validity (23, 24). The invitation to participate in the study was extended after a medical appointment at an endocrinology clinic and by health professionals at public health services.

c) Data Collection Procedures

Three instruments were used for data collection. The Questionnaire on Stress in Diabetic Patients - Revised (QSD-R) comprises 45 items divided into eight domains: leisure time; depression; hypoglycaemia issues; self-medication; physical complaints; work; partner relationships; and doctor-patient relationships (22). Reliability, measured by Cronbach's alpha, ranged from 0.69 to 0.81. Each item describes a potential negative effect on daily life and is rated on a numerical scale from 0 to 5. The total score spans from 0 to 225, with each item rated from "not applicable" to "a very big problem" for each statement (22).

The second instrument applied was the Diabetes-39 Quality of Life Assessment Questionnaire (D-39), which has been adapted and validated for use in the Brazilian context and was reduced to 21 items (D-21). The instrument is originally in English and specifically conceived to assess health-related QoL in individuals with DM2. The validation and adaptation for Brazil had good internal consistency, with Cronbach's alpha of 0.917. The instrument in its final version in Portuguese (25) has 21 items distributed among four dimensions of quality of life: energy and mobility (Items 9; 10; 11; 29; 32; 34; 36), diabetes control and social burden (Items 5; 15; 17; 24; 28; 19; 20; 26), anxiety and worry (Items 2; 8; 22), and sexual functioning (Items 21; 23; 30). The D-21 also has a general assessment domain (two items) that encompasses the self-perception of QoL and diabetes severity (26). The instrument enables respondents to state how much their QoL was affected in the previous month by a particular action or activity, which is expressed in each item by placing an X on a point of the scale represented by a continuous line, with spaces occupied by numbers from 1 to 7, with 1 corresponding to QoL absolutely unaffected and 7 corresponding to significantly affected (25, 26).

The Hospital Anxiety and Depression Scale (HADS) was also utilised. This scale is divided into two subscales, each comprising seven items. Using defined values, the subscales indicate different levels of anxiety or depression: 0-7 = normal; 8-10 = mild; 11-14 = moderate; 15-21 = severe (27).

The authors developed an instrument to characterise participants using 14 categorical variables and sociodemographic data, including age, sex, marital status, education, occupation, number of residents in the household, family income, age at diagnosis of DM, type of diabetes, duration of diabetes treatment, insulin use, comorbidities, risk factors, tobacco use, alcohol consumption, psychoactive substance use, weight, height, and body mass index. The average response time was 15 minutes.

For the assessment of temporal stability, a retest was conducted with 20 individuals. For this, the random selection method was used, and the instrument was completed a second time between 14 and 20 days after the first interview (28).

d) Statistical Analysis

i. Descriptive Analysis

All variables were analysed descriptively. Normality of the distribution was assessed using the Kolmogorov-Smirnovtest (29).

ii. Exploratory Factor Analysis

The Kaiser-Meyer-Olkin (KMO) test and Bartlett's test of sphericity were used to evaluate the suitability of the sample for factor analysis. These statistical procedures assess whether factor analysis is

appropriate, indicating the sample's adequacy and the data's factorability. The criteria for adequacy were a KMO value above 0.50 and a statistically significant result on Bartlett's test of sphericity ($p < 0.05$) (30).

Horn's parallel analysis was utilised to test the hypothesis regarding the number of factors adopted by each scale, with the minimum residual factorisation method (MINRES) summarised by a scree plot. The traditional correlation matrix and *Oblimin* rotation were used to identify the latent structure of the scale. Factor loadings, commonality, complexity of the original items, cumulative variance, and the objective function were derived from exploratory factor analysis (EFA). In the factor extraction step, two criteria were adopted for the retention of factors: an absolute factor loading greater than 0.30 and the presence of at least three items per factor.

iii. *Confirmatory Factor Analysis*

In confirmatory factor analysis (CFA), multiple fit indices were used to evaluate how well the model fits the observed data. The comparative fit index (CFI) and Tucker-Lewis index (TLI) were utilised, with values ranging from 0.90 to 0.95 indicating an acceptable fit, while values of 0.95 or higher suggest a good fit. The root mean square error of approximation (RMSEA) was considered to indicate good fit when between 0.05 and 0.08, with $p < 0.05$. The standardised root mean square residual (SRMR) was also analysed, with acceptable values set at ≤ 0.08. The confirmatory factor minimum discrepancy (CMIN) and the CMIN/degrees of freedom ratio were also estimated. Standardised loadings were deemed adequate when exceeding 0.30 (31).

iv. *Convergent Validity*

In the absence of an equivalent instrument that could be considered the "gold standard" and that met the criteria of methodological excellence required for the present study, the Quality of Life Scale for Patients with Diabetes and the Hospital Anxiety and Depression Scale were used as parameters for analysing convergent validity. The normal distribution of the data justified the use of Pearson's correlation coefficients, which were interpreted as follows: <0.4, 0.4 to 0.6, and >0.6, indicating weak, moderate, and strong correlations, respectively (29).

v. *Reliability*

Reliability was assessed using internal consistency with Cronbach's alpha coefficient (32) and test-retest stability with the intraclass correlation coefficient (ICC) (33), with values above 0.7 considered acceptable for both (32, 33).

The data were analysed using the Statistical Package for the Social Sciences (SPSS) and the R platform for statistical computing, version 4.1.2.The level of significance was set at 5% ($p < 0.05$).

e) Ethical Aspects

This study received authorisation from the authors of the original study and approval from the Human Research Ethics Committee (protocol number: 56981522.0.0000.5411; approval certificate number: 5.333.924). The study was conducted following the guidelines of the *Strengthening the Reporting of Observational Studies in Epidemiology* (STROBE) (34)and *Guidelines for the Process of Cross-Cultural Adaptation of Self-Report Measures*(35).

III. Results

a) Characterisation of the Sample

i. *Sociodemographic Data*

The sample comprised 315 participants diagnosed with diabetes. Most were women (n = 190; 60.3%). The average age was 59.10 ± 13.86 years. Married individuals (n = 202; 64.1%) and those with higher education (n = 137; 43.6%) were predominant. Slightly over half of the sample (n = 166; 52.7%) had a medical insurance plan or private healthcare. Regarding monthly family income, 84 (26.7%) earned between five and ten thousand Reais (Brazilian currency) (Table 1).

