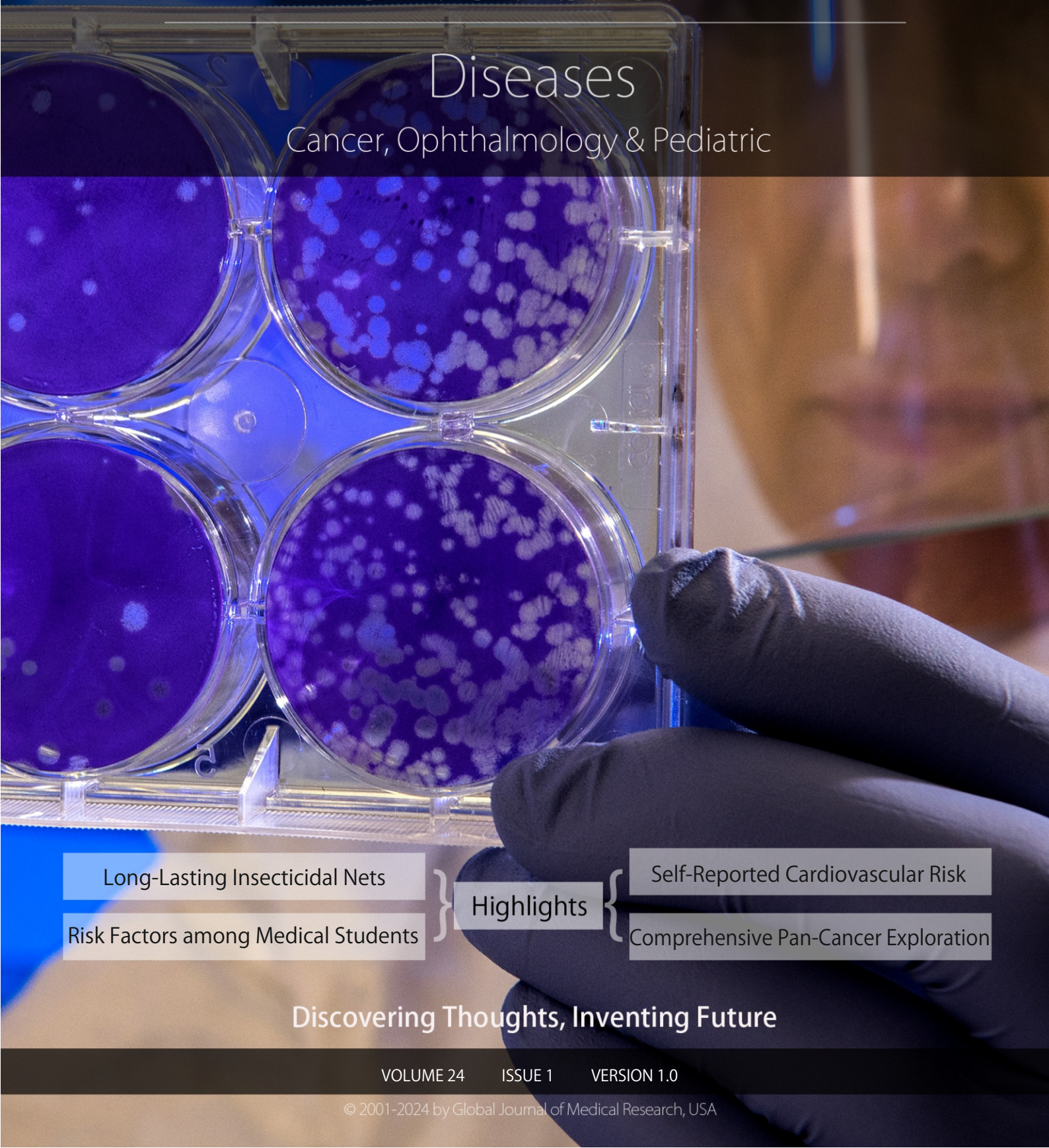
Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

# Global Journal

## of Medical Research: F

## Diseases

### Cancer, Ophthalmology & Pediatric



Long-Lasting Insecticidal Nets

Risk Factors among Medical Students

Highlights

Self-Reported Cardiovascular Risk

Comprehensive Pan-Cancer Exploration

Discovering Thoughts, Inventing Future

VOLUME 24 ISSUE 1 VERSION 1.0

Global Journal of Medical Research: F
Diseases
Cancer, Ophthalmology & Pediatric

Global Journal of Medical Research: F
Diseases
Cancer, Ophthalmology & Pediatric

Volume 24 Issue 1 (Ver. 1.0)

Open Association of Research Society

**Incorporation No.: 0423089**
**License No.: 42125/022010/1186**
**Registration No.: 430374**
**Import-Export Code: 1109007027**
**Employer Identification Number (EIN):**
**USA Tax ID: 98-0673427**

## Global Journals Inc.

*(A Delaware USA Incorporation with "Good Standing"; **Reg. Number: 0423089**)*

*Sponsors: Open Association of Research Society*
*Open Scientific Standards*

### *Publisher's Headquarters office*

Global Journals® Headquarters
945th Concord Streets,
Framingham Massachusetts Pin: 01701,
United States of America
*USA Toll Free: +001-888-839-7392*
*USA Toll Free Fax: +001-888-839-7392*

### *Offset Typesetting*

Global Journals Incorporated
2nd, Lansdowne, Lansdowne Rd., Croydon-Surrey,
Pin: CR9 2ER, United Kingdom

### *Packaging & Continental Dispatching*

Global Journals Pvt Ltd
E-3130 Sudama Nagar, Near Gopur Square,
Indore, M.P., Pin:452009, India

### *Find a correspondence nodal officer near you*

To find nodal officer of your country, please email us at *local@globaljournals.org*

### *eContacts*

Press Inquiries: *press@globaljournals.org*
Investor Inquiries: *investors@globaljournals.org*
Technical Support: *technology@globaljournals.org*
Media & Releases: *media@globaljournals.org*

### *Pricing (Excluding Air Parcel Charges):*

*Yearly Subscription (Personal & Institutional)*
250 USD (B/W) & 350 USD (Color)

# Editorial Board

## Global Journal of Medical Research

## Dr. Han-Xiang Deng

MD., Ph.D
Associate Professor and Research Department
Division of Neuromuscular Medicine
Davee Department of Neurology and Clinical Neurosciences
Northwestern University Feinberg School of Medicine
Web: neurology.northwestern.edu/faculty/deng.html

## Dr. Pina C. Sanelli

Associate Professor of Radiology
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic Radiology
M.D., State University of New York at Buffalo,
School of Medicine and Biomedical Sciences
Web: weillcornell.org/pinasanelli/

## Dr. Roberto Sanchez

Associate Professor
Department of Structural and Chemical Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University
Web: mountsinai.org/

## Dr. Michael R. Rudnick

M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and Hypertension Division (PMC)
Penn Medicine, University of Pennsylvania
Presbyterian Medical Center, Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of Internal Medicine
Web: uphs.upenn.edu/

## Dr. Feng Feng

Boston University
Microbiology
72 East Concord Street R702
Duke University
United States of America

## Dr. Seung-Yup Ku

M.D., Ph.D., Seoul National University Medical College,
Seoul, Korea Department of Obstetrics and Gynecology
Seoul National University Hospital, Seoul, Korea

## Dr. Hrushikesh Aphale

MDS- Orthodontics and Dentofacial Orthopedics.
Fellow- World Federation of Orthodontist, USA.

## Santhosh Kumar

Reader, Department of Periodontology,
Manipal University, Manipal

## Gaurav Singhal

Master of Tropical Veterinary Sciences, currently pursuing Ph.D in Medicine

## Dr. Aarti Garg

Bachelor of Dental Surgery (B.D.S.) M.D.S. in Pedodontics and Preventive Dentistr Pursuing Phd in Dentistry

### *Sabreena Safuan*

Ph.D (Pathology) MSc (Molecular Pathology and Toxicology) BSc (Biomedicine)

### *Getahun Asebe*

Veterinary medicine, Infectious diseases, Veterinary Public health, Animal Science

### *Dr. Suraj Agarwal*

Bachelor of dental Surgery Master of dental Surgery in Oromaxillofacial Radiology.
Diploma in Forensic Science & Oodntology

### *Osama Alali*

PhD in Orthodontics, Department of Orthodontics, School of Dentistry, University of Damascus. Damascus, Syria. 2013 Masters Degree in Orthodontics.

### *Prabudh Goel*

MCh (Pediatric Surgery, Gold Medalist), FISPU, FICS-IS

### *Raouf Hajji*

MD, Specialty Assistant Professor in Internal Medicine

### *Surekha Damineni*

Ph.D with Post Doctoral in Cancer Genetics

### *Arundhati Biswas*

MBBS, MS (General Surgery), FCPS, MCh, DNB (Neurosurgery)

### *Rui Pedro Pereira de Almeida*

Ph.D Student in Health Sciences program, MSc in Quality Management in Healthcare Facilities

### *Dr. Sunanda Sharma*

B.V.Sc.& AH, M.V.Sc (Animal Reproduction, Obstetrics & gynaecology), Ph.D.(Animal Reproduction, Obstetrics & gynaecology)

### *Shahanawaz SD*

Master of Physiotherapy in Neurology PhD- Pursuing in Neuro Physiotherapy Master of Physiotherapy in Hospital Management

### *Dr. Shabana Naz Shah*

PhD. in Pharmaceutical Chemistry

### *Vaishnavi V.K Vedam*

Master of dental surgery oral pathology

### *Tariq Aziz*

PhD Biotechnology in Progress

# Contents of the Issue

Global Journal of Medical Research: F
Diseases
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

# PPIB Unveiled: A Comprehensive Pan-Cancer Exploration Unraveling Immunological Signatures and Prognostic Implications

By Yan Ouyang, Qi Dai, Shengming Lai, Haiyan Huang, Yongsheng Huang & Shuwei Ren

*Sun Yat-Sen University*

*Abstract-* *Background:* Peptidylprolyl isomerase B (PPIB) has been shown to play an essential role in tumor initiation and progression. However, it lacks systematic analysis and evaluation of the effect of PPIB on pan-cancer.

*Methods:* The expression profile and survival analysis of PPIB in tumor tissues were demonstrated by the TIMER2.0, GEPIA2.0, and UALCAN online tools. The cBioportal, GSCA, TISDB, and TIMER2.0 databases were applied to analyze the correlation between PPIB and genetic variation, immune infiltration, and cancer-associated fibroblasts (CAFs), respectively. The STRING, GEPIA2.0, and TIMER2.0 databases were used to identify the co-expressed genes of PPIB. The DAVID online database was used for GO and KEGG pathway analysis.

*Results:* PPIB was highly expressed in 20 types of tumors. Upregulation of PPIB was associated with a poor prognosis of 6 types of tumors ($P<0.05$). In most cancers, the frequency of PPIB genetic variation is relatively low, and the common mutation types are missense mutations and splices.

*Keywords:* *PPIB, pan-cancer analysis, prognosis.*

*GJMR-F Classification:* *NLMC Code: QZ 202*


*Strictly as per the compliance and regulations of:*

# PPIB Unveiled: A Comprehensive Pan-Cancer Exploration Unraveling Immunological Signatures and Prognostic Implications

Yan Ouyang [α], Qi Dai [σ], Shengming Lai [ρ], Haiyan Huang [ω], Yongsheng Huang [¥] & Shuwei Ren [§]

*Abstract- Background:* Peptidylprolyl isomerase B (PPIB) has been shown to play an essential role in tumor initiation and progression. However, it lacks systematic analysis and evaluation of the effect of PPIB on pan-cancer.

*Methods:* The expression profile and survival analysis of PPIB in tumor tissues were demonstrated by the TIMER2.0, GEPIA2.0, and UALCAN online tools. The cBioportal, GSCA, TISDB, and TIMER2.0 databases were applied to analyze the correlation between PPIB and genetic variation, immune infiltration, and cancer-associated fibroblasts (CAFs), respectively. The STRING, GEPIA2.0, and TIMER2.0 databases were used to identify the co-expressed genes of PPIB. The DAVID online database was used for GO and KEGG pathway analysis.

*Results:* PPIB was highly expressed in 20 types of tumors. Upregulation of PPIB was associated with a poor prognosis of 6 types of tumors ($P<0.05$). In most cancers, the frequency of PPIB genetic variation is relatively low, and the common mutation types are missense mutations and splices. The expression of PPIB was significantly associated with copy number amplification and hypomethylation ($P<0.05$). PPIB expression was significantly correlated with infiltrating lymphocytes and CAFs ($P<0.05$). In the endoplasmic reticulum, PPIB and its co-expressed genes are mainly involved in protein processing. The molecular function of PPIB is especially protein folding.

*Conclusions:* It is revealed that the pan-cancer analysis of PPIB plays a pro-oncogenic role in tumorigenesis, which provides a new marker for the diagnosis and prognosis of cancers.

*Keywords: PPIB, pan-cancer analysis, prognosis.*

## I. Introduction

Cancers are now the leading cause of premature death in 127 countries. There is a trend that cancer may surpass cerebral vascular disease (CVD) as the leading cause of sudden death in most countries (Bray et al., 2021). According to the International Agency for Research on Cancer, 19.3 million new cancer cases and almost 10.0 million cancer deaths occurred in 2020. The global cancer burden is expected to be 28.4 million cases in 2040, a 47% rise from 2020 (Sung et al., 2021). Cancer is essentially a disease of the genome, which evolves and progresses with accumulations of somatic mutations, including copy-number alterations, structural variants, and epigenomic alterations (Nakagawa et al., 2018; Stratton et al., 2009). Thus, a pan-cancer analysis of cancer-associated genes will help understand their roles in cancer development.

We have identified abnormal expression of Peptidylprolyl isomerase B (PPIB) in various cancers through preliminary database analysis. PPIB is a member of the cyclosporine-binding protein and is mainly located within the endoplasmic reticulum. It is associated with the secretory pathway and released in biological fluids. This protein can bind to cells derived from T- and B-lymphocytes and may regulate the immunosuppressive drug cyclosporine A-mediated immunosuppression as a cell receptor protein. The molecular function of PPIB is to act as a peptidylprolyl anti-cis isomerase (PPIase), regulating the protein conformation of its substrate through propyl cis-trans isomerization in the endoplasmic reticulum lumen and nucleus, and participating in protein folding, secretion, and post-translational modification processes. PPIB could catalyze the cis-trans isomerization of xaa-proline bonds, a rate-limiting step in protein folding, which is required for proteome homeostasis (Hasel KW et al., 1991 Jul; Peddada LB et al., 1992).

There have been some relevant studies on the role of PPIB in cancer. A recent study found that UTMD-mediated miR-206 regulates the expression of apoptosis, migration, and invasion-related proteins by targeting PPIB, inhibiting the migration and invasion of hepatocellular cancer (LIHC) cells, and enabling cell apoptosis (Wu H et al., 2020 Jan-Dec). PPIB can enhance the JAK2/SAT3 signaling pathway in gastric cancer cells, thereby promoting the proliferation of cancer cells (Li T et al., 2017 Mar 1). Furthermore, PPIB can serve as a biomarker in the diagnosis and treatment of cancer. In ovarian cancer (OV) and head and neck squamous cell carcinoma (HNSC), PPIB is a potential prognostic marker (Pan X & X., 2020 Oct 15; Xin et al., 2021 Mar 29). PPIB induces chemotherapy resistance

*Author α ρ ω §: Department of Clinical Laboratory, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong 510655, China. e-mail: renshw3@mail.sysu.edu.cn*
*Author α ρ ω §: Biomedical Innovation Center, The Sixth Affiliated Hospital, Sun Yat-sen University.*
*Author σ: Department of Laboratory Medicine, School of Public Health, Xiamen University, Xiamen, Fujian, 361005, China.*
*Author ¥: Cellular & Molecular Diagnostics Center, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou 510120, China. e-mail: huangysh33@mail2.sysu.edu.cn*
*ORCID: 0000-0002-8449-7522*

by degrading wild-type p53 through its interaction with MDM2, which may serve as a predictive biomarker for chemotherapy resistance in the treatment of colorectal cancer (Choi et al., 2018 Sep). In addition, another study found that PPIB is also a novel indicator for evaluating lymph node metastasis in colorectal cancer (Yue et al., 2009 Oct).

This study focused on PPIB and utilized bioinformatics-related software and online databases to comprehensively analyze the differences and specific molecular mechanisms of PPIB in pan-cancer, providing a comprehensive understanding and sufficient basis for whether PPIB has the potential for therapeutic targets and detection markers in human cancers.

## II. Materials and Methods

*a) Research data sources*

The data for this study is sourced from TCGA and GTEx databases. We analyzed the expression level of PPIB and its correlation with CAFs using the TIMER2.0 database (http://timer.comp-genomics.org/). We studied the expression level of PPIB, expression differences in pathological staging, survival analysis, and correlation analysis of co-expressed genes using the GEPIA2 database (http://gepia.cancer-pku.cn/).We explored the protein expression levels of PPIB in different tumors using the UALCAN database (http://ualcan.path.uab.edu/). We investigated the genetic variation frequency and types of PPIB in various tumors using the cBioportal database (https://www.cbioportal.org/). We dissected the correlation between PPIB and methylation using the GSCA database (https://bioinfo.life.hust.edu.cn/gsca). We analyzed the correlation between PPIB and abundance of tumor-infiltrating lymphocytes by TISIDB database (http://cis.hku.hk/tisidb/index.php). We performed protein-protein interaction network (PPI) analysis using the STRING database (https://string-db.org/). We performed KEGG pathway and GO analysis of PPIB using the DAVID database (https://david.ncifcrf.gov/). The data and analysis provided by these databases and tools provide a foundation for us to comprehensively explore the role of PPIB in cancer.

*b) Analysis Methods*

i. *Expression Analysis of PPIB*

We used the "Gene-DE" module in TIMER2.0 to analyze the differential expression of PPIB in different tumors and adjacent normal tissues in the TCGA database (Li et al., 2020 Jul 2). Due to the loss of normal tissue samples of some tumors in the TIMER2.0 database, we used the GEPIA2 database for analysis and supplementation of tumors with differential expression of PPIB (Tang et al., 2019 Jul 2). By using the "Pathological Stage Plot" module tool and the "CPTAC" module in the UALCAN database, we analyzed the correlation between PPIB expression and clinical staging of different cancers and the protein expression of PPIB in various cancers (Chandrashekar et al., 2017 Aug). Comparisons between groups were performed using two independent sample t tests. The difference was statistically significant at $P<0.05$.

ii. *Survival Analysis of PPIB*

We analyzed the relationship between the high/low expression status of PPIB and overall survival (OS)/disease-free survival (DFS) using the "Survival Analysis" module of the GEPIA2 database(Tang et al., 2019 Jul 2).

iii. Genetic Variation of PPIB

We used the cBioportal database "TGCA Pan-Cancer Atlas Study" module to analyze the genetic variation of PPIB in different tumors and display specific domain mutation information. Simultaneously explored the relationship between PPIB mutations/non-mutations and survival (Cerami et al., 2012 May).

iv. *DNA methylation analysis of PPIB*

We analyzed the impact of methylation status on PPIB expression in different tumors through the "Mutation" module of the GSCA database (Liu et al., 2023 Jan 19).

v. *Analysis of the Correlation between PPIB and Tumor immune Microenvironment*

We used the "Gene" module of the "Immune" module in the TIMER2.0 database to analyze the relationship between PPIB and CAFs in different tumors. Using the "Lymphocyte" module of TISIDB database, we explored the relationship between the abundance of tumor infiltrating lymphocytes and PPIB expression in various tumors (Ru et al., 2019 Oct 15).

vi. *PPIB-related gene enrichment analysis*

We analyzed 50 proteins that interact with PPIB by using the STRING database "Protein by name" module (von Mering et al., 2003 Jan 1). Then, through the "Similar" module of the GEPIA2 database, we screened out the top 100 genes related to PPIB expression. We used the "Correlation Analysis" module to analyze the correlation between the top 5 genes with the highest correlation and PPIB expression. We analyzed the expression of the top 5 genes related to PPIB expression in different tumors using the "Gene Corr" module of the TIMER2.0 database. We used the DAVID database and the "OFFICIAL GENE SYN" module to screen PPIB expression-related genes from the GEPIA2 database for KEGG pathway analysis and GO analysis to provide a basis for exploring the specific molecular mechanisms and roles of PPIB in different tumors (Dennis et al., 2003).

## III. Results

*a) Analysis of expression patterns of PPIB*

To explore the clinical significance of PPIB in tumorigenesis, we utilized the "Gene-DE" module in the TIMER2.0 database. We supplemented it with the GEPIA2 database to analyze the expression profiles of PPIB at the mRNA level in 33 types of tumors. We found that PPIB was differentially expressed in 22 types of tumor tissues compared to corresponding normal tissues, up-regulation of PPIB happened in 20 types of tumors, including bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangio carcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma(KIRC), kidney renal papillary cell carcinoma (KIRP), Liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), uterine corpus endometrial carcinoma (UCEC), lymphoid neoplasm diffuse large B-cell lymphoma (DLBL), brain lower grade glioma (LGG), testicular germ cell tumors (TGCT), uterine carcinosarcoma (UCS). However, down-regulation of PPIB happened in 2 types of tumors, including kidney chromophobe (KICH) and thyroid carcinoma (THCA) (Figure 1A and 1B). This indicates that PPIB may play an inhibitory role in these two types of cancer, indicating the duality of PPIB in different cancers. BRCA1 is a protein factor that protects breast tissue cells from cancer invasion. However, a recent study suggests that this protein may also have the opposite effect: in another type of cancer-neuroblastoma, BRCA1 helps stabilize the tumor (Herold et al., 2019). Additionally, we also found that the protein level of PPIB was upregulated in four kinds of tumor tissues (COAD, KIRC, LUAD, UCEC). It was shown that PPIB plays a role as a carcinogen in the above tumors. Moreover, we analyzed the expression of PPIB in different tumor pathological stages. It was suggested that the upregulation of PPIB was significantly linked to the pathological stages of KICH, KIRC, UCS, TGCT, and THCA (Figure 1C). These results indicated that PPIB may be involved in promoting the metastasis progression in these cancers.

**Figure 1**

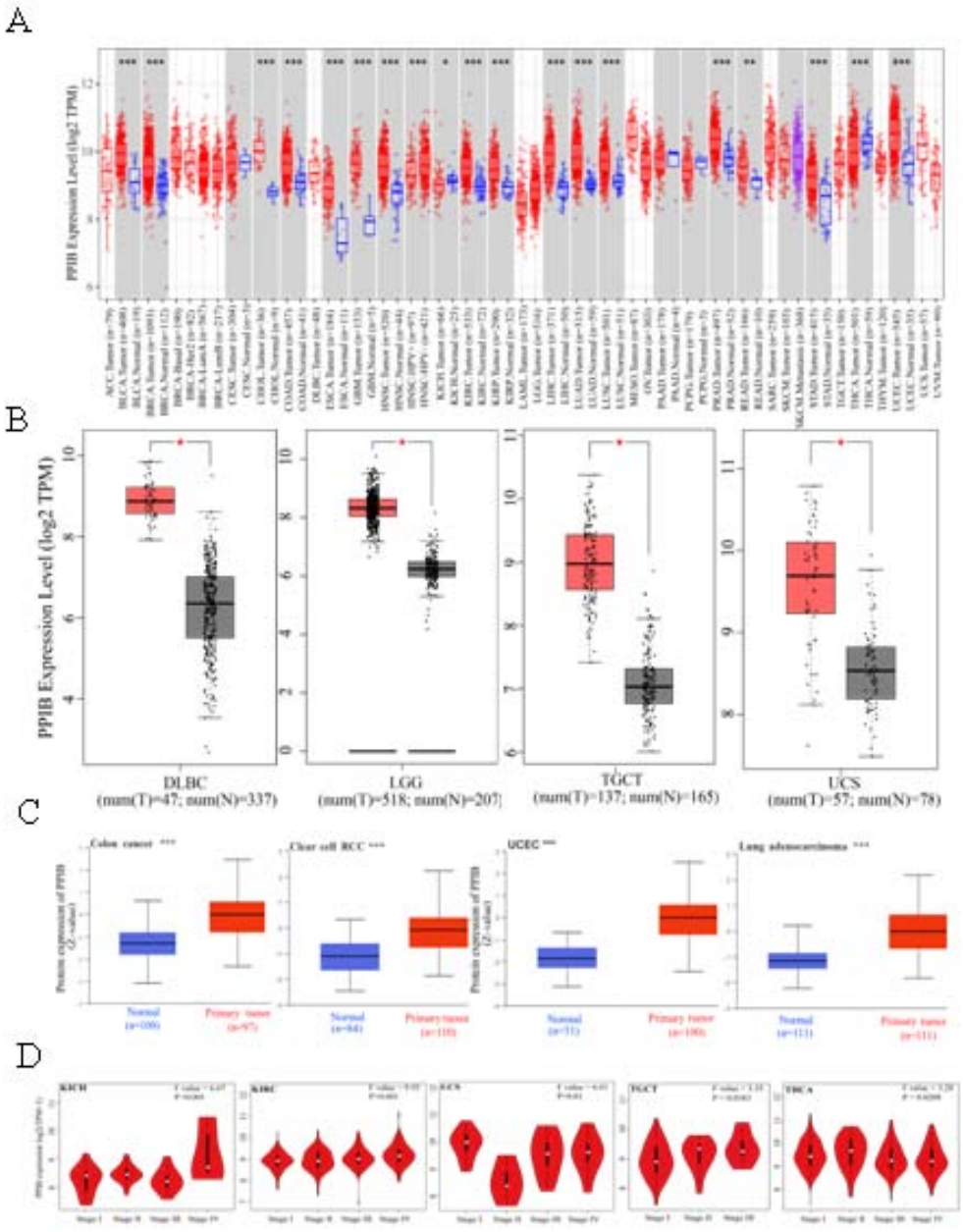



*Figure 1:* Expression profile of PPIB in different tumors

(A) Expression of PPIB in 33 types of tumor tissues.* P < 0.05; ** P <0.01; *** P <0.001.(B) Expression of PPIB in DLBC, THYM, TGCT and UCS tumor tissues and corresponding normal tissues.(C) Protein expression of PPIB in primary tumor tissues and corresponding normal tissues.(D) Relationship between expression of PPIB and pathological stage.

*b) Survival Analysis of PPIB*

We further explored the prognostic significance of PPIB among the 33 types of tumors based on the GEPIA2 database. It was found that high expression of PPIB is negatively correlated with overall survival (OS) in ACC, CESC, GBM, HNSC, KIRC, LGG, and UVM (Figure 2A). Simultaneously, disease-free survival (DFS) analysis showed that upregulation of PPIB expression is associated with poor prognosis in ACC, CESC, CHOL, HNSC, KIRC, LGG, and UVM (Figure 2B). These results indicated that PPIB is an independent prognostic marker of both DFS and OS in ACC, CESC, HNSC, KIRC, LGG, and UVM.

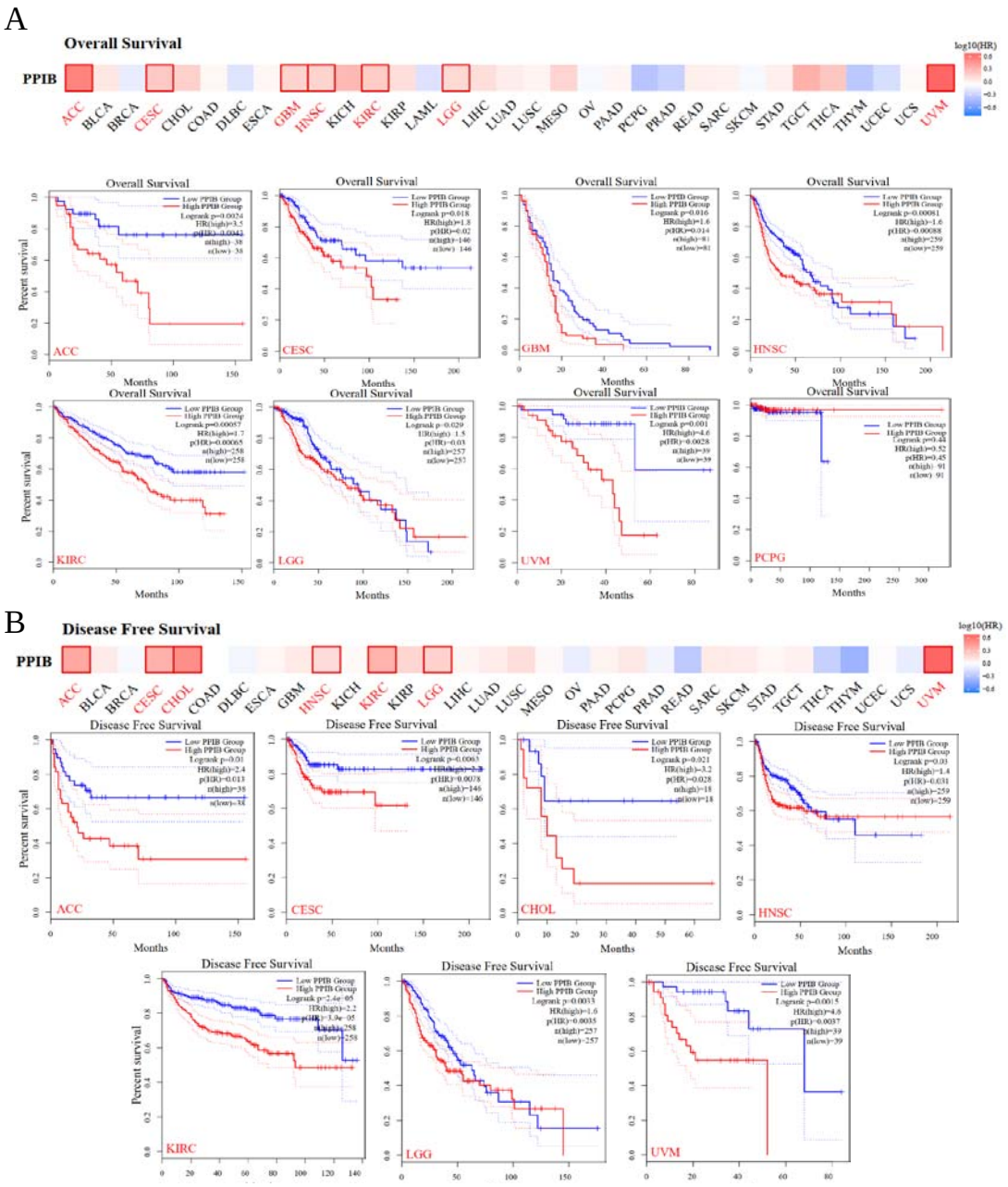



*Figure 2:* Survival analysis of PPIB in 33 types of tumors.
(A) Analysis of PPIB expression and overall survival in 33 types of tumors. (B)Analysis of PPIB expression and disease-free survival in 33 types of tumors.

*c) Genetic and Epigenetic Variation Analysis of PPIB*

We analyzed the genetic variation of PPIB in TCGA pan-cancer using the cBioportal database. As shown in Figure 3A, the overall genetic variation frequency of PPIB is relatively low. The highest alteration frequency of PPIB happened in UCEC (2.84%), with mutations (2.46%) being the predominant. However, it is worth noting that genetic variation is mainly amplification in MESO, KICH, SARC, and PRAD. In cases of gene variation in STAD, copy number deletion (0.68%) is the leading cause. Additionally, the genetic characteristics of PPIB were not observed in tumors such as LAML, ACC, CHOL, DLBC, GBM, and PCPG (Figure 3A).