Table 1: Sociodemographic Characterisation and Non-Demographic Data of 315 Participants

Variable	n	%
Type of Care		
Insurance/Private	166	52.7
Public	149	47.3
Age		
Mean	59.10	49**
SD*	±13.86	
Sex		
Male	125	60.3
Female	190	39.7
Marital Status		
Single	38	12.1
Married/Stable Union	202	64.1
Separated/Widowed	75	23.8
Monthly Family Income		
<R$ 1,000	7	2.2
R$ 1,000 to R$ 3,000	82	26.0
R$ 3,000 to R$ 5,000	82	26.0
R$ 5,000 to R$ 10,000	84	26.7
>R$ 10,000	60	19.0
Schooling		
Primary School	77	24.5
High School	100	31.8
Higher Education	137	43.6
Non-demographic variables	**n**	**%**
Residents in same home		
Lives alone	49	15.7
Lives with partner	111	35.5
Three to five residents	148	47.3
Six or more residents	5	1.6
Time since diagnosis		
< 1 year	21	6.7
1 to 5 years	48	15.3
6 to 10 years	69	22.0
11 to 20 years	97	30.9
> 21 years	79	25.2
Duration of treatment for DM		
< 1 year	30	9.6
1 to 5 years	57	18.2
6 to 10 years	66	21.0
>11 years	161	51.3
Type of DM		
Type 1	54	17.1
Type 2	261	82.9
Use de insulin		
Not used	199	63.2
< 1 year	18	5.7
1 to 5 years	27	8.6
6 to 10 years	18	5.7
> 11 years	53	16.8
Comorbidities		
None	126	40.0
Some type of comorbidity	189	60.0
Risk Factor		
None	134	42.5
Some risk factor	181	57.5

Standard deviation/median*

b) Construct Validity

1st Step: Confirmatory Factor Analysis

Confirmatory factor analysis was performed using an initial model with five dimensions, based on robust error estimates. However, the final model did not fit well: comparative fit index (CFI): 0.786; robust Tucker-Lewis index (TLI): 0.768; RMSEA: 0.083. Reliability estimated by Cronbach's Alpha for the entire instrument was 0.96.

2nd Step: Exploratory Factor Analysis (EFA)

The results of the Kaiser-Meyer-Olkin (KMO) test (0.95) and Bartlett's test of sphericity (X^2 = 9645.746, d.f. = 990, p < 0.000001) confirmed the adequacy of the sample for EFA. Among the 315 individuals, 44 were identified as outliers based on the distribution of the Mahalanobis distance. However, removing these individuals did not affect the KMO value. Horn's parallel analysis identified five oblique factors with eigenvalues greater than 1.0 (Figure 1).

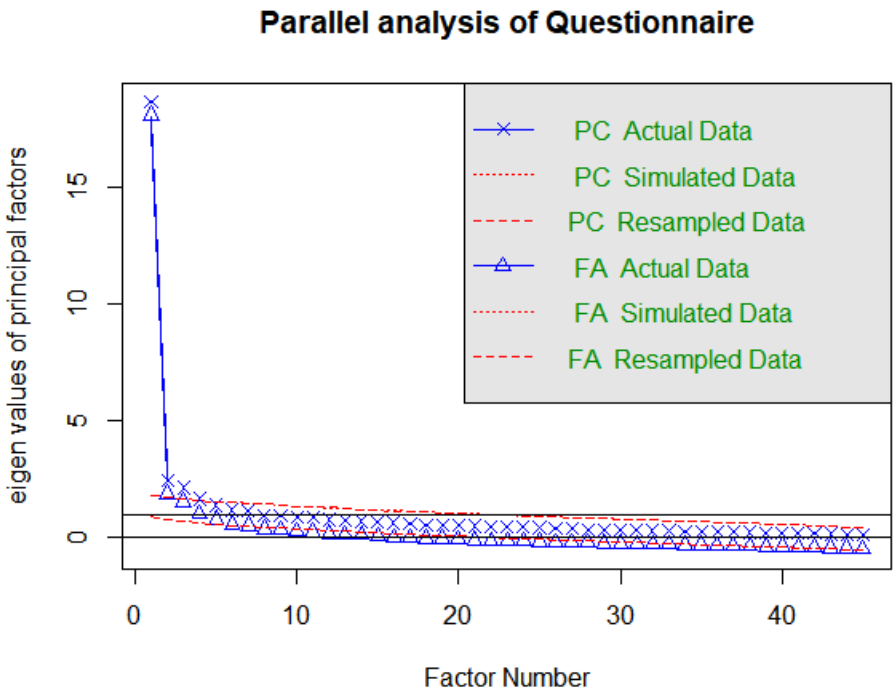

Figure 1: Scree Plot of Horn's Parallel Analysis of 45 Items of QSD-R

EFA was performed to identify how the items clustered across the five factors (Table 2). After the first round of exploratory analysis, Items 10, 17, 18, 4, 14, and 20 were removed because they had loadings above 0.3 on more than one factor, and Items 11, 24, 25, 39, and 40 were eliminated due to loadings below 0.3. As a result, 34 questions remained, each with loadings of 0.3 or higher on only one factor, distributed across five domains with at least three items in each domain. Factor 1 contained the highest number of variables, with a total of eight items.

The instrument accounted for 55.38% of the variance in the data. Communality values ranged from 0.26 (Q40) to 0.79 (Item 42), indicating varying levels of explanation for the factors within the items. Table 2 shows the factor loadings, commonality (h2), and complexity (com) of the EFA.

Table 2: Factor Loadings, Commonality (h2), and Complexity (com) of EFA

	ML 1	ML 2	ML 3	ML 4	ML 5	H 2	com	% Variance
Item 1	0.03	0.08	0.00	0.04	**0.66**	0.53	1.0	4.34%
Item 2	0.20	-0.03	0.14	0.12	**0.50**	0.55	1.6	2.73%
Item 3	-0.20	0.14	0.25	0.23	**0.30**	0.35	4.1	1.94%
Item 4	**0.39**	-0.11	**0.72**	0.23	0.28	0.42	2.7	1.73%
Item 5	0.03	-0.07	**0.72**	0.13	0.07	0.63	1.1	1.60%
Item 6	-0.05	0.29	0.05	0.03	**0.36**	0.32	2.0	2.20%
Item 7	0.13	-0.14	0.20	**0.48**	0.14	0.46	1.9	4.43%
Item 8	0.14	-0.02	**0.79**	-0.08	-0.06	0.63	1.1	1.58%
Item 9	0.22	0.00	0.21	-0.01	**0.41**	0.45	2.1	1.98%
Item 10	0.11	-0.01	**0.37**	0.09	**0.37**	0.54	2.3	1.72%
Item 11	0.29	0.13	0.12	0.18	0.23	0.50	3.5	1.17%
Item 12	0.22	0.17	0.00	0.06	**0.46**	0.52	1.8	1.98%
Item 13	0.17	0.14	0.24	-0.11	0.44	0.52	2.3	2.05%
Item 14	**0.43**	**0.56**	0.11	-0.04	0.21	0.56	2.3	1.75%
Item 15	**0.63**	0.05	0.17	0.04	-0.04	0.56	1.2	2.75%
Item 16	**0.54**	0.14	0.12	0.05	0.08	0.57	1.3	2.03%
Item 17	-0.06	**0.51**	-0.11	0.11	**0.31**	0.46	1.9	2.24%
Item 18	**0.54**	0.15	-0.07	-0.05	**0.32**	0.57	1.9	2.47%
Item 19	0.18	**0.60**	0.15	0.00	0.07	0.68	1.4	2.60%
Item 20	**0.53**	**0.47**	0.15	-0.06	0.22	0.69	2.3	2.12%
Item 21	-0.06	-0.04	**0.84**	0.11	0.02	0.73	1.1	5.16%
Item 22	0.25	**0.36**	0.03	0.15	0.16	0.53	2.7	1.67%
Item 23	-0.09	0.12	0.27	**0.47**	0.12	0.50	2.0	2.38%
Item 24	0.27	0.16	0.19	0.29	-0.05	0.47	3.4	1.33%
Item 25	0.23	0.18	0.24	0.07	0.19	0.46	4.0	1.07%
Item 26	0.06	0.13	0.06	**0.38**	0.17	0.37	1.8	1.22%
Item 27	**0.57**	-0.08	-0.02	0.26	0.03	0.51	1.4	2.64%
Item 28	0.13	**0.82**	-0.08	0.02	0.01	0.75	1.1	5.10%
Item 29	0.07	0.06	0.00	**0.78**	-0.05	0.69	1.0	4.60%
Item 30	**0.70**	-0.04	-0.03	0.16	0.16	0.71	1.2	4.20%
Item 31	**0.63**	0.18	0.16	0.06	-0.10	0.65	1.4	3.25%
Item 32	0.05	0.07	-0.05	**0.71**	0.01	0.57	1.0	3.94%
Item 33	**0.35**	0.11	0.12	0.29	0.03	0.49	2.4	1.72%
Item 34	0.02	**0.86**	-0.01	0.04	-0.07	0.74	1.0	5.26%
Item 35	0.26	0.28	0.04	**0.36**	-0.22	0.44	3.5	2.04%
Item 36	**0.58**	0.02	0.11	0.10	0.00	0.50	1.1	2.26%
Item 37	-0.01	0.06	**0.60**	0.05	0.13	0.51	1.1	2.58%
Item 38	-0.13	**0.53**	0.14	0.06	0.24	0.52	1.7	2.69%
Item 39	0.15	0.15	0.14	0.13	0.21	0.34	4.3	0.73%
Item 40	-0.08	0.18	0.23	0.02	0.27	0.26	2.9	0.96%
Item 41	**0.36**	0.03	0.27	0.17	0.12	0.54	2.6	1.79%
Item 42	-0.09	**0.87**	0.05	0.08	0.00	0.79	1.0	5.33%
Item 43	0.11	0.18	**0.65**	-0.06	-0.03	0.60	1.2	3.37%
Item 44	0.02	**0.47**	0.27	0.03	0.05	0.48	1.6	2.10%
Item 45	-0.03	0.24	**0.56**	-0.10	-0.01	0.42	1.4	2.70%

c) Confirmatory Factor Analysis (CFA)

After excluding items, the instrument's structure was organised into five factors (F1, F2, F3, F4, and F5) based on the analysis of the factor clusters obtained in the initial steps. This analysis confirmed a redistribution of items that differed from the proposed model. The adjustment items indicated a good fit of the model to the data, as shown by CFI = 0.91, TLI = 0.87, RMSEA = 0.06, and SRMR = 0.067; X^2 = 1520.64; df = 517; p < 0.0001, with a χ^2/df ratio of 2.76. These results empirically support the theoretical structure of the instrument, which retained 34 variables across five factors, as illustrated in Figure 2.

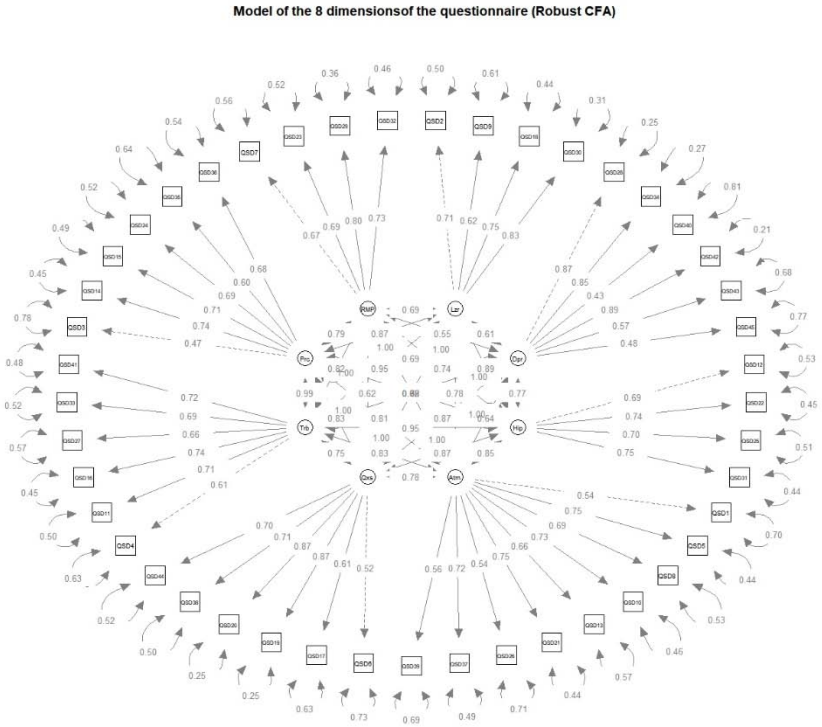

Figure 2: Diagram of Confirmatory Factor Trajectory and Standardised Loading of Items

Among the items of the new instrument related to the domains of the original instrument, Items 1, 5, 8, 13, 21, 26, and 37 form the “self-medication” domain. Items 28, 34, 42, 43, and 45 form the “depression” domain. Items 12, 22, and 31 belong to the “hypoglycaemia” domain. Items 2, 9, and 30 make up the “leisure time” domain. Items 3, 15, 35, and 36 belong to the “relationship with partner” domain. Items 6, 19, 38, and 44 are in the “physical complaints” domain. Items 7, 23, 29, and 32 fall within the “doctor-patient relationship” domain. Lastly, Items 16, 27, 33, and 41 comprise the “work” domain of the original instrument.

d) Convergent Validity

Table 3 displays the Pearson correlation matrix between factors derived from the exploratory analysis and latent variables identified in the confirmatory analysis of the original instrument, which the author refers to as domains. The “leisure time” domain showed a strong correlation with Factor 1. The “depression” domain was strongly correlated with Factor 2. The “self-medication” domain showed a strong correlation with Factor 3. The “doctor-patient relationship” domain was strongly correlated with Factor 4. These robust correlations between factors and domains suggest consistency and validity in the measures used, confirming internal coherence within the instrument.