We further investigated the gene mutation types, loci, and number of cases of PPIB. As shown in Figure 3B, the highest number of cases is missense mutations among the kinds of gene mutations of PPIB. The data represented that there have been cases of missense mutations in R95C/G in BLCA, ESCA, and COAD. The arginine (R) at position 95 in the "PRO_isomerase" domain is mutated to cysteine (C) or glycine (G). In LGG, SKCM, and STAD cases, X115 spliceosomes were found on the "PRO_isomerase" domain (Figure 3B).

According to the high proportion of copy number amplification in mutations, we evaluated the

relationship between PPIB expression and copy number amplification. We found a significant positive correlation between the expression levels of PPIB and copy number amplification in 20 types of cancer (FDR<0.05), including UCEC, READ, COAD, OV, LUAD and LUSC (FDR < 0.05) (Figure 3C). Research has shown that DNA promoter methylation plays a crucial role in tumor progression (Kulis & Esteller, 2010). We evaluated the DNA methylation patterns of PPIB based on the GSCA database. The expression level of PPIB is significantly negatively correlated with DNA methylation in 22 types of tumors (FDR < 0.05), including PRAD, BRCA, LGG, COAD, LUAD, HNSC (Figure 3D). According to the above data, DNA copy number amplification and methylation are the two underlying causes of PPIB upregulation in cancers.

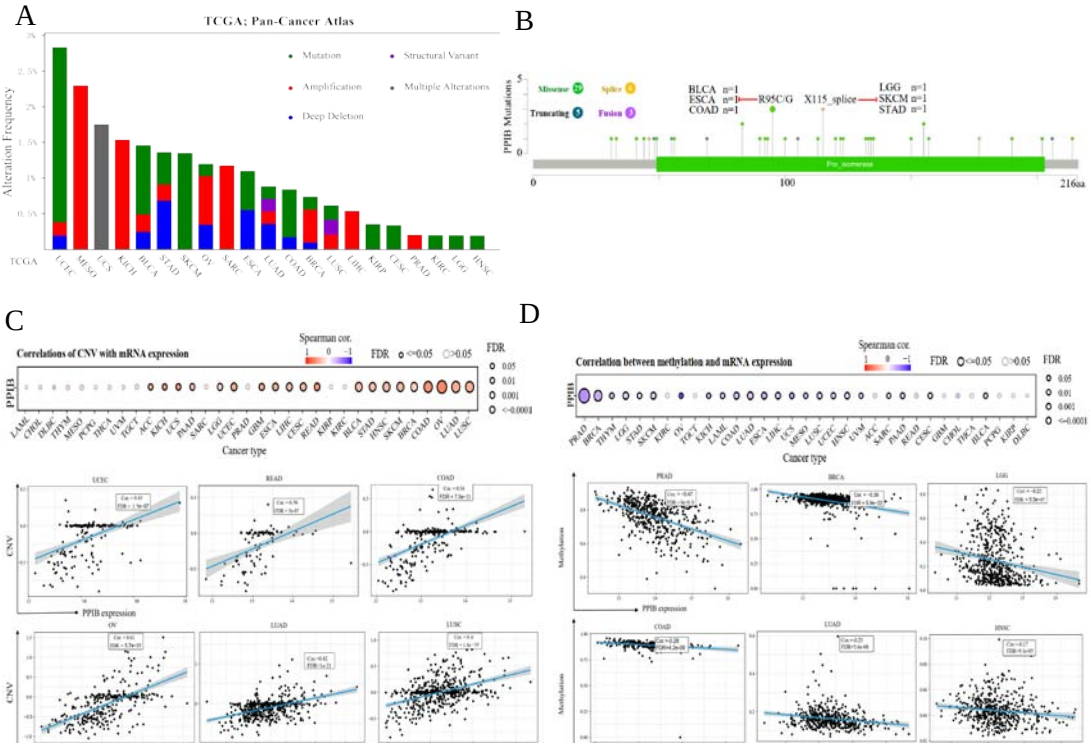



*Figure 3:* Genetic variation analysis of PPIB in tumors.

(A) Genetic variation types of PPIB in tumors. (B) Mutations in various domains of PPIB. (C) Correlation between copy number variation and PPIB expression. (D)Correlation between DNA methylation and PPIB expression.

*d) Correlation analysis between PPIB and the immune microenvironment*

Malignant solid tumor tissue contains not only cancer cells, but also normal stromal, infiltrating immune, epithelial, and vascular cells. It has been reported that infiltrating immune cells play essential roles in regulating tumor growth, metastasis, and drug resistance (Han et al., 2020 Jun). In this study, we used the TISIDB platform to explore the correlation between PPIB expression and infiltrating immune cells. It was found that PPIB expression was positively associated with activated CD8+ T cell(Act CD8), central memory CD8+ T cell (Tcm CD8), effect memory CD8+T cells (Tem CD8), effect memory (Tcm CD4), gamma delta T cells (Tgd), CD56bright/CD56dim NK cells, myeloid suppressive cells (MDSCs) and activated dendritic cells (Act DCs). Nevertheless, we observed a statistically negative correlation between PPIB expression and activated/immature/memory B cells, natural killer cells (NK), and eosinophils (EOS) (Figure 4A). Further analysis revealed that most types of adaptive immune cells (activated/central memory/effector memory CD4+/CD8+T cells, Tgd cells, Th1 cells, Treg cells, follicular helper T cells (Tfh), and activated/immature/ memory B cells) and cell types related to innate immunity (neutrophils, monocytes, macrophages, mast cells, activated, immature dendritic cells (DCs), NK cells, natural killer T cells (NKT), and MDSCs) are positively correlated with PPIB expression levels in LGG (Figure 4B). These results imply that NUTF2 promotes tumor progression by regulating infiltrating immune cells in LGG.

A kind of highly heterogeneous and hyper-activated fibroblast named CAFs has been demonstrated to promote tumor initiation, migration, inflammation, and drug resistance via the secretion of chemokines and cytokines, such as VEGFA and CXCL12(Guo et al., 2008 Jul 11; Nurmik et al., 2020 Feb 15). Our study utilized four algorithms (TIDE, XCELL, MCPCOUNTER, EPIC) based on the TIMER 2.0 tool to

analyze the correlation between PPIB expression and CAFs in various tumors. The results revealed that the expression level of PPIB is positively correlated with CAFs in BLCA, GBM, HNSC, HNSC-HPV-, KIRC, LGG, and TGCT (appeared in 4 algorithms). By contrast, the expression level of PPIB is negatively correlated with CAFs in PRAD (Figure 4C). These results implied that the correlation between PPIB expression and CAFs varies among different types of tumors.

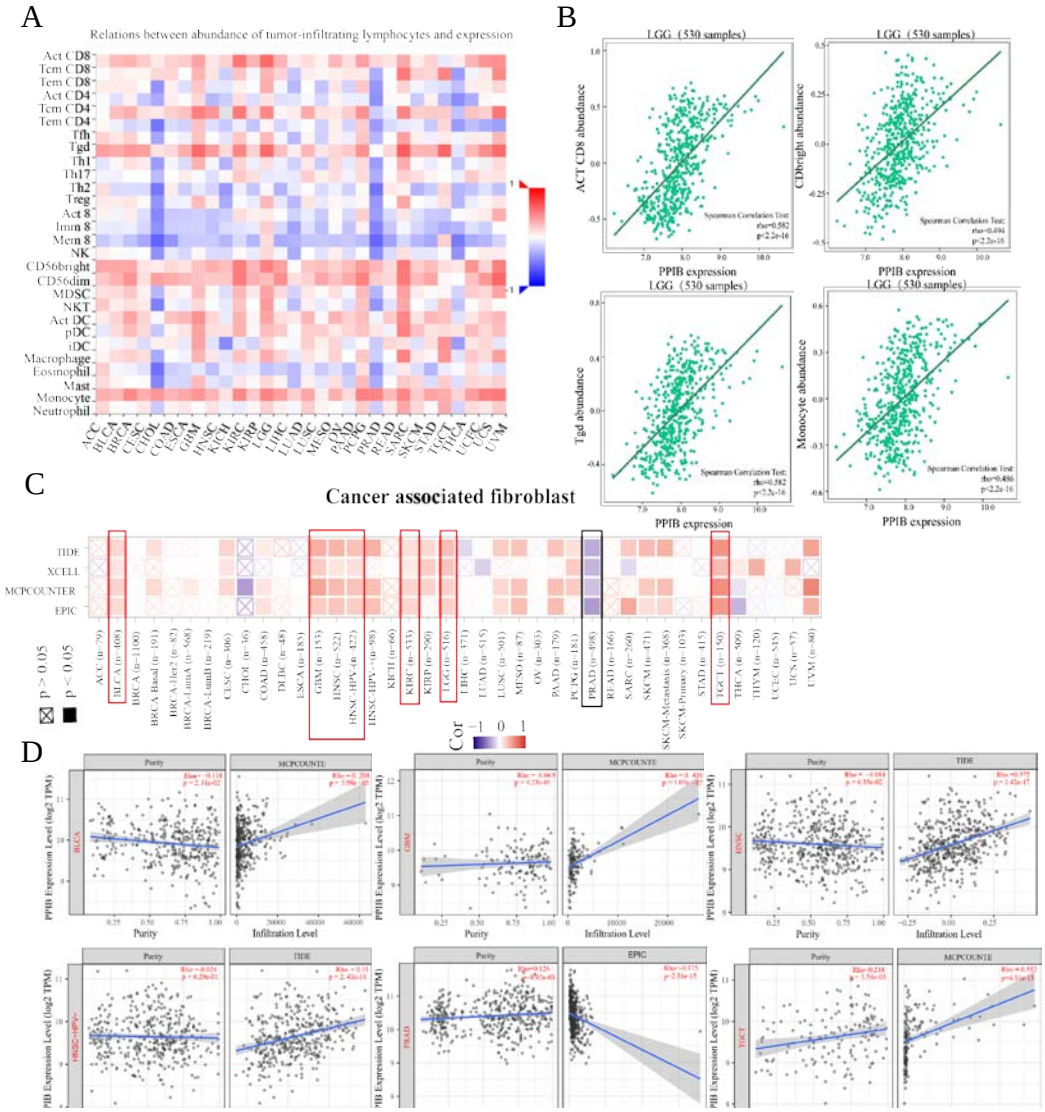



*Figure 4:* Analysis of PPIB in immune microenvironment.

(A) Correlation analysis between tumor infiltrating lymphocyte abundance and PPIB expression in tumors. (B) The expression of PPIB in LGG is correlated with the activation of CD8+T cells, central memory CD8+T cells, Tgd cells, CD56 positive cells, NKT cells, and monocytes. (C) Correlation between PPIB and CAFs calculated by four algorithms. (D) The top 6 tumors were positively correlated with expression level of PPIB.

*e) Enrichment Analysis of PPIB co-expression genes*

To investigate the potential molecular mechanisms of PPIB in tumor occurrence and development. In this study, we conducted protein-protein interaction network analysis (PPI) using the string online tool. As shown in Figure 5A, we obtained 50 PPIB binding proteins (Figure 5A). In addition, by combining the expression data of all TCGA tumors, we identified the top 100 genes most related to PPIB expression. Among them, five genes with high correlation with PPIB were screened: PDIA3 (R=0.64), MANF (R=0.63), SERF2 (R=0.58), CALR (R=0.57), and SEC61B (R=0.54). Moreover, we substituted the top 5 genes with high correlation into the TIMER2.0 database and analyzed that PPIB is positively correlated with their expression levels (Figure 5B).

We identified KEGG pathways related to cancer through functional enrichment analysis of the top 100 genes, such as protein processing in the endoplasmic reticulum, biosynthesis of N-glycans, and protein output

(Figure 5C). In addition, we conducted a Gene Ontology (GO) analysis on the relationship between PPIB and biological processes, cellular components, and molecular functions. Research has found that protein folding and protein disulfide isomerase activity may be involved in the role of PPIB in the cancer pathogenesis. These results revealed the possible molecular mechanisms and roles of PPIB in tumor pathogenesis (Figure 5D).

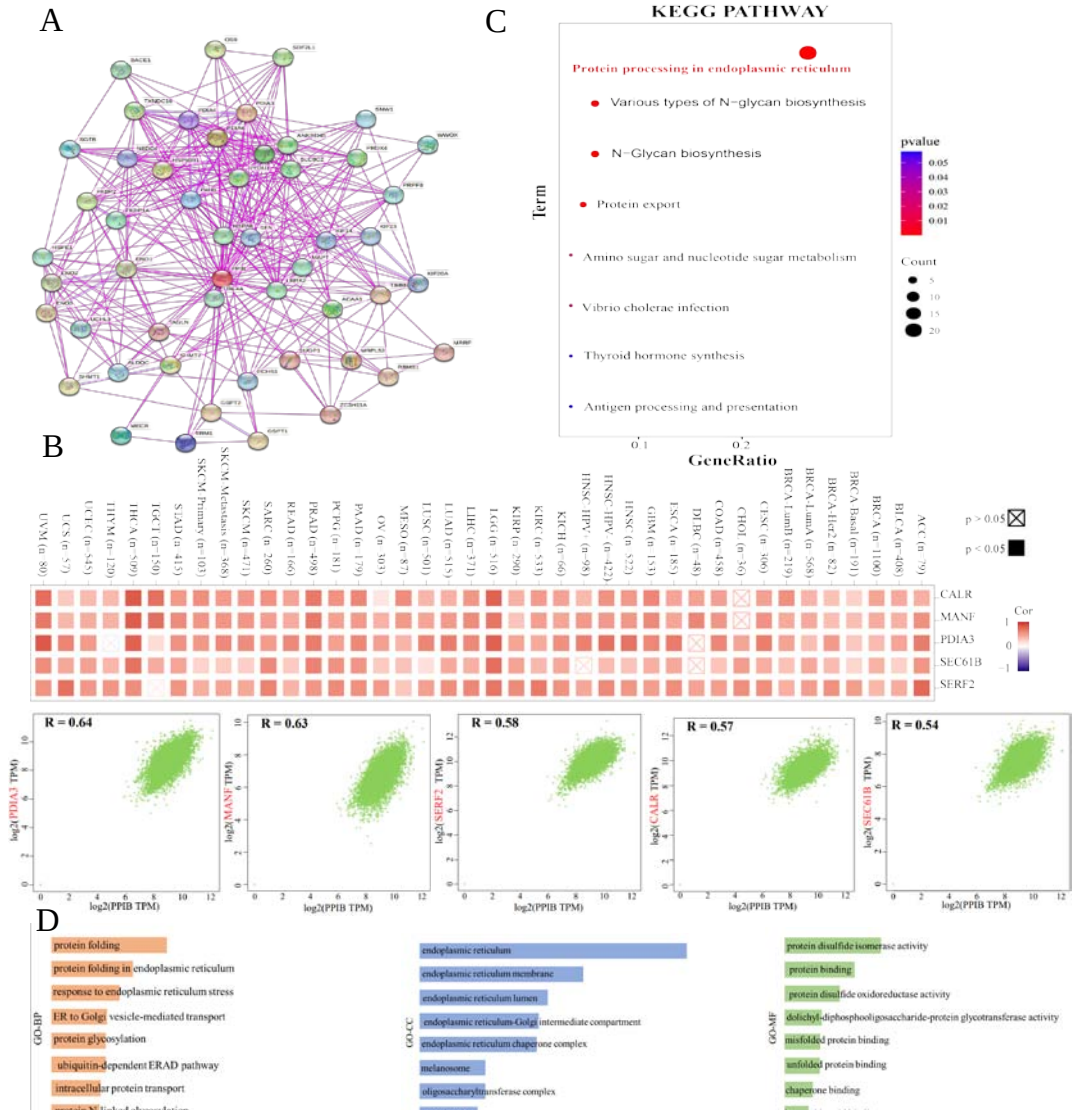



*Figure 5:* Analysis of molecular mechanism of PPIB in tumorigenesis.