Table 3: Correlations between Factors of Exploratory Analysis and Latent Variables of Confirmatory Analysis of Adapted Instrument

		QDS Total	QSD F1	QSD F2	QSD F3	QSD F4	QSD F5	HADS A	HADS D	D39 Total	D39 D1	D39 D2	D39 D3	D39 D4
QDS Total	Rho	—												
	p	—												
QSD F1	Rho	0.826***	—											
	p	<.001	—											
QSD F2	Rho	0.879***	0.660***	—										
	p	<.001	<.001	—										
QSD F3	Rho	0.852***	0.655***	0.628***	—									
	p	<.001	<.001	<.001	—									
QSD F4	Rho	0.752***	0.694***	0.606***	0.571***	—								
	p	<.001	<.001	<.001	<.001	—								
QSD F5	Rho	0.863***	0.681***	0.695***	0.655***	0.627***	—							
	p	<.001	<.001	<.001	<.001	<.001	—							
HADS A	Rho	0.672***	0.527***	0.684***	0.557***	0.467***	0.543***	—						
	p	<.001	<.001	<.001	<.001	<.001	<.001	—						
HADS D	Rho	0.580***	0.473***	0.623***	0.388***	0.407***	0.485***	0.744***	—					
	p	<.001	<.001	<.001	<.001	<.001	<.001	<.001	—					
D39 Total	Rho	0.881***	0.732***	0.826***	0.726***	0.638***	0.745***	0.701***	0.634***	—				
	p	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	—				
D39_D1	Rho	0.804***	0.688***	0.801***	0.628***	0.573***	0.661***	0.679***	0.635***	0.933***	—			
	p	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	—			
D39_D2	Rho	0.845***	0.707***	0.723***	0.712***	0.631***	0.776***	0.615***	0.535***	0.933***	0.829***	—		
	p	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	—		

		QDS Total	QSD F1	QSD F2	QSD F3	QSD F4	QSD F5	HADS A	HADS D	D39 Total	D39 D1	D39 D2	D39 D3	D39 D4
D39_D3	Rho	0.770***	0.586***	0.786***	0.624***	0.504***	0.624***	0.681***	0.585***	0.854***	0.745***	0.732***	—	
	p	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	—	
D39_D4	Rho	0.558***	0.514***	0.488***	0.485***	0.504***	0.436***	0.410***	0.400***	0.644***	0.526***	0.551***	0.446***	—
	p	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	—

*Note: * p < .05, ** p < .01, *** p < .001*

e) Reliability

The reliability analysis was based on the internal consistency of the instrument using Cronbach's alpha and its temporal stability (test-retest) using the intraclass correlation coefficient (ICC). The results showed satisfactory internal consistency for all factors, ranging from 0.86 (Factor 5) to 0.93 (Factor 4), except for Factor 1, which had an ICC of 0.68. The overall scale achieved an ICC of 0.97.

Table 4 shows the mean values for the total QSD-R score at two assessment points, used to evaluate temporal stability. Minimal changes between the two assessments indicate good levels of stability over time, nearing the threshold for moderate reliability.

Table 4: Distribution of Temporal Stability (test-retest) of Adapted QSD-R and Subscales (n=20).

Variable I	Test	Retest	ICC (95% CI)	*P*
QSD total	53 (34-77)	55 (34-76)	0.97 (0,95-1.02)	0.04
F1	10 (8-14)	9 (7-13)	0.68 (0.48- 0.97)	0.26
F2	12 (7-18)	14 (7-19)	0.91 (0.83-1.02)	<0.001
F3	12 (6-17)	12 (7-17)	0.88 (0.73-1.09)	<0.001
F4	7 (6-15)	6 (6-15)	0.93 (0.90-1.06)	<0.001
F5	10 (7-18)	11 (7-16)	0.86 (0.67-1.07)	<0.001

IV. Discussion

The present study aimed to analyse the psychometric properties of the Brazilian Portuguese version of the Questionnaire on Stress in Diabetic Patients – Revised (QSD-R). The sample of adults with a medical diagnosis of type 1 or type 2 diabetes was predominantly women (60.3%), differing from the study that originally developed the QSD-R instrument (22) as well as a subsequent study (36), but similar to findings reported in a study that used the QSD-R to explore self-perceived stress in relation to hair cortisol (20) and another study that validated the instrument for the Portuguese adolescent population (37). Regarding other sociodemographic variables, married individuals (64.1%), those living with the disease for over ten years (30.9%), individuals with some risk factor (57.5%), and those with at least one comorbidity (60.9%) were predominant in the sample, as reported in other international studies (20,22,36).

To assess the adequacy of the data, an initial analysis was conducted using the Kaiser-Meyer-Olkin (KMO) index and Bartlett's Test of Sphericity. The KMO value was 0.95, indicating suitability for factor analysis, based on the criteria proposed by Kaiser (1974) (38), who considers values above 0.80 as indicative of satisfactory quality. Bartlett's Test of Sphericity yielded a statistically significant result ($X^2 = 9645.746$, d. f. = 990, $p < 0.000001$), suggesting that the correlations between the items are significantly different from zero. These findings suggest that the data are suitable for identifying an underlying factorial structure, justifying further factor analysis to evaluate the construct validity of the instrument.

In the initial step (confirmatory factor analysis based on the original eight-dimensional, 45-item model), the values obtained for the CFI (0.786) and TLI (0.768) fell below the recommended minimum ($\geq$ 0.90), indicating that the proposed model did not fit the data adequately. Although the RMSEA (0.083) was within the acceptable range, the other indices did not suggest a good fit, highlighting the need to explore the model further through EFA. This was performed to develop a more reasonable and reliable model (39), based on the minimum retention criterion of three items per factor and factor loadings above 0.3. Consequently, 11 items were excluded from the original instrument (Items 4, 10, 11, 14, 17, 18, 20, 24, 25, 39, and 40), similar to the validation study of the instrument in Portuguese for Portugal (37). The new distribution of items within each factor was as follows: FACTOR 1 (QSD-R Items 15, 16,

27, 30, 31, 33, 36, and 41), FACTOR 2 (QSD-R Items 19, 22, 28, 34, 38, 42, and 44), FACTOR 3 (QSD-R Items 5, 8, 21, 37, 43, and 45), FACTOR 4 (QSD-R Items 7, 23, 26, 29, 32, and 35), and FACTOR 5 (QSD-R Items 1, 2, 3, 6, 9, 12, and 13).