(A) Analysis of protein-protein interaction network of PPIB. (B) Correlation between PPIB and the first five genes in different types of tumors. (C) KEGG pathway analysis of the top 200 genes that associated with PPIB expression. (D) Gene ontology analysis of the top 200 genes.

## IV. Discussion

Cancer has become one of the leading causes of increased human mortality. The process of cancer transformation is the result of cellular dysfunction. This is due to the accumulation of many genetic and epigenetic changes within cells, manifested as the accumulation of chromosomal or molecular abnormalities, leading to genetic instability (Peters & Gonzalez, 2018 Oct 1). For example, DNA methylation of SDC2 promotes tumor progression in colorectal cancer (Galamb et al., 2016). Exogenous and endogenous factors, as well as individual factors, including genetic susceptibility, contribute to the occurrence and development of cancer. Environmental chemicals interact with chemical metabolism and endogenous signaling. Exogenous chemicals can form reactive intermediates with DNA through toxic metabolic enzymes. If not repaired by DNA repair enzymes, it can cause mutations in critical genes, such as tumor suppressors or oncogenes, ultimately leading to the formation of precancerous cells (Lewandowska et al.). Amplification is mechanism of

activation of oncogenes such as fibroblast growth factor receptor 1 (FGFR1) and discoidin domain receptor 2 (DDR2) in SCC. Intriguingly, many of these genetic alternations are associated with smoking status (Cooper et al., 2013). The intrinsic molecular mechanisms of tumors are complex, and there is still a need to explore more potential molecular markers to study the mechanisms of tumor occurrence further.

This study conducted a multi-omics study on PPIB through pan-cancer analysis and found that based on the TGCA and GTEx databases, the expression level of PPIB was significantly upregulated in 20 types of cancer compared to normal tissues. Moreover, high expression levels of PPIB are associated with a poor prognosis. There are differences in the expression levels of PPIB among different pathological stages of KICH, KIRC, UCS, TGCT, and THCA. According to the above research, PPIB has a cancer-promoting effect. The high expression of PPIB in tumors indicates its potential as a tumor marker.

In the genetic variation study of PPIB, the expression level of PPIB is positively correlated with copy number amplification. But there is no significant correlation between PPIB mutations and prognosis. Therefore, we focused on the epigenetic mechanism analysis of PPIB. Methylation disorders are involved in many diseases, including human cancer. DNA methylation regulates the expression of target genes during transcription. Loss or silencing of promoter methylation leads to tumor progression (Dai et al., 2021 Mar 31; Ehrlich, 2019 Dec). Compared with normal tissues, the methylation level of PPIB is lower in multiple tumor types, and the expression level of PPIB is negatively correlated with the methylation level. Therefore, it can be considered that methylation of PPIB may be one of the important epigenetic mechanisms in tumor progression.

With the emergence and development of tumors, a series of soluble factors promote the influx of non-malignant cells (immune cells), blood vessels, and stroma, collectively becoming the tumor immune microenvironment (TME) (Bilotta et al., 2022 Oct 20). According to Weinberg et al, among the top ten characteristics of tumors, promoting angiogenesis, activating infiltration and metastasis, avoiding immune attacks, and promoting tumor inflammation are correlated with TME. These become essential factors in cancer cell behavior and disease progression (Hanahan & Weinberg, 2011 Mar 4). Immune cells and CAFs are essential components of TME. The importance of dynamically regulating cancer progression and influencing treatment outcomes is now widely recognized, and multiple treatment methods targeting various components of TME have been developed in recent years. Immunotherapy can inhibit the occurrence and development of tumors (Bejarano et al., 2021 Apr). The expression level of PPIB in most tumors was significantly correlated with most infiltrating immune cells in this study. Studies have shown that infiltration of CD8+T cells exerts anti-tumor effects, while B cell nuclear plasma cells can synergistically exert anti-tumor effects(Han et al., 2020 Jun; Wouters et al., 2018 Dec 15). Egelston et al. proved that CD8+tumor infiltrating lymphocytes are related to the excellent prognosis of triple-negative breast cancer (TNBC) (Egelston et al., 2022 Feb 8). CAFs are significant prognostic factors and therapeutic targets (Chen et al., 2021 Dec). In this study, the expression levels of PPIB were positively correlated with CAFs in BLCA, GBM, HNSC, HNSC-HPV-, KIRC, LGG, and TGCT. PPIB may be involved in the transformation and activation of CAFs. Contrary to the fact that PPIB plays anoncogenic role in most tumors, the results above indicate that PPIB in PRAD is negatively correlates with Treg cells and CAFs. It can be inferred that PPIB may play an anti-cancer role in PRAD. However, further specific research is needed.

This study explored the potential molecular mechanisms of PPIB's role in tumors through enrichment analysis of PPIB co-expressed genes. It was found that there was a significant correlation between PPIB and endoplasmic reticulum-related activities. Hasel et al. proposed that PPIB is located in the endoplasmic reticulum and is inhibited by cyclosporine A. Inhibition of PPIB in the endoplasmic reticulum may reduce the number of correctly folded proteins that ultimately reach the cell surface (Hasel KW et al., 1991 Jul). This research result is consistent with the PPIB mechanism research results. Wei X et al. found that cyclosporine A-mediated downregulation of PPIB, which can induce β apoptosis of pancreatic islet cells and occurrence of endoplasmic reticulum stress (Wei et al., 2018 Nov). It can be inferred that PPIB has a potential role in the endoplasmic reticulum stress pathway, and its value as a targeted factor for promoting cancer mechanisms is highly worthy of further research.

This study is the first to explore the mechanism of PPIB in tumors through pan-cancer analysis, statistical analysis of differential expression of PPIB, clinical prognosis, genetic analysis, immune infiltration analysis, and enrichment analysis. PPIB is differentially expressed in most tumors and is associated with a poor prognosis. This study links its mechanism of action in the endoplasmic reticulum and infers its potential and value as a targeted factor in tumor treatment. Based on the high correlation between PPIB and endoplasmic reticulum stress pathway, we will further explore the specific location and mechanism of PPIB in the endoplasmic reticulum stress pathway.

## Acknowledgments

We would like to thank the authors of previous studies and the staff members of the cBioPortal and

TCGA for providing available data. We thank the authors of previous studies for providing available data.

*Authors' contributions*

Study administration, validation, and design: Shuwei Ren, Yongsheng Huang and Haiyan Huang. Methodology, acquisition, and interpretation of data: Yan Ouyang, QiDai and Shengming Lai.

*Writing-original manuscript:* Yan Ouyang.

*Study supervision:* Shuwei Ren.

All authors read and approved the final manuscript.

*Funding*

This study was supported by funding from the National Natural Science Foundation of China (No.82203703 to Shuwei Ren) and the Guangdong Basic and Applied Basic Research Foundation (2021A1515111138 to Yongsheng Huang).

*Data availability*

Data are available in a public, open access repository.

*Declarations*

Ethics approval and informed consent

The study was conducted in accordance with the Helsinki Declaration.

*Consent for publication*

Not applicable.

*Competing interest*

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

*Availability of data and materials*

All of the data in this study were described in the "Methods" section.

## References Références Referencias

1. Bejarano, L., Jordão, M. J. C., & Joyce, J. A. (2021 Apr). Therapeutic Targeting of the Tumor Microenvironment. Cancer Discov, 11(4), 933-959. https://doi.org/10.1158/2159-8290.CD-20-1808
2. Bilotta, M. T., Antignani, A., & Fitzgerald, D. J. (2022 Oct 20). Managing the TME to improve the efficacy of cancer therapy. Front Immunol, 13, 954-992. https://doi.org/10.3389/fimmu.2022.954992
3. Bray, F., Laversanne, M., Weiderpass, E., & Soerjomataram, I. (2021). The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer, 127(16), 3029-3030. https://doi.org/10.1002/cncr.33587
4. Cerami, E., Gao J Fau - Dogrusoz, U., Dogrusoz U Fau - Gross, B. E., Gross Be Fau - Sumer, S. O., Sumer So Fau - Aksoy, B. A., Aksoy Ba Fau - Jacobsen, A., Jacobsen A Fau - Byrne, C. J., Byrne Cj Fau - Heuer, M. L., Heuer Ml Fau - Larsson, E., Larsson E Fau - Antipin, Y., Antipin Y Fau - Reva, B., Reva B Fau - Goldberg, A. P., Goldberg Ap Fau - Sander, C., Sander C Fau - Schultz, N., & Schultz, N. (2012 May). The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov, 2(5), 401-404. https://doi.org/10.1158/2159-8290.CD-12-0095
5. Chandrashekar, D. S., Bashel, B., Balasubramanya, S. A. H., Creighton, C. J., Ponce-Rodriguez, I., Chakravarthi, B., & Varambally, S. (2017 Aug). UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia, 19(8), 649-658. https://doi.org/10.1016/j.neo.2017.05.002. Epub 2017 Jul 18
6. Chen, Y. A.-O., McAndrews, K. A.-O., & Kalluri, R. A.-O. X. (2021 Dec). Clinical and therapeutic relevance of cancer-associated fibroblasts. Nat Rev Clin Oncol, 18(12), 792-804. https://doi.org/10.1038/s41571-021-00546-5
7. Choi, T. G., Nguyen, M. N., Kim, J., Jo, Y. H., Jang, M., Nguyen, N. N. Y., Yun, H. R., Choe, W., Kang, I., Ha, J., Tang, D. G., & Kim, S. A.-O. (2018 Sep). Cyclophilin B induces chemoresistance by degrading wild-type p53 via interaction with MDM2 in colorectal cancer. J Pathol, 246(1)(115-126). https://doi.org/10.1002/path.5107. Epub 2018 Aug 6
8. Cooper, W. A., Lam Dc Fau - O'Toole, S. A., O'Toole Sa Fau - Minna, J. D., & Minna, J. D. (2013). Molecular biology of lung cancer. Journal of thoracic disease, vol. 5(2072-1439 (Print)). https://doi.org/10.3978/j.issn.2072-1439.2013.08.03
9. Dai, X. A.-O., Ren, T., Zhang, Y., & Nan, N. (2021 Mar 31). Methylation multiplicity and its clinical values in cancer. Expert Rev Mol Med, 23, e2. https://doi.org/10.1017/erm.2021.4
10. Dennis, G., Jr., Sherman Bt Fau - Hosack, D. A., Hosack Da Fau - Yang, J., Yang J Fau - Gao, W., Gao W Fau - Lane, H. C., Lane Hc Fau - Lempicki, R. A., & Lempicki, R. A. (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol, 4(5), P3. https://doi.org/10.1093/nar/gkm415
11. Egelston, C. A., Guo, W., Tan, J., Avalos, C., Simons, D. L., Lim, M. H., Huang, Y. J., Nelson, M. S., Chowdhury, A., Schmolze, D. B., Yim, J. H., Kruper, L., Melstrom, L., Margolin, K., Mortimer, J. E., Yuan, Y., Waisman, J. R., & Lee, P. P. (2022 Feb 8). Tumor-infiltrating exhausted CD8+ T cells dictate reduced survival in premenopausal estrogen receptor-positive breast cancer. JCI Insight, 7(3), 2379-3708 https://doi.org/10.1172/jci.insight.153963
12. Ehrlich, M. (2019 Dec). DNA hypermethylation in disease: mechanisms and clinical relevance. Epigenetics, 14(12), 1141-1163. https://doi.org/10.1080/15592294.2019.1638701
13. Galamb, O., Kalmár, A., Barták, B. K., Patai Á, V., Leiszter, K., Péterfia, B., Wichmann, B., Valcz, G., Veres, G., Tulassay, Z., & Molnár, B. (2016). Aging related methylation influences the gene expression

of key control genes in colorectal cancer and adenoma. World journal of gastroenterology, 22 (47), 10325-10340. https://doi.org/10.3748/wjg.v22.i47.10325

14. Guo, X., Oshima H Fau - Kitmura, T., Kitmura T Fau - Taketo, M. M., Taketo Mm Fau - Oshima, M., & Oshima, M. (2008 Jul 11). Stromal fibroblasts activated by tumor cells promote angiogenesis in mouse gastric cancer. J Biol Chem, 283(28), 19864-19871. https://doi.org/10.1074/jbc.M800798200
15. Han, J., Khatwani, N., Searles, T. G., Turk, M. J., & Angeles, C. V. (2020 Jun). Memory CD8(+) T cell responses to cancer. Semin Immunol, 49( ), 1096-3618. https://doi.org/10.1016/j.smim.2020.101435
16. Hanahan, D., & Weinberg, R. A. (2011 Mar 4). Hallmarks of cancer: the next generation. Cell, 144(5), 646-674. https://doi.org/10.1016/j.cell.2011.02.013
17. Hasel KW, Glass JR, Godbout M, & JG., S. (1991 Jul). An endoplasmic reticulum-specific cyclophilin. Mol Cell Biol, 11(7), 3484-3491. https://doi.org/10.1128/mcb.11.7.3484-3491.1991
18. Herold, S., Kalb, J., Büchel, G., Ade, C. P., Baluapuri, A., Xu, J., Koster, J., Solvie, D., Carstensen, A., Klotz, C., Rodewald, S., Schülein-Völk, C., Dobbelstein, M., Wolf, E., Molenaar, J., Versteeg, R., Walz, S., & Eilers, M. (2019). Recruitment of BRCA1 limits MYCN-driven accumulation of stalled RNA polymerase. Nature, 567(7749), 545–549. https://doi.org/10.1038/s41586-019-1030-9
19. Kulis, M., & Esteller, M. (2010). DNA methylation and cancer. Adv Genet, 70, 27-56. https://doi.org/10.1016/B978-0-12-380866-0.60002-2
20. Lewandowska, A. M., Rudzki, M., Rudzki, S., Lewandowski, T., & Laskowska, B. Environmental risk factors for cancer - review paper. Ann Agric Environ Med, 26(1), 1-7. https://doi.org/10.26444/aaem/94299
21. Li T, Guo H, Zhao X, Jin J, Zhang L, Li H, Lu Y, Nie Y, Wu K, Shi Y, & D, F. (2017 Mar 1). Gastric Cancer Cell Proliferation and Survival Is Enabled by a Cyclophilin B/STAT3/miR-520d-5p Signaling Feedback Loop. Cancer Res, 77(5), 1227-1240. https://doi.org/10.1158/0008-5472.CAN-16-0357
22. Li, T., Fu, J., Zeng, Z., Cohen, D., Li, J., Chen, Q., Li, B., & Liu, X. S. (2020 Jul 2). TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res, 48(W1), W509-W514. https://doi.org/10.1093/nar/gkaa407
23. Liu, C. A.-O., Hu, F. A.-O., Xie, G. A.-O., Miao, Y. A.-O., Li, X. W., Zeng, Y., & Guo, A. A.-O. (2023 Jan 19). GSCA: an integrated platform for gene set cancer analysis at genomic, pharmacogenomic and immunogenomic levels. Brief Bioinform, 24(1). https://doi.org/10.1093/bib/bbac558
24. Nakagawa, Hidewaki, Fujita, & Masashi. (2018). Whole genome sequencing analysis for cancer genomics and precision medicine. Cancer Science, 109(3), 513-522. https://doi.org/10.1111/cas.13505
25. Nurmik, M., Ullmann, P., Rodriguez, F., Haan, S., & Letellier, E. A.-O. (2020 Feb 15). In search of definitions: Cancer-associated fibroblasts and their markers. Int J Cancer, 146(4), 895-905. https://doi.org/10.1002/ijc.32193
26. Pan X, & X., M. (2020 Oct 15). A Novel Six-Gene Signature for Prognosis Prediction in Ovarian Cancer. Front Genet, 11, 1006. https://doi.org/10.3389/fgene.2020.01006
27. Peddada LB, McPherson JD, Law R, W. J., Youderian P, & RJ, D. (1992). Somatic cell mapping of the human cyclophilin B gene (PPIB) to chromosome 15. Cytogenct Cell Genet, 60(3-4), 219-221. https://doi.org/10.1159/000133343
28. Peters, J. M., & Gonzalez, F. J. (2018 Oct 1). The Evolution of Carcinogenesis. Toxicol Sci, 165(2), 272-276. https://doi.org/10.1093/toxsci/kfy184
29. Ru, B., Wong, C. N., Tong, Y., Zhong, J. Y., Zhong, S. S. W., Wu, W. C., Chu, K. C., Wong, C. Y., Lau, C. Y., Chen, I., Chan, N. W., & Zhang, J. (2019 Oct 15). TISIDB: an integrated repository portal for tumor-immune system interactions. Bioinformatics, 35(20), 4200-4202. https://doi.org/10.1093/bioinformatics/btz210
30. Stratton, R., M., Campbell, J., P., Futreal, & Andrew, P. (2009). The cancer genome. Nature, 458(7239), 719-724. https://doi.org/10.1038/nature07943
31. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 71(3), 209-249. https://doi.org/10.3322/caac.21660
32. Tang, Z., Kang, B., Li, C., Chen, T., & Zhang, Z. (2019 Jul 2). GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res, 47(W1), W556-W560. https://doi.org/10.1093/nar/gkz430
33. von Mering, C., Huynen M Fau - Jaeggi, D., Jaeggi D Fau - Schmidt, S., Schmidt S Fau - Bork, P., Bork P Fau - Snel, B., & Snel, B. (2003 Jan 1). STRING: a database of predicted functional associations between proteins. Nucleic Acids Res, 31(1), 258-261. https://doi.org/10.1093/nar/gkg034
34. Wei, X., Zhu, D., Feng, C., Chen, G., Mao, X., Wang, Q., Wang, J., & Liu, C. (2018 Nov). Inhibition of peptidyl-prolyl cis-trans isomerase B mediates cyclosporin A-induced apoptosis of islet β cells. Exp Ther Med, 16(5), 3959-3964. https://doi.org/10.3892/etm.2018.6706
35. Wouters, Auid-Orcid, M., Nelson, & H., B. (2018 Dec 15). Prognostic Significance of Tumor-Infiltrating B

Cells and Plasma Cells in Human Cancer. Clin Cancer Res, 24(24), 6125-6135. https://doi.org/10.1158/1078-0432.CCR-18-1481

36. Wu H, Xie D, Yang Y, Yang Q, Shi X, & R, Y. (2020 Jan-Dec). Ultrasound-Targeted Microbubble Destruction-Mediated miR-206 Overexpression Promotes Apoptosis and Inhibits Metastasis of Hepatocellular Carcinoma Cells Via Targeting PPIB. Technol Cancer Res Treat, 19(1533-0338). https://doi.org/10.1177/1533033820959355
37. Xin, W., Zhao, C., Jiang, L., Pei, D., Zhao, L., & Zhang, C. (2021 Mar 29). Identification of a Novel Epithelial-Mesenchymal Transition Gene Signature Predicting Survival in Patients With HNSCC. Pathol Oncol Res, 27, 585192. https://doi.org/10.3389/pore.2021.585192
38. Yue, F., Wang Ls Fau - Xia, L., Xia L Fau - Wang, X.-L., Wang Xl Fau - Feng, B., Feng B Fau - Lu, A.-G., Lu Ag Fau - Chen, G.-Q., Chen Gq Fau - Zheng, M.-H., & Zheng, M. H. ( 2009 Oct). Modulated T-complex protein 1 ζ and peptidyl-prolyl cis-trans isomerase B are two novel indicators for evaluating lymph node metastasis in colorectal cancer: Evidence from proteomics and bioinformatics. Proteomics Clin Appl, 3(10), 1225-1235. https://doi.org/10.1002/prca.200900028. Epub 2009 Aug 4

Global Journal of Medical Research: F
Diseases
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

# Self-Reported Cardiovascular Risk Factors among Medical Students

By Jéssica de Paula Chalup Junqueira, Lais de Souza Rodrigues,
Maria Aparecida de Almeida Souza Rodrigues & Ivana Picone Borges de Aragão

*University of Vassouras*

*Summary-* Cardiovascular diseases are the leading cause of death in the world, with smoking, diabetes, high blood pressure, dyslipidemia and a sedentary lifestyle being among the main risk factors. The objective of this study was to evaluate the self knowledge about cardiovascular risk factors among medical students.

*Methodology:* Observational and cross-sectional study on students from the first to the eighth semester of medicine using anonymous questionnaires containing questions about cardiovascular risk factors.

*Results:* A total of 288 students paticipeted. There was a higher percentage of self-declared emotional stress ($p<0.001$), and high blood pressure ($p=0.007$), family history of coronary heart disease (CAD) or stroke (0.049) in the female group; The group under 20 years old practiced more physical activity ($p=0.023$) and reported a balanced diet ($p=0.047$), the age group between 20 and 29 years old reported a higher percentage of smoking ($p=0.001$) and alcohol consumption ($p=0.048$), those older than 30 years old reported a higher knowledge about family history of CAD or stroke.

*Keywords: cardiovascular diseases, primary cardiovascular prevention, cardiovascular risk factors, risk assessment.*

*GJMR-F Classification: NLMC Code: WG 120*


*Strictly as per the compliance and regulations of:*

# Self-Reported Cardiovascular Risk Factors among Medical Students

Jéssica de Paula Chalup Junqueira [α], Lais de Souza Rodrigues [σ],
Maria Aparecida de Almeida Souza Rodrigues [ρ] & Ivana Picone Borges de Aragão [ω]

*Summary-* Cardiovascular diseases are the leading cause of death in the world, with smoking, diabetes, high blood pressure, dyslipidemia and a sedentary lifestyle being among the main risk factors. The objective of this study was to evaluate the self knowledge about cardiovascular risk factors among medical students.

*Methodology:* Observational and cross-sectional study on students from the first to the eighth semester of medicine using anonymous questionnaires containing questions about cardiovascular risk factors.

*Results:* A total of 288 students paticipeted. There was a higher percentage of self-declared emotional stress ($p<0.001$), and high blood pressure ($p=0.007$), family history of coronary heart disease (CAD) or stroke (0.049) in the female group; The group under 20 years old practiced more physical activity ($p=0.023$) and reported a balanced diet ($p=0.047$), the age group between 20 and 29 years old reported a higher percentage of smoking ($p=0.001$) and alcohol consumption ($p=0.048$), those older than 30 years old reported a higher knowledge about family history of CAD or stroke. Students at the clinical level of the medical course, between the fifth and eighth semesters, demonstrated a higher percentage of smoking ($p=0.001$) and alcohol consumption ($p=0.030$).

*Conclusion:* This study demonstrated that smoking and alcohol consumption are higher among those who were older than 30 years and who were attending more advanced semesters of the medical course. Female students and those older than 30 years demonstrated higher knowledge about family history of coronary artery disease or stroke. Female medical students self-reported emotional stress and higher blood pressure levels. It is important to have curricular activities with the participation of students in activities related to health promotion aiming at primary prevention.

*Keywords: cardiovascular diseases, primary cardiovascular prevention, cardiovascular risk factors, risk assessment.*

*Author α σ: Medical student at the University of Vassouras (UNIVASSOURAS), Vassouras, RJ – Brazil.*
*e-mails: jessicachalup90@gmail.com, laissrodrigues205@hotmail.com*
*ORCID: https://orcid.org/0009-0009-6541-7281*
*ORCID: https://orcid.org/0009-0008-3496-1885*
*Author ρ ω: Professor at the Faculty of Medicine of the University of Vassouras (UNIVASSOURAS), Vassouras, RJ – Brazil.*
*e-mails: mariasouza@cardiol.br, ivanapbaragao@gmail.com*
*ORCID: https://orcid.org/0009-0007-4200-9766*
*ORCID https://orcid.org/0000-0002-4295-0165*

## I. Introduction

Coronary heart disease (CAD) has a high prevalence throughout the world, representing a global public health challenge. Its incidence varies between regions and countries, influenced by socioeconomic factors, lifestyles, and genetic characteristics. In industrialized nations, the prevalence is often higher due to unhealthy diets, increased obesity, a sedentary lifestyle, and a higher incidence of cardiovascular risk factors such as hypertension and diabetes. However, there is growing concern in developing countries, where urbanization, and the Westernization of lifestyle habits contribute to the increase in the prevalence of coronary disease. Understanding this worldwide distribution is crucial to direct prevention strategies, early detection and effective treatment, seeking to reduce the impact of CAD on population health globally. (WHO, 2024)

Atherosclerosis is a condition of significant importance, given its relationship with cardiovascular diseases, which are one of the leading causes of morbidity and mortality worldwide. The vascular endothelium is the starting point for the formation of the atherosclerotic plaque, through its aggregation caused by cardiovascular risk factors such as dyslipidemia, high blood pressure or smoking, among others. Consequently, there is an increase in the permeability of the intima to plasma lipoproteins, mainly LDL, which undergo oxidation. Configuring the beginning of atherogenesis, it occurs in a proportional to the concentration of these lipoproteins in plasma. (VISSEREN et al, 2021) (FALUDI et al, 2017) (PRÉCORA et al, 2021) (ARNETT et al, 2019)

Would medical students be able to recognize cardiovascular risk factors? The present study had the objective of evaluate self-knowledge about atherosclerotic risk factors among medical students. As they will be able to contribute early to primary cardiovascular prevention, functioning as knowledge multipliers, in addition to increasing their awareness on the topic.

## II. Methods

Observational and cross-sectional study through the application of an anonymous questionnaire on cardiovascular risk factors in medical students,

carried out between October and December 2023 due to the inter-exam period of the academic semester, after signing the free and informed consent form (FICF) by the participants. This study was pproved by the Research Ethics Committee, registered on Plataforma Brasil (PLATAFORMA BRAIL), number 4,482,980.

Those who did not agree to sign were excluded. The participants included in this study were all medical students enrolled at the university, regardless of gender and age, who were studying medicine between the first and eighth semesters.

The total group was divided into three types of groups to carry out statistical analysis: 1- according to the level of the medical course: basic level, between the first and fourth semester of medicine, and the clinical level, between the fifth and eighth semester of medical course; 2- according to the age: below 20 years old, between 20 and 29 years old, over 30 years old; 3- according to biological sex: female and male. The level of the course was chosen taking into account the level of knowledge of medical students. The age ranges were chosen based on the predominance in the course and the possible degree of academic maturity. Biological sex was chosen due to the difference in the prevalence of cardiovascular disease in men and women.

Anonymous questionnaires were used to collect data to preserve the identity of the participants, The questionnaire was filled out by the volunteer student and immediately delivered to one of the research authors. The FICF was signed in advance and handed over to another author, in order to guarantee confidentiality and to be unlinked. Anonymity aimed to guarantee the spontaneity and reliability of responses, avoiding embarrassment for those who ignored them. However, it may have contributed to less care in the responses.

The questionnaire was inspired by previous work on raising awareness of cardiovascular risk factors in pregnant women in the gynecology, S and obstetrics outpatient clinic. (JENNIFER et al, 2020) Questionnaire contains 20 quick answer questions about age, sex, declared ethnicity, weight, height, dyslipidemia, diabetes, high blood pressure, family history of coronary artery disease (CAD), or stroke, physical activity, own perception of emotional stress, personal history of CAD or stroke, smoking, alcoholism, balanced diet, considered DASH diet. The answers were based on the student's perception, and knowledge. (BRICANELLO et al, 2020)

For the sample calculation, the total number of students enrolled at the medical school was considered equal to 1,138, an error of 5%, reliability of 95% and percentage frequencies estimated at 50%, a value that maximizes the sample size. The calculation was carried out using the EPI-INFO program version 7.3.2.1. The research was carried out with 288 students who answered the questions in full, except for references to the memory of the values of the biochemical parameters from the last exam carried out.

The only variables with missing information were recorded in the biochemical test values remembered by the students and statistical calculations were performed with the exact number of biochemical test values reported.

Data were calculated using the number of questionnaires available, regardless of whether they were complete. There was double typing to avoid errors and the available data was used, without replacing missing answers. Data was entered into the Microsoft EXCEL (https://www.microsoft.com/pt-br/microsoft-365/free-office-online-for-the-web) spreadsheet and the computer program used to obtain the statistical calculations was IBM SPSS in version 25 (https://www.ibm.com/docs/en/spss-statistics/25.0.0).

Categorical variables were expressed by absolute frequencies and percentages, and in the numerical variables by statistical measures: mean, standard deviation, minimum value, P25, median, P75 and maximum value. Association between two categorical variables Pearson's Chi-square test or Fisher's Exact test was used when the condition for using the Chi-square test was not verified. In the case of the association study between cardiac risk presented dichotomously (yes or no) with the variables of interest, the OR (Odds Ratio) and respective confidence interval were obtained in each cross. Confidence intervals were also obtained for the mean of each numerical variable. The confidence level used in the decision of the statistical tests was 5.0%, with a significant association being considered if $p < 0.05$ and the intervals were obtained with 95% confidence. The only variables with missing information were those recorded relating to the values of biochemical tests, SBP and DBP not mentioned by the interviewees. Statistical calculations were performed with the exact number of values reported. (DOUGLAS, 1991) (CONOVER, 1999).

## III. Results

The age of the patients studied ranged from 18 to 53 years, mean 23.12 years, standard deviation of 5.19 years, confidence interval for mean age varied between 22,52 and 23, 72 years, and median of 22.00 years. Table 1 shows that two-thirds (66.7%) of the sample were between 20 and 29 years old, the majority (65.6%) were in the basic level of the course of medicine, and the remaining 34.4% the clinical level; the majority (63.9%) were female; the majority (75.3%) were those self-declared as white.

*Table 1:* Self-declared datas about of age, level of medical course, biological sex and color by medical students

| Variable | n (%) |
|---|---|
| Total | 288 (100,0) |
| **Age range** | |
| 18 to 19 | 65 (22,6) |
| 20 to 29 | 192 (66,7) |
| 30 or more | 31 (10,8) |
| **Cycle** | |
| Basic | 189 (65,6) |
| Clinical | 99 (34,4) |
| **Sex** | |
| Masculine | 104 (36,1) |
| Feminine | 184 (63,9) |
| **Self-declared color** | |
| White | 217 (75,3) |
| Brown | 54 (18,8) |
| Black | 17 (5,9) |

*Source: the author*

As shown in table 2, the majority (62.5%) of the group reported practicing physical activity at least 150 minutes per week; the majority (59.4%) considered themselves stressed person; execept for three patients with CAD and one with stroke, the remaining 98.6% denied a personal history of CAD or stroke; a little more than half (51.0%) had a family history of CAD or stroke and 8.3% did not know whether or not they had a family history; the percentage that had a smoking habit was 14.9% and that of alcoholism was 13.2%. It was possible to verify that the majority (67.7%) stated that they had a balanced diet; the percentage who stated that they had total cholesterol greater than 190 mg/dL was 6.3% and 42.0% did not know how to provide information; the prevalence of HDL lower than 40 mg/dL was 5.2% and the majority (54.5%) did not know how to inform the HDL value; the percentage frequency with blood pressure greater than 120 x 80 mmHg was 4.5%; it was recorded that only one patient stated that he had diabetes mellitus, of which 8.3% did not know.