The factorial structure obtained through EFA revealed five factors that explained 55.38% of the total variance, demonstrating a coherent and appropriate theoretical organisation of the QSD-R instrument for the Brazilian population diagnosed with type 1 and type 2 diabetes. This result reinforces the construct validity of the scale and aligns with findings described in previous studies that employed the same instrument in different cultural contexts (20, 22, 36, 37).

Confirmatory factor analysis was then conducted by restructuring the instrument into five factors. All quality criteria for the model's fit were deemed satisfactory, with the final model showing high goodness of fit (CFI = 0.91; TLI = 0.87; RMSEA = 0.06; SRMR = 0.067; X^2 = 1520.64; df = 517; $p < 0.0001$, with $\chi 2$/df ratio = 2.76), aligning with data reported in the literature (30). The results confirmed the construct validity of the instrument, indicating that the items are appropriately organised into five theoretical factors that allow a comprehensive assessment of factors related to stress in patients with diabetes.

The reliability of the scale was evaluated using Cronbach's alpha coefficient for internal consistency and the intraclass correlation coefficient (ICC) for assessing temporal stability (test-retest) (40, 41).

The results demonstrated good internal consistency of the QSD-R, with high Cronbach's alpha coefficients, especially for the "complaints" and "self-medication" domains (0.86 and 0.87, respectively), aligning with findings from a previous study that used the same instrument for similar purposes and the original research (22,37). Regarding the other scales used, Cronbach's alpha was 0.97 for the Diabetes-21 (final version in Portuguese) and 0.91 for the HADS. All factors met or surpassed the minimum acceptable threshold of 0.70, recommended as a cutoff for instruments under development or initial validation (42). It is important to note that the number of items significantly influences Cronbach's alpha coefficients in a measurement instrument (40).

The temporal stability of the scale was evaluated using the intraclass correlation coefficient (ICC), as recommended by COSMIN (43). The sample of 20 participants provided sufficient methodological evidence for overall ICC estimates of 0.91 (44). There was minimal variation between the test and retest results, indicating satisfactory levels of temporal stability and moderate reliability.

To assess convergent validity, correlations between the domains of the QSD-R and both the Diabetes-21 Scale and HADS were examined using Pearson's correlations between the factors derived from the exploratory analysis and the latent variables from the confirmatory analysis of the original instrument. Strong correlations were identified between the factors and domains of the original scale and the D39 scale, whereas a weaker correlation was observed between Factor 5 and HADS. These strong correlations between factors and domains support the consistency and validity of the measures used, confirming the internal coherence of the instrument.

V. Conclusion

The results obtained in this study show that the Brazilian version of the QSD-R has a structure comprising five factors and 34 items, organised with reliable consistency and a good fit to the proposed factorial model. This confirms the methodological robustness of the cross-cultural adaptation, ensuring the instrument's validity for use in Brazilian contexts.

The availability of this instrument – unprecedented in Brazil – marks a significant advance for the healthcare field, particularly in clinical settings focused on care for individuals living with diabetes. Its design aligns with institutional guidelines for implementing evidence-based interventions in health promotion, treatment, and therapies for the target population. Furthermore, the practical use of the instrument can help healthcare providers achieve more accurate diagnoses and monitor the effects of therapeutic strategies, thereby supporting improvements in clinical and research practices across Brazil.

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Epidemiological, Clinical, and Therapeutic Aspects of Tumors and Vascular Malformations in Senegal: about 52 Cases

By Sow N. F, Sall A. M, Gaye M, Gold I, Dieng P. A & Cissa. G

Abstract- *Objectives:* Vascular tumors and malformations (VTMs) are characterized by their diversity, making their study complex. Our objective was to describe the different aspects of these vascular anomalies in Senegal.

Patients and Methods: This was a retrospective, analytical and descriptive studyfrom 2004 to 2022. It included all patients admitted for tumors or vascular malformations at our center.

Results: Fifty-two patients were included with a male-to-female ratio of 1:2 (sex ratio = 0.5). The mean age was 24 years. Consanguinity was found in 8% and polymalformative syndrome in 2%. The meantime to the consultation was 8 years, and the main reason was a mass in 94% of the cases. The neck (23%) was the predominant location followed by the cheek (17%)and then the head (13%).

Keywords: *tumors and vascular malformations, classification, treatment, senegal.*

GJMR-F Classification: *NLMC: W 26.5*

Strictly as per the compliance and regulations of:

Epidemiological, Clinical, and Therapeutic Aspects of Tumors and Vascular Malformations in Senegal: about 52 Cases

Sow N. F [α], Sall A. M [σ], Gaye M [ρ], Gold I [Ↄ], Dieng P. A [¥] & Cissa. G [§]

Abstract- Objectives: Vascular tumors and malformations (VTMs) are characterized by their diversity, making their study complex. Our objective was to describe the different aspects of these vascular anomalies in Senegal.

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Results: Fifty-two patients were included with a male-to-female ratio of 1:2 (sex ratio = 0.5). The mean age was 24 years. Consanguinity was found in 8% and polymalformative syndrome in 2%. The meantime to the consultation was 8 years, and the main reason was a mass in 94% of the cases. The neck (23%) was the predominant location followed by the cheek (17%)and then the head (13%). A vascular character of the mass was found in 25%, and trophic disorders in 8%. Vascular imaging revealed an arteriovenous malformation (48%), a venous malformation (17%), an angioma (11%), a hemangioma (10%), a lymphangioma(8%), a glomus tumor (4%), and an angiosarcoma (2%). Treatment consistedof excision (75%), single or primary ligation of the feeding artery (29%), and primary embolization (6%). Morbidity rate was 12%, consisting of hemorrhage (4%), surgical site infection (6%), and limb ischemia (2%). Secondary amputation was performed in 2%. Recurrence was noted in 8%. No deaths were observed.

Conclusion: VMTs are diverse and varied. They are mostly benign, with a few rare malignancies being described. The prognosis of certain complex anomalies can be poor. Classifications are thus made to facilitate the choice of themost appropriate treatment for each type of VMT.

Keywords: tumors and vascular malformations, classification, treatment, senegal.

I. Introduction

Vascular anomalies are divided into two groups: tumors and vascular malformations. They constitute a polymorphic set of pathologies whose etiologies are not well elucidated. The classification of these VMTs, formerly grouped under the name angiomas remains unclear [1]. The work of Mulliken and Glowacki made it possible to distinguish vascular tumors (involving cellular proliferation) from malformations (structural abnormalities of the vessels due to a disruption of vascular morphogenesis during embryonic development). The management of VMTs requires a multidisciplinary approach involving several specialists (pediatrician, dermatologist, radiologist, vascular-surgeon, plastic surgeon, psychologist) [2].

Our objective was to report our observations on patients with tumors or vascular malformations undergoing surgery in the Thoracic and Cardiovascular Surgery Department of the Fann National University Hospital, in Dakar, Senegal.

II. Patients and Methods

This was a descriptive and retrospective analytical study of all cases of tumors andvascular malformations operated on between 2004 and 2022. The parameters studied were age, sex, history, clinical presentation, imaging data (ultrasound, CT angiography), surgical protocol, morbidity, and mortality. The mean follow-up time was 3 years.

III. Results

The total number of patients was 52 over the 18 years. The sex ratio was 0.5. Themean age was 24 years, with a range of 2 to 72 years. Consanguinity was found in 8%. Klippel-Trenauney syndrome was found in 2%. Trauma or recent surgery was noted in 9 patients (17%). The mean time to consultation was 8 years, ranging from 3 months to 20 years. The reason for consultation was a mass in 94% of cases, pain (37%), a skinmark (2%), a sensation of trembling (4%), or a skin ulceration (8%). The physical examination revealed a mass in 47 patients. It was associated with vascular characters in 13 cases, skin ulceration in 4 cases, collateral venous circulation in 2 cases, and limbdeformity in 2 cases. The location of the lesions (Figure 1) predominated in the neck (Figure 2) and the head (54%). No multiple locations were noted. Vascular Doppler ultrasound was performed in 28 patients and found an arteriovenous malformation (n=11), an angioma (n=4), a venous malformation (n=2), alymphangioma (n=2), a glomus tumor (n=2), a hemangioma (n=2) or a vascular mass of undetermined nature (n=4). The ultrasound data were sufficient to make the diagnosis in 12 patients (23%). CT

Author σ ρ Ↄ ¥ §: Department of Thoracic and Cardiovascular Surgery, CHNU-Fann, Dakar, Senegal.

Author α: Ndeye Fatou Sow; Department of Thoracic and Cardiovascular Surgery, CHNU-Fann, Dakar, SenegalBP 5035, Tel: (+221) 77 681 01 31. e-mail: zenefes@gmail.com

angiography was performed in 40 patients, 24 of which (46%)were performed immediately without prior ultrasound. It revealedan arteriovenous malformation (n=22), a venous malformation (n=5), an angioma(n=4), a lymphangioma (n=4), a hemangioma (n=2), a glomus tumor (n=2) orangiosarcoma (n=1) (Figure 3). The therapeutic procedure consisted of excision (n=39), ligation of the feeding artery (n=15) or primary embolization (n=3). An additional procedure was performed in 6 patients. This consisted of elastic compression, sclerotherapy, additional ligation of the feeding artery, or embolization. The postoperative course was satisfactory in 88% of cases (Figure 4). Else where, theywere marked by hemorrhage with hematoma formation (4%) requiring revision, suppuration of the surgical site (6%) controlled by antibiotic therapy, and severe limbischemia (2%) for which forearm amputation was performed. Recurrence was noted in 8%. The average time to recurrence was 2 years and varied from 1 to 5 years. No deaths were observed.

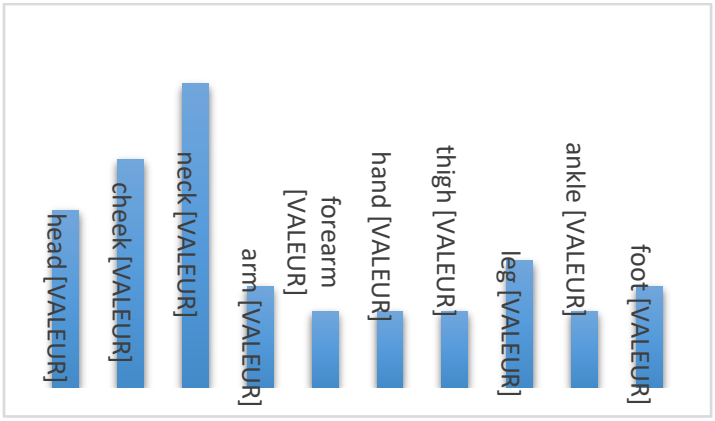

Figure 1: Topography of Lesions

Figure 2: Cervical AVM with Large Draining Vein

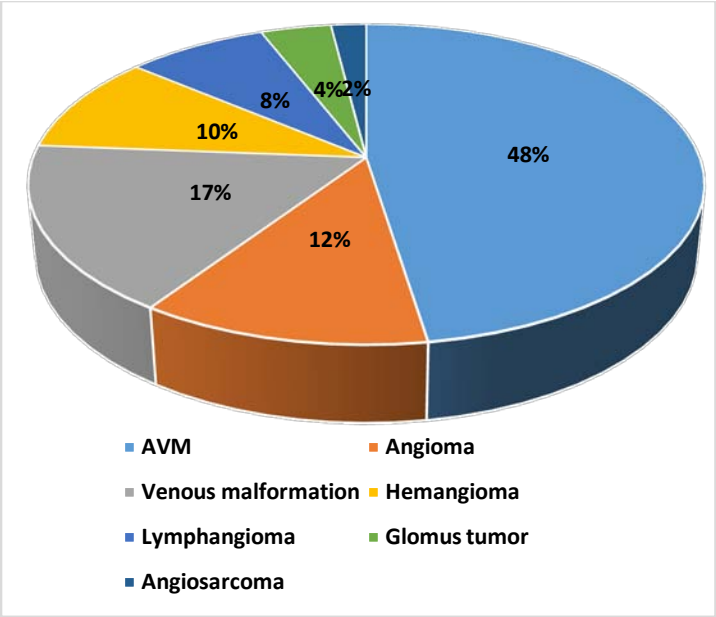

Figure 3: Ultrasound Data Combined with CT Angiography

Figure 4: Before and After Surgical Excision of a Labial AVM

IV. Discussion

Until 1970, the term angioma referred to all vascular anomalies. It was not until 1996 that the classification established by Mulliken and Glowacki in 1982 was validated bythe ISSVA (International Society for the Study of Vascular Anomalies), thus making itpossible to separate malformations from vascular tumors [2]. Depending on the vessel involved, there is an arterial, venous, or lymphatic anomaly. A vascular tumor is aproliferation of endothelial cells, while a vascular malformation results from an anomaly in the embryogenesis of the vessels, leading to wall alterations [3]. One in three childrenis born with a vascular spot, red, blue, or purple, which for the most part will disappear. One in a hundred children will retain this vascular anomaly, which will warrant medicaladvice [4]. The average age of our patients was 24 years. This result is different from those found in other literature where the average age varied between 3 and 5 years [5,6] forvascular tumors and 15 years for vascular malformations [7]. This age difference isexplained by the fact that in our country, patients tend to consult late. But also, for most surgical series like ours, the age of the surgical indication is retained and the latter is pushed back as much as possible. The female predominance reported in the literature is found in our

patients [7,8,9]. This female predominance could be explained by the fact that mothers of female infants consult more because of the aesthetic impactof the condition [10]. For both tumors and vascular malformations, we found a hereditary character. Couriveau in a retrospective study reports that vascular malformations are transmitted in an autosomal dominant manner [11]. Vascular anomalies can be latent for a long time, and their development can be triggered by factors such as trauma, surgery, or puberty, which was observed in 17% of our patients.