*Table 2:* Self-declared clinical data by medical students

| Variable | n (%) |
|---|---|
| Total | 288 (100,0) |
| **Practice physical activity at least 150 minutes/week** | |
| Yes | 180 (62,5) |
| No | 108 (37,5) |
| **Self-declared as a stressed person** | |
| Yes | 171 (59,4) |
| No | 117 (40,6) |
| **Personal history of cardiovascular disease** | |
| CAD | 3 (1,0) |
| STROKE | 1 (0,3) |
| Deny | 284 (98,6) |
| **Family history of cardiovascular disease** | |
| Yes | 147 (51,0) |
| No | 117 (40,6) |
| **Smoking** | 24 (8,3) |
| Yes | 43 (14,9) |
| No | 245 (85,1) |
| **Alcoholism** | |
| Yes | 38 (13,2) |
| No | 250 (86,8) |
| **Medical evaluation** | |
| Yes | 36 (12,5) |
| No | 252 (87,5) |
| **Balanced diet** | |
| Yes | 195 (67,7) |
| No | 93 (32,3) |
| **Reports total cholesterol more than 190 mg/dL** | |
| Yes | 18 (6,3) |
| No | 149 (51,7) |

| | |
|---|---|
| Do not know | 121 (42,0) |
| **Report HDL less than 40 mg/dL** | |
| Yes | 15 (5,2) |
| No | 116 (40,3) |
| Do not know | 157 (54,5) |
| **Reports LDL greater than 130 mg/dL** | |
| Yes | 5 (1,7) |
| No | 122 (42,4) |
| Do not know | 161 (55,9) |
| **Reports blood pressure greater than 120/80** | |
| Yes | 13 (4,5) |
| No | 261 (90,6) |
| Do not know | 14 (4,9) |
| **Diabetes Mellitus** | |
| Yes | 1 (0,3) |
| No | 263 (91,3) |
| Do not know | 24 (8,3) |

*Source: the author*

Data of weight, height and body mass index (BMI), total cholesterol, HDL cholesterol and LDL cholesterol, systolic and diastolic blood pressure (SBP) (DBP) and glucose were mentioned in table 3. The variability of the standard deviation was reduced in analyzed variables of table 3, since the value was less than one-third of the corresponding means, except for HDL cholesterol.

*Table 3:* Self-reported data on anthropometric and laboratory measurements by medical students.

| Variable | Average | SD | CI 95% to mean | Minimum | P25 | Median | P75 | Maximum |
|---|---|---|---|---|---|---|---|---|
| Weight (n = 288) (kilogram) | 69,74 | 15,29 | 67.97 to 71.51 | 43,00 | 59,25 | 67,00 | 78,00 | 140,00 |
| Height (n = 288) | **1.69** | 0,09 | 1,68 to 1.70 | 1,48 | 1,62 | 1,69 | 1,75 | 1,97 |
| BMI (n = 288) | 24,37 | 4,24 | 23.88 to 24.86 | 16,42 | 21,36 | 23,62 | 26,30 | 43,21 |
| Total cholesterol (n = 43) mg/dL | 177,72 | 42,76 | 164.56 to 190.88 | 90 | 150,00 | 180,00 | 205,00 | 290 |
| HDL (n = 37) mg/dL | 57,78 | 19,40 | 51.32 to 64.25 | 29 | 46,00 | 51,00 | 64,50 | 120 |
| LDL col (n=26) mg/dL | 109,28 | 33,78 | 95.63 to 122.92 | 20 | 89,00 | 114,00 | 125,50 | 170 |
| SBP (n = 125) mmHG | 113,81 | 10,55 | 111,94 to 115.68 | 90 | 110,00 | 110,00 | 120,00 | 150 |
| DBP (n = 125) mmHg | 75,95 | 8,58 | 74.43 to 77.47 | 50 | 70,00 | 80,00 | 80,00 | 100 |
| Glucose (n = 75) mg/dL | 84,52 | 17,62 | 80.47 to 88.57 | 2 | 80,00 | 86,00 | 90,00 | 150 |

*Source: the author.*

The results of crossing each of the questions: practice of physical activity, consider yourself stressed, family history, smoking, alcoholism, balanced diet, high total cholesterol, low HDL cholesterol, high LDL cholesterol and high blood pressure with gender, age, and level of the course of medicine groups are described in tables 4 to 7.

Table 4 demonstrated significant associations ($p < 0.05$) were verified for the level of significance considered (5%) between sex and self-reported stress and family history. percentage of self-report stress was higher among female than male respondents (67.4% x 45.2%); the percentage who responded positively to family history was higher in the group of male students than female students (56.0% x 42.3%) and the opposite happened with those who responded negatively to the question, which was higher among males (50 .0% x 35.3%).

Information on the level of high blood pressure demonstrated an association with sex, with a higher prevalence in males than females (9.6% x 1.6%) and, on the contrary, it occurred with those who did not have the problem, with a higher value in females (93.5% x 85.6%).

*Table 4:* Assessment of variables: physical activity, stress, family history, smoking, alcoholism, cholesterol data, according to biological sex

| variable | biological sex | | | P value |
|---|---|---|---|---|
| | male n (%) | female n (%) | Total group n (%) | |
| Total | 104 (100,0) | 184 (100,0) | 288 (100,0) | |
| **Practice physical activity** | | | | p (1) = 1,000 |
| Yes | 65 (62,5) | 115 (62,5) | 180 (62,5) | |
| No | 39 (37,5) | 69 (37,5) | 108 (37,5) | |
| **Self-declared stress** | | | | p (1) < 0,001* |
| Yes | 47 (45,2) | 124 (67,4) | 171 (59,4) | |
| No | 57 (54,8) | 60 (32,6) | 117 (40,6) | |
| **Family history CV disease** | | | | p (1) = 0,049* |
| Yes | 44 (42,3) | 103 (56,0) | 147 (51,0) | |
| No | 52 (50,0) | 65 (35,3) | 117 (40,6) | |
| Do not know | 8 (7,7) | 16 (8,7) | 24 (8,3) | |
| **Smoking** | | | | p (1) = 0,060 |
| Yes | 21 (20,2) | 22 (12,0) | 43 (14,9) | |
| No | 83 (79,8) | 162 (88,0) | 245 (85,1) | |
| **Alcoholism** | | | | p (1) = 0,121 |
| Yes | 18 (17,3) | 20 (10,9) | 38 (13,2) | |
| No | 86 (82,7) | 164 (89,1) | 250 (86,8) | |
| **Balanced diet** | | | | p (1) = 0,678 |
| Yes | 72 (69,2) | 123 (66,8) | 195 (67,7) | |
| No | 32 (30,8) | 61 (33,2) | 93 (32,3) | |
| **High cholesterol** | | | | p (1) = 0,100 |
| Yes | 3 (2,9) | 15 (8,2) | 18 (6,3) | |
| No | 51 (49,0) | 98 (53,3) | 149 (51,7) | |
| Do not know | 50 (48,1) | 71 (38,6) | 121 (42,0) | |
| **low HDL** | | | | p (1) = 0,054 |
| Yes | 3 (2,9) | 12 (6,5) | 15 (5,2) | |
| No | 35 (33,7) | 81 (44,0) | 116 (40,3) | |
| Do not know | 66 (63,5) | 91 (49,5) | 157 (54,5) | |
| **Elevated LDL** | | | | p (2) = 0,077 |
| Yes | 1 (1,0) | 4 (2,2) | 5 (1,7) | |
| No | 36 (34,6) | 86 (46,7) | 122 (42,4) | |
| Do not know | 67 (64,4) | 94 (51,1) | 161 (55,9) | |
| **High blood pressure** | | | | p (1) = 0,007* |
| Yes | 10 (9,6) | 3 (1,6) | 13 (4,5) | |
| No | 89 (85,6) | 172 (93,5) | 261 (90,6) | |
| Do not know | 5 (4,8) | 9 (4,9) | 14 (4,9) | |

*(*) Significant association at 5%. (1) Pearson's Chi-square test. (2) Fisher's Exact Test.*

*Source: the author*

Table 5 shows a significant association between age group with practice of physical activity, family history, smoking, alcoholism, showing significant differences: the percentage who said they practiced physical activity was lower (41.9%) among students aged 30 or over, it was higher among those aged 18 to 19 (70.8%); the prevalence of reported family history was lower (43.1%) among students aged 18 to 19 and ranged from 53.1% to 54.8% in the other two age groups; the percentage of smoking was highest (21.4%) in the 20 to 20 age group and ranged from 1.5% to 3.2% in the other two age groups; the percentage of those with a drinking habit was higher (16.7%) in the 20 to 29 age group. Balanced eating was lowest among students aged 30 or over (48.4%) and ranged from 69.3% to 72.3% in the other two age groups.

*Table 5:* Assessment of variables: physical health, personal and family history, smoking, alcoholism, according to age group

| | age group | | | | |
|---|---|---|---|---|---|
| variable | 18 to 19 | 20 to 29 | 30 ou more | Total group | P value |
| | n (%) | n (%) | n (%) | n (%) | |
| Total | 65 (100,0) | 192 (100,0) | 31 (100,0) | 288 (100,0) | |
| Practice physical activity | | | | | p (1) =0,023* |
| Yes | 46 (70,8) | 121 (63,0) | 13 (41,9) | 180 (62,5) | |
| No | 19 (29,2) | 71 (37,0) | 18 (58,1) | 108 (37,5) | |
| Self-declared stress | | | | | p (1) =0,587 |
| Yes | 35 (53,8) | 117 (60,9) | 19 (61,3) | 171 (59,4) | |
| No | 30 (46,2) | 75 (39,1) | 12 (38,7) | 117 (40,6) | |
| Family history | | | | | p (1) =0,020* |
| Yes | 28 (43,1) | 102 (53,1) | 17 (54,8) | 147 (51,0) | |
| No | 25 (38,5) | 79 (41,1) | 13 (41,9) | 117 (40,6) | |
| Do not know | 12 (18,5) | 11 (5,7) | 1 (3,2) | 24 (8,3) | |
| Smoking | | | | | p (1) <0,001* |
| Yes | 1 (1,5) | 41 (21,4) | 1 (3,2) | 43 (14,9) | |
| No | 64 (98,5) | 151 (78,6) | 30 (96,8) | 245 (85,1) | |
| Alcoholism | | | | | p (1) =0,048* |
| Yes | 4 (6,2) | 32 (16,7) | 2 (6,5) | 38 (13,2) | |
| No | 61 (93,8) | 160 (83,3) | 29 (93,5) | 250 (86,8) | |
| Balanced diet | | | | | p (1) =0,047* |
| Yes | 47 (72,3) | 133 (69,3) | 15 (48,4) | 195 (67,7) | |
| No | 18 (27,7) | 59 (30,7) | 16 (51,6) | 93 (32,3) | |
| High cholesterol | | | | | p (2) =0,317 |
| Yes | 3 (4,6) | 13 (6,8) | 2 (6,5) | 18 (6,3) | |
| No | 31 (47,7) | 97 (50,5) | 21 (67,7) | 149 (51,7) | |
| Do not know | 31 (47,7) | 82 (42,7) | 8 (25,8) | 121 (42,0) | |
| low HDL | | | | | p (2) =0,116 |
| Yes | 5 (7,7) | 9 (4,7) | 1 (3,2) | 15 (5,2) | |
| No | 25 (38,5) | 72 (37,5) | 19 (61,3) | 116 (40,3) | |
| Do not know | 35 (53,8) | 111 (57,8) | 11 (35,5) | 157 (54,5) | |
| Elevated LDL | | | | | p (2) =0,433 |
| Yes | 0 (0,0) | 4 (2,1) | 1 (3,2) | 5 (1,7) | |
| No | 29 (44,6) | 77 (40,1) | 16 (51,6) | 122 (42,4) | |
| Do not know | 36 (55,4) | 111 (57,8) | 14 (45,2) | 161 (55,9) | |
| High blood pressure | | | | | p (2) =0,759 |
| Yes | 3 (4,6) | 8 (4,2) | 2 (6,5) | 13 (4,5) | |
| No | 59 (90,8) | 173 (90,1) | 29 (93,5) | 261 (90,6) | |
| Do not know | 3 (4,6) | 11 (5,7) | 0 (0,0) | 14 (4,9) | |

*(*) Significant association at 5%. (1) Pearson's Chi-square test. (2) Fisher's Exact Test.*

*Source: the author*

Table 6 showed a significant association between the level of medical school with smoking and alcohol consumption, percentages were higher among students in the clinical level, when compared with basic level (24.2% x 10.1% for smoking and 19.2% x 10.1% for alcohol consumption). No significant associations ($p > 0.05$) were recorded between the cycle and self-reported clinical variables.

*Table 6:* Assessment of data variables according to the stage of the medical course.

| Variable | basic level n (%) | clinical level n (%) | Total group n (%) | P value |
|---|---|---|---|---|
| Total | 189 (100,0) | 99 (100,0) | 288 (100,0) | |
| Practice physical activity | | | | p (1) = 0,321 |
| Yes | 122 (64,6) | 58 (58,6) | 180 (62,5) | |
| No | 67 (35,4) | 41 (41,4) | 108 (37,5) | |
| Self-declaration of stress | | | | p (1) = 0,116 |
| Yes | 106 (56,1) | 65 (65,7) | 171 (59,4) | |
| No | 83 (43,9) | 34 (34,3) | 117 (40,6) | |
| Family history | | | | p (1) = 0,122 |
| Yes | 97 (51,3) | 50 (50,5) | 147 (51,0) | |
| No | 72 (38,1) | 45 (45,5) | 117 (40,6) | |
| Do not know | 20 (10,6) | 4 (4,0) | 24 (8,3) | |
| Smoking | | | | p (1) = 0,001* |
| Yes | 19 (10,1) | 24 (24,2) | 43 (14,9) | |
| No | 170 (89,9) | 75 (75,8) | 245 (85,1) | |
| Alcoholism | | | | p (1) = 0,030* |
| Yes | 19 (10,1) | 19 (19,2) | 38 (13,2) | |
| No | 170 (89,9) | 80 (80,8) | 250 (86,8) | |
| Balanced diet | | | | p (1) = 0,601 |
| Yes | 126 (66,7) | 69 (69,7) | 195 (67,7) | |
| No | 63 (33,3) | 30 (30,3) | 93 (32,3) | |
| High cholesterol | | | | p (1) = 0,826 |
| Yes | 11 (5,8) | 7 (7,1) | 18 (6,3) | |
| No | 100 (52,9) | 49 (49,5) | 149 (51,7) | |
| Do not know | 78 (41,3) | 43 (43,4) | 121 (42,0) | |
| low HDL | | | | p (1) = 0,808 |
| Yes | 11 (5,8) | 4 (4,0) | 15 (5,2) | |
| No | 76 (40,2) | 40 (40,4) | 116 (40,3) | |
| Do not know | 102 (54,0) | 55 (55,6) | 157 (54,5) | |
| Elevated LDL | | | | p (2) = 1,000 |
| Yes | 3 (1,6) | 2 (2,0) | 5 (1,7) | |
| No | 80 (42,3) | 42 (42,4) | 122 (42,4) | |
| Do not know | 106 (56,1) | 55 (55,6) | 161 (55,9) | |
| High blood pressure | | | | p (2) = 0,340 |
| Yes | 11 (5,8) | 2 (2,0) | 13 (4,5) | |
| No | 168 (88,9) | 93 (93,9) | 261 (90,6) | |
| Do not know | 10 (5,3) | 4 (4,0) | 14 (4,9) | |

*(*) Significant association at 5%. (1) Pearson's Chi-square test.*
*Source: the author.*

Table 7 contains the results of cross-checks between the cardiovascular risk factors of cholesterol levels and blood pressure, reported by the students, expressed with at least one of the four factors (High

cholesterol, low HDL, high LDL, and high blood pressure, being considered "no" if all four factors are negative and the category "does not know" if in any of the four the answer was "does not know") with each of the characterization variables: sex, age group and cycle in addition to the practical variables of physical activity, is considered stressed and has a balanced diet. In Table 7, no significant associations ($p > 0.05$) were recorded between cardiac risk and the variables analyzed.