The main reason for consultation for vascular anomalies is the observation of a mass, posing a cosmetic problem or a source of social embarrassment. Sometimes,it is an ulceration, often linked to a delay in consultation. This was the case in 94% ofour patients. Vascular tumors and malformations are ubiquitous. Cervicocephalic locations are the most common as found in our study (53%) and those of several Western or African authors such as Belzunce (50%), Diarra (50%), and Casanova (75%) [7,8,12].

The size of vascular anomalies varies, ranging from a few millimeters to several centimeters. However, according to Casanova, hemangiomas smaller than 3 cm arethe most frequent and extensive forms are the rarest [12]. Certain vascular anomalies such as arteriovenous malformations (AVMs) can be revealed by rarer complications. Heartfailure in a healthy heart revealing an AVM has been described by Sow [13].

Diagnosing certain vascular tumors such as hemangiomas is essentially clinical, the contribution of medical imaging remains essential in other cases. Ultrasoundis the first-lineimaging modality. It is non-invasive, reproducible and inexpensive. It allows diagnosis in themajority of cases, but it remains operator-dependent. Twelve patients (23%) were operated on based on Doppler ultrasound. CT angiography is indicated in cases where ultrasound does not allow a decision. It provides additional information, particularly for AVMs, by outlining the feeding and drainage vessels, as well as the topography of the nidus. Johnson recommends starting with an MRI for the diagnosis of vascular malformations in general and arteriovenous malformations in particular [14]. He argued that MRI would allow treatment planning by characterizing the flow rates and the number of vessels involved in the malformation. In addition, it is a technique that visualizes soft tissues well and reduces radiation, especially in young subjects requiring follow-up. The criticism he reported for Doppler ultrasound was that itcould not accurately study deep lesions near bone structures or lesions containing air. Inour series, all patients benefited from either Doppler ultrasound, CT angiography, or both, in cases where Doppler ultrasound was not helpful. MRI was not performed in anycase because it is an expensive examination, sometimes unavailable, and the twoprevious examinations were sufficient for a positive diagnosis.

The therapeutic arsenalis broad with specific indications for each form. Therapeutic abstention with surveillanceis adopted in cases of hemangiomas by many teams [15,16]. This attitude is explainedby their potential for spontaneous regression. Ceballo describes a spontaneous regression of 30% of hemangiomas in 3 years, 50% in 5 years and 70% in 7 years [17]. Alazard in a study on 44 lymphatic malformations found a spontaneous regression in15% of cases [18]. Medical treatment with Bisoprolol is proposed as a first-line treatmentfor complex infantile hemangiomas. Other drugs such as corticosteroids, antimitotics (vincristine) can be used. Surgery in VMT is most often indicated after failure of well-conducted medical treatment or in case of major functional or vital risk for the patient [19]. In our series, primary excision was the main procedure for all anomalies combined, followed by primary ligation of the feeding artery for AVMs. Primary embolization was performed in 3 patients.

Complications can occur postoperatively. Diop describes hemorrhagic accidents and peripheral facial paralysis during MAV treatment of the face[19]. Mortality is zero in our series as in most surgical series [20,21].

V. Conclusion

MVDs are diverse and varied, sometimes little understood. They exhibit clinical, anatomical, and progressive polymorphism. They are mostly benign, with a few rarecases of malignancy described. While in the majority of cases, diagnosis is primarily clinical, medical imaging remains essential in cases of doubt. The prognosis of certaincomplex anomalies can be poor. Classifications are thus established tofacilitate the choice of the most appropriate treatment for each type of VMD.

Management by a multidisciplinary team consisting of a dermatologist, radiologist, vascular surgeon and plastic surgeon help reassure parents and ensure optimal treatment.

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Longitudinal Follow-Up to Assess Knowledge Retention and Practice Change of Mothers and Caregivers on Childhood Diarrhea in Zanzibar, Tanzania

By Dr. Kheir Makame

Objectives: To assess knowledge retention and practice change 6–12 months after initial training among mothers and caregivers of under-five children in Zanzibar, and to identify factors associated with sustained adoption.

GJMR-F Classification: *NLM Code: RJ218, RA440.5, RA427.8*

Longitudinal Follow-Up to Assess Knowledge Retention and Practice Change of Mothers and Caregivers on Childhood Diarrhea in Zanzibar, Tanzania

Dr. Kheir Makame

Objectives: To assess knowledge retention and practice change 6–12 months after initial training among mothers and caregivers of under-five children in Zanzibar, and to identify factors associated with sustained adoption.

I. Design and Setting

We will conduct a longitudinal follow-up of mothers and caregivers previously reached by a caregiver education and WASH promotion programme across multipledistricts in west urban region in Zanzibar (Urban district, West 'A' district and West 'B' district).

II. Population and Eligibility

The mothers/caregivers of under-five children reached by the programme.

III. Sampling and Sample size

Population proportion formula will employ using desired characteristics of 50% (Kheir et al, 2025) from knowledge and practice of mothers and caregivers on childhood diarrhea cases as calculated below.

Fishers' formula: n = Z2pq/r2 (Singh, Ajay & Masuku, 2014)

Where: n = Desired sample size; p = Proportion of the population with a desired characteristics which will be 50% (Edwin & Azage, 2019); q = 1; z = standard deviation desired degree of accuracy. Where z is 1.96 if the degree of confidence is 95%; r = Degree of error which will be 5%. Therefore: n was found to be 384. The reason of chosen 50% is same even there are no past studies that already did the same line, also 384 sample size is ethical to the study area are greater than 5000 population. Purpose random sampling is the type of method which will be used in the study toinvolves selectively individual or elements from a population based on specific criteria or purpose.

IV. Outcomes and Measures

- Correct oral rehydration solution preparation and use, recognition of dehydration/danger signs.
- Handwashing at critical times such as after using the toilet, before eating or preparing food, and after changing a child's diaper.
- Safe water storage and treatment methods such as boiling water.
- Timely care-seeking for diarrhea with danger signs. Danger signs include lethargy or unconsciousness, inability to drink or breastfeed, and vomiting everything
- Knowledge will be measured with a structured questionnaire, and practices by self-report corroborated with spot checks where feasible. Changes from baseline to follow-up will be analysed using mixed-effects models for repeated measures, accounting for clustering at community level and adjusting for key covariates. Subgroup analyses by district and caregiver characteristics are planned. Ethical approval and written informed consent will be obtained.

V. Data Collection Tools and Translations

The structured questionnaires, observation checklists and consent will be used as tools for data collection. The all-data collection tools will be prepared in English and translate into Swahili language which is mother tongue of mothers and caregivers.

VI. Field Procedures

A set of standardized instructions for conducting fieldwork is enumerator training, pilot, daily debriefs and referral protocol for danger signs.

VII. Data Quality Assurance

A systematic process for ensuring data is accurate, complete, consistent, and reliableis skip logic, supervisor review, re-interview rate and secure data handling.

VIII. Ethics

Ethical approval will be grant from the Zanzibar medical research ethics committee. Permission to conduct the study will be sought from the respective health centre authorities. The information about the study was given in writings, and study representative

Author: e-mail: zube.makame@gmail.com

explained the benefits, participation rights and freedom to withdraw from the study at any time. The consent will be obtained from mothers and caregivers aged above 18 years of age before collection of information. With regards to interview mothers and caregivers aged 15 to 17 years, a written informed consent will be obtained from a legal guardian for participants below 18 years. Both mothers and caregivers who above 18 years will be provided signed consents and the legal guardians sign assent form. The participants will assure of the confidentiality of the information of knowledge retention and practice change in the household prevention and management of childhood diarrhea. The information will be obtained from the participant will not intend to be used for any other purpose except for research study.

IX. Analysis plan

A detailed blueprint for a research study that outlines how data will be collected, organized, and analyzed to answer specific questions is repeated measures with clustering, covariate adjustment, planned subgroup analyses.

X. Timeline and Responsibilities

The study expected to be completed in 6 months and following activities will be carried out:

Research Activities	Two months				Two months				Two months			
	First month		Second month		Third month		Fourth month		Fifth month		Sixth month	
	First 2 weeks of Nov	Last 2 weeks ofNov	First 2 weeks ofDec	Last 2 weeks ofDec	First 2 weeks of Jan	Last 2 weeks ofJan	First 2 weeks of Feb	Last 2 weeks of Feb.	First 2 weeks of Mar	Last 2 weeks of Mar	First 2 weeks ofApr	Last 2 weeks of Apr
Completion ofrapid methodological												
Procurement and deploy materials for field work												
Enumerator training and pilot study												
Collection of data and field work practice												
Data analysis and interpretation												
Report preparation and Publication												

Questionnare

Longitudinal follow-up to assess knowledge retention and practice change of mothers and caregivers on childhood diarrhea in Zanzibar, Tanzania.

Clinics	Name	District	Date

	Address		
	Telephone No.		
	Questions	Categories	Coding
1	Mother or Caregivers	Mother Caregivers	1 2

2	Age	15 – 20 21 - 25 26 - 30 31 - 35 36 - 40 41 – 45	1 2 3 4 5 6
3	Level of mother education	Primary education Secondary education Tertiary education None	1 2 3 4
4	Mother occupation	Farmer Animals keeper Public employed Private employed Housewife Self employed	1 2 3 4 5
	Knowledgeretention		
5	Diarrheal disease	Frequent passing watery stool (3 or more stool) Frequent passing normal stool Blood in stools	1 2 3
6	Causes of Diarrheal disease	Teething Contaminated water Contaminated food Eaten faecal matter / faeces Evil eye	1 2 3 4 5
7	Danger sign of diarrheal disease	Becoming weak Repeated vomiting Fever and blood in the stool Marked thirst for water Other specify_____	1 2 3 4
8	Respondents' knowledge about the correct use of ORS	Do you understand to prepare ORS (homemade solution? Yes No If Yes how is ORS prepared? 1 sachet of ORS 300ml (1 coke bottle) of water 1sachet of ORS-500 ml (1small size of mineral bottle) of water 1 sachet of ORS – 600 ml (1 beer bottle) of water 1 sachet of ORS -1000 ml (11) of water 1 sachet of ORS – 1500 ml (1.5 or large size of mineral bottle) of water How often should ORS be given? Once a day 2-3 times a day Whatever child wants to drink After the passing of very loose stool How long should be mixed ORS last? 24 hours (1 day) 48 hours (2 days) 72 hours (3 days) 96 hours (4 days) Don't known	1 2 1 2 3 4 5 1 2 3 4 1 2 3 4 5
	Practices Change		
9	Drinking Water	Drinking treated or boiled water Not drinking treated or boiled water	1 2

10	Disposal of child waste in latrine	Disposal of child waste in latrine Not disposal child waste in latrine	1 2
11	Maternal feeding practices during child diarrheal disease	When (Name) had diarrhea, did you breastfeed him/her less than usual, about the same amount, or more than usual? Less Same More Child not breastfed Don't known When (Name) had diarrhea, was he/she offered less than usual to drink, about the same amount, or more than usual to drink? Less Same More Nothing to drink Don't known Was (Name) offered less than usual to eat, about the same amount, or more than usual to eat? Less Same More Nothing to eat Don't known When do you wash hands with soap? Before prepare food Before feeding children After helping children defecation Never Other_____	 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4
12	Mother care seeking behavior and place sought for care in this region	Did you seek advice or treatment from someone outside of the home for (Names) diarrhea? Yes No Where did you first go for advice or treatment? Hospital Health center Traditional practitioner Other specify_____	 1 2 1 2 3

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A FMRC member gets access to a closed network of Tier 1 researchers and scientists with direct communication channel through our website. Fellows can reach out to other members or researchers directly. They should also be open to reaching out by other.

Career | Credibility | Exclusive | Reputation

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Career | Credibility | Reputation

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Career | Financial

GJ Internal Account

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Career | Credibility | Reputation

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Financial

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Career | Credibility | Financial

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Early invitations to all the symposiums, seminars, conferences

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Exclusive

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ASSOCIATE OF MEDICAL RESEARCH COUNCIL is the membership of Global Journals awarded to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.

Benefits

To the institution

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Career | Credibility | Exclusive | Reputation

Certificate

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Career | Credibility | Exclusive | Reputation

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Associates can publish articles (limited) without any fees. Also, they can earn up to 30-40% of sales proceeds from the sale of reference/review books/literature/publishing of research paper

Exclusive | Financial

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Associate	Fellow	Research Group	Basic
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Certificate, LoR and Momento **2** discounted publishing/year **Gradation** of Research **10** research contacts/day **1 GB** Cloud Storage **GJ** Community Access	**Certificate,** LoR and Momento **Unlimited** discounted publishing/year **Gradation** of Research **Unlimited** research contacts/day **5 GB** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**Certificates,** LoRs and Momentos **Unlimited** free publishing/year **Gradation** of Research **Unlimited** research contacts/day **Unlimited** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**GJ** Community Access

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

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Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
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7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

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Plagiarism is not acceptable in Global Journals submissions at all.

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- Findings
- Writings
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- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

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1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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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.

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	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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