*Table 7:* Assess Analysis of the characteristics studied according to the level of the medical course

| | level of medical course | | | |
|---|---|---|---|---|
| Variable | basic level | clinical level | Total group | P value |
| | n (%) | n (%) | n (%) | |
| Total | 189 (100,0) | 99 (100,0) | 288 (100,0) | |
| Practice physical activity | | | | p (1) = 0,321 |
| Yes | 122 (64,6) | 58 (58,6) | 180 (62,5) | |
| No | 67 (35,4) | 41 (41,4) | 108 (37,5) | |
| Self-declaration of stress | | | | p (1) = 0,116 |
| Yes | 106 (56,1) | 65 (65,7) | 171 (59,4) | |
| No | 83 (43,9) | 34 (34,3) | 117 (40,6) | |
| Family history | | | | p (1) = 0,122 |
| Yes | 97 (51,3) | 50 (50,5) | 147 (51,0) | |
| No | 72 (38,1) | 45 (45,5) | 117 (40,6) | |
| Do not know | 20 (10,6) | 4 (4,0) | 24 (8,3) | |
| Smoking | | | | p (1) = 0,001* |
| Yes | 19 (10,1) | 24 (24,2) | 43 (14,9) | |
| No | 170 (89,9) | 75 (75,8) | 245 (85,1) | |
| Alcoholism | | | | p (1) = 0,030* |
| Yes | 19 (10,1) | 19 (19,2) | 38 (13,2) | |
| No | 170 (89,9) | 80 (80,8) | 250 (86,8) | |
| Balanced diet | | | | p (1) = 0,601 |
| Yes | 126 (66,7) | 69 (69,7) | 195 (67,7) | |
| No | 63 (33,3) | 30 (30,3) | 93 (32,3) | |
| High cholesterol | | | | p (1) = 0,826 |
| Yes | 11 (5,8) | 7 (7,1) | 18 (6,3) | |
| No | 100 (52,9) | 49 (49,5) | 149 (51,7) | |
| Do not know | 78 (41,3) | 43 (43,4) | 121 (42,0) | |
| Low HDL | | | | p (1) = 0,808 |
| Yes | 11 (5,8) | 4 (4,0) | 15 (5,2) | |
| No | 76 (40,2) | 40 (40,4) | 116 (40,3) | |
| Do not know | 102 (54,0) | 55 (55,6) | 157 (54,5) | |
| Elevated LDL | | | | p (2) = 1,000 |
| Yes | 3 (1,6) | 2 (2,0) | 5 (1,7) | |
| No | 80 (42,3) | 42 (42,4) | 122 (42,4) | |
| Do not know | 106 (56,1) | 55 (55,6) | 161 (55,9) | |
| High blood pressure | | | | p (2) = 0,340 |
| Yes | 11 (5,8) | 2 (2,0) | 13 (4,5) | |
| No | 168 (88,9) | 93 (93,9) | 261 (90,6) | |
| Do not know | 10 (5,3) | 4 (4,0) | 14 (4,9) | |

*(*) Yes if it was registered by one of the yes factors, no if all four factors had a negative answer and don't know if at least one of the factors didn't know and the other factors had a no answer. (1) Pearson's Chi-square test. (2) Fisher's Exact Test*
*Source: the author*

*Table 8:* Assessment four risk factors according to the sample characteristics

| Four cardiovascular risk factors (high cholesterol, low HDL, high LDL, high blood pressure) | | | | | |
|---|---|---|---|---|---|
| variable | yes * | no * | Total | OR (%, a 0,95) | P value |
| | n (%) | n (%) | n (%) | | |
| Total group | 41 (31,8) | 88 (68,2) | 129 (100,0) | | |
| **Age range** | | | | | p (1) = 0,855 |
| 18 to 19 | 9 (32,1) | 19 (67,9) | 28 (100,0) | 1,33 (0,36 a 4,83) | |
| 20 to 29 | 27 32,9) | 55 (67,1) | 82 (100,0) | 1,37 (0,45 a 4,21) | |
| 30 or more | 5 (26,3) | 14 (73,7) | 19 (100,0) | 1,00 | |
| **Cycle** | | | | | p (1) = 0,343 |
| Basic | 30 34,5) | 57 (65,5) | 87 (100,0) | 1,48 (0,66 a 3,36) | |
| Clinical | 11 26,2) | 31 (73,8) | 42 (100,0) | 1,00 | |
| **Sex** | | | | | p (1) = 0,284 |
| Masculine | 15 (38,5) | 24 (61,5) | 39 (100,0) | 1,54 (0,69 a 3,39) | |
| Feminine | 26 (28,9) | 64 (71,1) | 90 (100,0) | 1,00 | |
| **Practice physical activity** | | | | | p (1) = 0,463 |
| Yes | 27 (34,2) | 52 (65,8) | 79 (100,0) | 1,34 (0,62 a 2,89) | |
| No | 14 (28,0) | 36 (72,0) | 50 (100,0) | 1,00 | |
| **Self-declaration of stress** | | | | | p (1) = 0,950 |
| Yes | 24 (32,0) | 51 (68,0) | 75 (100,0) | 1,02 (0,48 a 2,17) | |
| No | 17 (31,5) | 37 (68,5) | 54 (100,0) | 1,00 | |
| **Family history** | | | | | p (2) = 0,328 |
| Yes | 25 (37,9) | 41 (62,1) | 66 (100,0) | 1,52 (0,27 a 8,46) | |
| No | 14 (25,0) | 42 (75,0) | 56 (100,0) | 0,83 (0,14 a 4,78) | |
| Do not know | 2 (28,6) | 5 (71,4) | 7 (100,0) | 1,00 | |
| **Balanced diet** | | | | | p (1) = 0,107 |
| Yes | 24 (27,3) | 64 (72,7) | 88 (100,0) | 1,00 | |
| No | 17 (41,5) | 24 (58,5) | 41 (100,0) | 1,89 (0,87 a 4,11) | |

*(*) "Yes" if at least one of the factors was recorded and "no" if all four factors had a negative response. (1) Pearson's Chi-square test. (2) Fisher's Exact Test*

*Source: the author*

In Table 8 we analyzed 129 responses to the questionnaire due to the exclusion of the "didn't know" answers. No significant associations ($p > 0.05$) were recorded between the data reported on knowledge of cholesterol and blood pressure levels, blood lipids, and the variables analyzed.

## IV. Discussion

Understanding the main risk factors for the development of atherosclerotic disease plays a vital role in disease prevention, and control. It is essential training of health professionals, especially medical students, contributing to early knowledge of atherosclerotic risk factors since the first years of medical school. This is particularly relevant given that cardiovascular diseases often begin in childhood and adolescence, making it crucial that future doctors are well-informed from the beginning of their academic journey. Furthermore, this study seeks to contribute with awareness about the importance of primary prevention of cardiovascular diseases for of medical students, in the context of the risk factors involved. By assessing the level of knowledge and monitoring its evolution, we will be able to identify areas that require greater emphasis in the educational curriculum and develop strategies to strengthen the training of future health professionals. The knowledge obtained from this research will benefit medical students themselves, and will contribute with promoting population's health, as well-informed doctors are essential for the prevention and adequate treatment of cardiovascular diseases. The sample included aged patients between 18 and 53 years, with an average of 23.12 years, majority of patients were between 20 and 29 years old, representing 66.7% of the sample. 65.6% of respondents were in the basic level of medical

school. Females predominated, representing 63.9% of the sample. These sociodemographic characteristics of the sample are important to contextualize the research results and understand how different groups may present variations in their knowledge of atherosclerotic risk factors. The analysis of these variables can provide valuable insights for discussing the results and their clinical and educational implications. These results provide a comprehensive view of the sample's characteristics regarding physical health, risk behaviors, and personal and family histories. These variables are essential for understanding atherosclerotic risk factors and their implications for the prevention and early diagnosis of cardiovascular diseases, aspects that will be discussed in detail.

Regarding the practice of physical activity, there was a difference between age groups, which was more significant in younger groups. Around 62.5% of all participants reported practicing at least 150 minutes of physical activity per week, while 37.5% reported not performing this minimum amount of exercise. This data is relevant as regular physical activity is associated with a reduced risk of cardiovascular diseases. (PRÉCOMA et al. 2021) (MOSCA et al. 2011) (BRYAN et al, 2018) (BARROSO et al, 2021)

The perception of stress was also investigated, with significant difference between the sexes. A greater number of women, 67.4%, reported considering themselves stressed, compared to 45.2% of men. Difference between female and male sexes was statistically significant ($p < 0.001$). In the total group, 59.4% of patients self-declared emotional stress, while 40.6% did not. Stress is a factor that can influence the development of cardiovascular diseases, and self-perception is important aspect to be considered. Regarding the perception of stress, there was also no significant difference between the groups according to the level in the medical faculty. Both groups had similar proportions of students who considered themselves stressed regarding knowledge of a family history of cardiovascular diseases. However, in smoking, a significant difference was observed between the groups with a higher prevalence in the basic level group (24.2% vs. 10.1%, $p = 0.001$), as alcoholism (19.2% vs. 10.1%, $p = 0.030$). (STEPTOE et al. 2012) (DAR et al. 2019)

Smoking and alcohol consumption were more prevalent among aged group between 20 and 30 years and those who were studying the clinical level of medical school. Regarding smoking, 14.9% of patients reported being smokers, while the vast majority, 85.1%, stated they did not smoke. Smoking is a main risk factors for atherosclerosis and cardiovascular diseases. Regarding alcoholism, 13.2% of participants reported consuming alcohol in a way that could be considered alcoholism, while 86.8% denied this behavior. Excessive alcohol consumption is also associated with cardiovascular health risks. (ROY et al. 2017) (PRÉCOMA et al. 2021) (GALLUCCI et al. 2020) (HOEK et al. 2022)

The average of weight was 69.74 kg, with a wide range from 43.00 kg to 140.00 kg. Body Mass Index (BMI) demonstrated average of 24.37 (16.42 to 43.21). Most patients had a BMI between 21.36 and 26.30. (SIMÃO et al, 2013) (VISSEREN et al, 2023) (PRÉCOMA et al, 2021) (BARROSO et al, 2021) (BRYAN et al, 2018)

The majority of the group reported adequate cholesterol levels, as well as blood pressure levels, with an average systolic pressure (SBP) of 113.81 mmHg and an average diastolic pressure (DBP) of 75.95 mmHg; glycemia, average was 84.52 mg/dL. Regarding family history, there were more reports among female and older groups. The percentage of 51% of students interviewed reported a family history of cardiovascular diseases. A family history of cardiovascular disease may increase an individual's risk of these conditions. The high prevalence of female knowledge regarding family history of cardiovascular diseases may possibly be related to the social values of caring. Added to this, it can be attributed to the role of the gynecologist in women's health care, during prevention consultations. (PREISLER et al, 2018) (FALUDI et al, 2017) (LEON et al, 2025) (NICOLAUS et al, 2023) (UNGER et al, 2020) (PRÉCOMA et al, 2021) (BARROSO et al, 2021) (BRYAN et al, 2018)

In relation to balanced nutrition, a significant difference was observed between age groups. The youngest group (18 to 19 year old) showed a higher prevalence of students reporting a balanced diet (72.3%), followed by the group between 20 to 29 (69.3%), and the older group had the lowest proportion (48.4 %). The difference between age groups was statistically significant ($p = 0.047$). These results indicate that the age group has a significant influence only on the variable related to a balanced diet. This information is relevant to understanding how eating habits can vary according to age and will be discussed in the Discussion section to evaluate its clinical and educational implications. (NICOLAUS et al, 2023) (BARROSO et al, 2021) (BRYAN et al, 2018)

It was observed that, overall, 14.2% of participants presented at least one of the four cardiac risk factors, while 30.6% did not present any of the factors, and 55.2% did not know or did not respond. However, there was no difference in relation to the age group. The distribution of risk factors was similar across all age groups (18 to 19 years, 20 to 29 years and 30 or more years).

When analyzed according to the level of the medical school, basic, and clinical level, no statistically significant differences were observed of risk factors. Both groups had similar proportions of participants with at least one of these factors. Regarding sex, there was no statistically significant difference in the presence of

risk factors between men and women. Both groups had similar proportions of participants with at least one of the factors.

The presence of at least one of the four cardiac risk factors (high cholesterol, low HDL, high LDL, and high blood pressure) based on several characteristics of the sample, including age group, medical course level, gender, practice of physical activity, perception of stress, family history and balanced diet. The table also includes the Odds Ratio (OR) and p-value for each association. It was observed that, overall, 31.8% of participants presented at least one of the four cardiac risk factors, while 68.2% did not present any of the factors.

When analyzing risk factors according to age group, no significant differences were found between the groups. Odds Ratio of the youngest group (18 and 19 years old), the intermediate group (20 to 29 years old), and the oldest group were not statistically significant, suggesting no association with aged group and risk factors.

When analyzed according to the phase of the medical course, basic and clinical level, the Odds Ratio was also not statistically significant, indicating that the level in medical school was not associated with risk factors. Regarding gender, the Odds Ratio for male group compared to female group was 1.54, but this difference was not statistically significant.

The practice of physical activity also did not show a significant association with the presence of cardiac risk factors. The Odds Ratio between participants who practiced physical activity and those who did not was 1.34, but this difference was not statistically significant. Likewise, the perception of stress was not associated with the presence of cardiac risk factors, with an Odds Ratio of 1.02 between participants who considered themselves stressed and those who did not.

When evaluating family history, the Odds Ratio for those with a family history of cardiovascular disease compared to those without a family history was 1.52, but this difference was not statistically significant. Finally, in relation to a balanced diet, the Odds Ratio for those who did not follow a balanced diet compared to those who did was 1.89, but this difference was also not statistically significant. This analysis did not identify significant associations between the presence of at least one of the four cardiac risk factors and the characteristics of the sample examined, including age group, medical course cycle, gender, practice of physical activity, perception of stress, family history and balanced diet. This suggests that these risk factors may affect medical students independently of these characteristics.

Future research can investigate the gap between the findings found in this research and correlate with parallel situations, such as different populations, but with the same profile. University extension activities in a community or hospital environment that explore the importance of primary cardiovascular prevention, emphasizing self-knowledge about risk factors, as well as active methodologies and workshops on laboratory dosages and blood pressure measurements could contribute to greater assimilation about the theme. The study demonstrated that students in the clinical cycle and those aged between 20 and 29 years reported a higher prevalence of smoking and alcohol consumption, and there may be preventive measures to promote co-incentives in this group.

As limitations of this study, we could mention the possible lack of reliability of the values provided by the students, referring to blood tests and blood pressure measurements, since they were reported and not collected for laboratory analysis. However, the objective was to evaluate students' knowledge of their own risk factors and their values, therefore, the relevance lies in this aspect.

As limitations of this study, we could mention the possible lack of reliability of the values provided by the students, referring to blood tests and blood pressure measurements, since they were reported and not collected for laboratory analysis. However, the objective was to evaluate students' knowledge of their own risk factors and their values, therefore, the relevance lies in this aspect. As much as anonymity may have contributed to the spontaneity of responses, it may generate less loyalty

## V. Conclusion

This study demonstrated that smoking and alcohol consumption were more prevalent among students with the highest age group studied, over 30 years old, and were also more frequent in the clinical cycle phase of medical school. There was a higher prevalence of knowledge regarding the family history of CAD and CVA among female students and those over 30 years of age. Reports of self-declared emotional stress and higher blood pressure levels were more frequent among female medical students.

## References Références Referencias

1. ARNETT DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019; 140:e596–e646. DOI: 10.1161/CIR.0000000000000678
2. BARROSO WKS, Rodrigues CIS, Bortolotto LA, Mota-Gomes MA, Brandão AA, Feitosa ADM, et al. Diretrizes Brasileiras de Hipertensão Arterial – 2020. Arq Bras Cardiol. 2021; 116(3): 516-658

3. BRICARELLO, L P, Retondario A, Poltronieri F, Souza A S, Vasconcelos F A G. A dietary approach to control hypertension: reflections on adherence to and possible impacts on public health. Ciênc. saúde coletiva 25 (4) 06 Abr 2020Mar 2020
4. BRYAN WILLIAMS, Giuseppe Mancia, Wilko Spiering, Enrico Agabiti Rosei, Michel Azizi, Michel Burnier et al. ESC Scientific Document Group , 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH), *European Heart Journal*, Volume 39, Issue 33, 01 September 2018, Pages 3021–3104, https://doi.org/10.1093/eurheartj/ehy 339
5. CONOVER W J. Practical Nonparametric Statistics. New York. Texas Tech University: Thrird Edition. Editora John Wiley & Sons,1999, 608 p
6. DAR T, Radfar A, Abohashem S, Pitman RK, Tawakol A, Osborne MT. Psychosocial Stress and Cardiovascular Disease. Curr Treat Options Cardiovasc Med. 2019 Apr 26; 21(5): 23. doi: 10.1007/s11936-019-0724-5. PMID: 31028483; PMCID: PMC6568256.
7. DOUGLAS G. Altman, Chapman and Hall. Practical Statistics for Medical Research. Great Britain, London, 1991. 611 p
8. EXCEL MICROSOFT ONLINE. https://www.microsoft.com/pt-br/microsoft-365/free-office-online-for-the-web
9. FALUDI, A. A. et al. Atualização da diretriz Brasileira de dislipidemias e prevenção da aterosclerose. Arquivo Brasileiro de Cardiologia, São Paulo, v. 109, n.2, 2017
10. GALLUCCI G, Tartarone A, Lerose R, Lalinga AV, Capobianco AM. Cardiovascular risk of smoking and benefits of smoking cessation. J Thorac Dis. 2020 Jul; 12(7): 3866-3876. doi: 10.21037/jtd.2020.02.47. PMID: 32802468; PMCID: PMC7399440.
11. HOEK AG, van Oort S, Mukamal KJ, Beulens JWJ. Alcohol Consumption and Cardiovascular Disease Risk: Placing New Data in Context. Curr Atheroscler Rep. 2022 Jan; 24(1): 51-59. doi: 10.1007/s11883-022-00992-1. Epub 2022 Feb 7. PMID: 35129737; PMCID: PMC8924109.
12. IMB SPSS. https://www.ibm.com/docs/en/spss-statistics/25.0.0.
13. JENNIFER Yu, Ki Park, Jaya Chandrasekhar, Deborah N Kalkman, Jerri A. Johnson et al. Feasibility and Utility of a Cardiovascular Risk Screening Tool in Women Undergoing Routine Gynecology Evaluation. JOURNAL OF WOMEN'S HEALTH Volume 00, Number 00, 2020.
14. LEON BM, Maddox TM. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. World J Diabetes. 2015 Oct 10; 6(13): 1246-58. doi: 10.4239/wjd.v6.i13.1246. PMID: 26468341; PMCID: PMC4600176.
15. MOSCA L, Benjamin EJ, Berra K et al. Effectiveness-Based Guidelines for the Prevention of Cardiovascular Disease in Women—2011 Update: A Guideline From the American Heart Association. Circulation. 2011 March 22; 123(11): 1243–1262.
16. NIKOLAUS Marx, Massimo Federici, Katharina Schütt, Dirk Müller-Wieland, Ramzi A Ajjan, Manuel J Antunes et al. ESC Scientific Document Group, 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes: Developed by the task force on the management of cardiovascular disease in patients with diabetes of the European Society of Cardiology (ESC), *European Heart Journal*, Volume 44, Issue 39, 14 October 2023, Pages 4043–4140, https://doi.org/10.1093/eurheartj/ehad192
17. PLATAFORMA BRASIL. https://plataformabrasil.saude.gov.br/login. Acesso em 11 de janeiro de 2024
18. PRÉCOMA DB, Oliveira GMM, Simão AF, Dutra OP, Coelho OR, Izar MCO et al. Updated Cardiovascular Prevention Guideline of the Brazilian Society of Cardiology - 2019. Arq Bras Cardiol. 2019 Nov 4; 113(4): 787-891. doi: 10.5935/abc.20190204. Erratum in: Arq Bras Cardiol. 2021 Apr; 116(4): 855. PMID: 31691761; PMCID: PMC7020870.
19. PREISLER Y, Ziv-Baran T, Chorin E, Margolis G, Khoury S, Shacham Y. Family history of coronary artery disease and adverse clinical outcomes in patients suffering from acute ST-segment elevation myocardial infarction. Coron Artery Dis. 2018 Dec; 29(8): 657-662. doi: 10.1097/MCA.0000000000000667. PMID: 30308587.
20. ROY A, Rawal I, Jabbour S, et al. Tobacco and Cardiovascular Disease: A Summary of Evidence. In: Prabhakaran D, Anand S, Gaziano TA, et al., editors. Cardiovascular, Respiratory, and Related Disorders. 3rd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2017 Nov 17. Chapter 4. Available from: https://www.ncbi.nlm.nih.gov/books/NBK525170/ doi: 10.1596/978-1-4648-0518-9_ch4
21. SIMÃO AF, Precoma DB, Andrade JP et al. I Diretriz de Prevenção Cardiovascular. Arq Bras Cardiol. 2013; 101(6Sup2): 1-63.
22. STEPTOE A, Kivimäki M. Stress and cardiovascular disease. Nat Rev Cardiol. 2012 Apr 3; 9(6): 360-70. doi: 10.1038/nrcardio.2012.45. PMID: 22473079.
23. UNGER T, Borghi C, Charchar F, Khan N A, Poulter N R, Prabhakaran D et al. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. Hypertension Volume 75, Issue 6, June

2020; Pages 1334-1357. https://doi.org/10.1161/HYPERTENSIONAHA.120.15026
24. VISSEREN FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M et al. ESC Scientific Document Group. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021 Sep 7; 42(34): 3227-3337. doi: 10.1093/eurheartj/ehab484. Erratum in: Eur Heart J. 2022 Nov 7; 43(42):4468. PMID: 34458905.
25. WHO. World Heath Organization. https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1, disponível em 08 de janeiro de 2024

This page is intentionally left blank

Global Journal of Medical Research: F
Diseases
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

# Durability of Long-Lasting Insecticidal Nets (Yorkool) under Operational Conditions, Anseba Zone, Eritrea

By Mensur Yenus, Amanuel Mhreteab, Henos Kiflom, Amanuel Kflemariam & Awet Mebrahtu

*Abstract-* *Background:* Investment in malaria control has dramatically reduced transmission. An estimated 663 million cases have been averted worldwide between 2000 and 2015, with 68% attributed to insecticide-treated nets. In recent years, prevention in Eritrea has primarily relied on two main methods of intervention: mass distribution of Long-Lasting Insecticide Nets (LLINs) and Indoor Residual Spraying (IRS) in specific areas prone to epidemics. These are complemented by other supporting strategies like larval source management (LSM). While LLINs remain the primary prevention strategy in the study area, the durability of nets distributed at different times has not been comprehensively assessed for their impact on: 1) survival rate, 2) fabric integrity, and 3) insecticidal activity. So, this study was designed to address whether the nets distributed have been durable for three years under operational conditions.

*Keywords:* *LLINs, durability, attrition or survivorship, physical integrity, and bio-efficacy.*

*GJMR-F Classification:* *NLM: WC 765*


*Strictly as per the compliance and regulations of:*

# Durability of Long-Lasting Insecticidal Nets (Yorkool) under Operational Conditions, Anseba Zone, Eritrea

Mensur Yenus [α], Amanuel Mhreteab [σ], Henos Kiflom [ρ], Amanuel Kflemariam [ω] & Awet Mebrahtu [¥]

*Abstract-* ***Background:*** Investment in malaria control has dramatically reduced transmission. An estimated 663 million cases have been averted worldwide between 2000 and 2015, with 68% attributed to insecticide-treated nets. In recent years, prevention in Eritrea has primarily relied on two main methods of intervention: mass distribution of Long-Lasting Insecticide Nets (LLINs) and Indoor Residual Spraying (IRS) in specific areas prone to epidemics. These are complemented by other supporting strategies like larval source management (LSM). While LLINs remain the primary prevention strategy in the study area, the durability of nets distributed at different times has not been comprehensively assessed for their impact on: 1) survival rate, 2) fabric integrity, and 3) insecticidal activity. So, this study was designed to address whether the nets distributed have been durable for three years under operational conditions.

***Method:*** A community-based, prospective longitudinal study was conducted in the Asmat, Habero, Hagaz, and Elabered sub-zones to evaluate the survivorship, physical integrity, and insecticidal activity of newly distributed Yorkool-type LLINs. A total of 270 nets were included in the study.

***Result:*** After 12 months of use, the study found a survivorship rate of 95.05% for nets reported as used and 97.9% for stored nets. Over 95% of the LLINs found in households were reportedly used for sleeping every night, with 82% used throughout the year. However, 19% of the nets were used outside the primary residence, such as in gardens, farmland, or other locations. While 20% of the nets had holes, they were all deemed repairable, and none required replacement.

***Conclusion:*** The newly distributed Yorkool LLINs are expected to have a lifespan of approximately 3 years, maintaining their physical integrity, survivorship, attrition resistance, and bio-efficacy. However, the present study identified holes in nearly 20% of the nets after only 12 months of use. Fortunately, none required immediate replacement. Encouragingly, over 95% of the nets in the study remained intact despite the insecticide not meeting WHO's bio-efficacy criteria for knock-down or mortality rates. Further evaluation of net durability is recommended to determine the ongoing protective effect these nets provide to the community.

*Keywords: LLINs, durability, attrition or survivorship, physical integrity, and bio-efficacy.*

*Author α: Entomologist at ministry of Health Anseba Region.*
*e-mail: mensuryenus@gmail.com*
*Author σ: Entomologist at ministry of health Gash-Barka Region.*
*Author ρ ω: Public health officer at ministry of Health Anseba Region.*
*Author ¥: Public health officer at ministry of health Gash-Barka Region.*

## I. Introduction

Malaria is one of the world's most deadly diseases, and it is perilous for pregnant women and children under 5 years of age. In 2018, 67% of all the mortality due to malaria was attributed to pregnant mothers and children <5 years (WHO, 2020). Africa is greatly affected by malaria more than the other continents that more than 93% of morbidity and 94% of mortality were from Africa (WHO, 2020). Apart from the consequences in mortality and the subsequent reduction in life expectancy, malaria affects people's physical conditions, making them more vulnerable to other diseases (Blanco, 2018). Anemia, malnutrition, and other health problems can significantly increase one's vulnerability to malaria. This option emphasizes that these factors directly contribute to being more susceptible to malaria.

Malaria-endemic countries are heavily impacted by the economic burden of the disease, primarily due to: -1- Reduced productivity: Malaria affects the workforce by causing absenteeism and decreased work performance due to illness. -2-). Reduces students' attendance at school, affecting their education and productivity in the long run: -3-) Large of money spent for its prevention and treatment that could otherwise be used for investment in productive activities (Blanco, 2018).

Investment in malaria control has greatly reduced transmission. An estimated 663 million cases have been averted worldwide between 2000 and 2015, with 68% of them attributed to insecticide-treated nets (Bhatt, *et al.*, 2015). Long-lasting insecticidal nets (LLINs) protect against malaria by acting as a physical barrier between mosquitos and humans, and by the insecticide repelling or killing susceptible mosquitoes (Darriet *et al*., 1984; Lengeler, 2004 and Davies *et al*., 2007). Insecticide enhances public health by reducing mosquito density and helping maintain the net's effectiveness after holes develop (Derriet *et al.,* 1984)).

In Eritrea, found in Sub-Saharan Africa (SSA), malaria is still endemic in four of the six zones (regions) despite the rate of endemicity is different among these regions. However, malaria is greatly reduced in Eritrea over the past two decades (Mihreteab *et al*, 2020).

During the last years, prevention in Eritrea has been based on two main methods of intervention: LLINs mass distribution and indoor residual spraying (IRS) in selective epidemic-prone areas with other supporting strategies like larval source management (LSM) and larviciding using temephos.

According to WHO Global Malaria Program (a system to improve value for money in LLIN procurement through market competition based on cons per year of adequate coverage), the annual cost of LLINs for malaria control is more than 500 million US dollars; it is the largest commodity category in the malaria control budget, so do in Eritrea mainly in Anseba Zone uses only LLINs with other supporting activity in malaria prevention nowadays every individual is provided with the net with high cost.

The same study was conducted in Benin, West Africa, to assess the physical integrity and survivorship of LLINs distributed to households in a community with similar socio-cultural characteristics. The study found that only 1.73% of the LLINs exhibited visible integrity loss after six months (Gnanguenon *et al*. 2014). After a year, the damaged nets were increased by 10.41%. This study revealed that the survival rate of the nets in households was lower than expected. However, the nets surprisingly met the WHO standard for physical integrity for a period of one year (Ahogni *et al.* 2020). Different studies from the same country found almost the same result in 12 months of utilization, that more than 70% of the nets survived (Azondekon *et al,* 2014 and Gnanguenon *et al*, 2014).

Ahogni's study found that, out of 1,134 bed nets, only 1.41% needed replacement, while 3.44% required repairs. In contrast, another study from Uganda reported a significantly higher rate of net damage, with over 33% of the nets having one or more holes (Kilian *et al*, 2011).

LLINs are expected to remain effective for at least three years under typical use conditions (WHO, 2011; Kilian *et al*. 2011). This recommendation from the WHO informs the practice of mass distribution campaigns every three years. However, numerous studies (Ahogni *et al*. 2020; Massue *et al.* 2016; Tan *et al.* 2016; Randriamaherijaona, 2017) have shown that various factors can compromise net lifespan, preventing them from reaching the expected three years.

Universal coverage campaigns often lack strategies to address net survivorship (resistance to damage and loss), attrition (nets falling out of use), and physical integrity (tears and holes). While the WHO emphasizes the importance of monitoring national coverage and durability (WHO, 2017), a more comprehensive approach is needed. Studies evaluating LLIN durability typically assess survivorship and attrition, physical integrity, and bio-efficacy (insecticide effectiveness).

In Eritrea, LLINs combined with IRS and larviciding remain the primary tools for vector control (Mihreteab et al., 2020). However, despite increased coverage of IRS and LLINs in recent years, malaria cases and deaths haven't shown a consistent decline (Mihreteab et al., 2020). Notably, LLINs have been the mainstay of malaria prevention for many years, distributed freely throughout the country. While the Eritrean government's commitment to tackling communicable diseases and providing free bed nets is commendable, a critical gap exists – a lack of recent studies on net survivorship, fabric integrity, and insecticidal activity (bio-efficacy).

## II. Methodology

*a) Study design*

This study employed a community-based, prospective longitudinal design in the Asmat, Habero, Hagaz, and Elabered sub-zones (January 2021 - December 2020) – a period with an error. During net distribution, researchers identified participants and followed them for six months. The study focused on the durability of Yorkool-type LLINs, distributed one year prior, where each household member received a net (100% coverage). A total of 101 households were selected, enrolling 405 Yorkool nets (263 reported as used and 142 stored). The nets marked for use were assessed for durability every six months over three consecutive years.

Separately, 40 Yorkool LLINs were tested for bio-efficacy at baseline (day zero) and distributed to families in four sentinel sites. After one year, the tagged nets were collected and transported to the Elabered malaria entomology laboratory for further biological testing. Importantly, these families did not receive new nets during the study, as they were considered additional to the standard one-to-one distribution.

*b) Study area*

The study was conducted at 4-sentinel sites of Anseba region*:* Asneda (Asmat Sub-zone), Filfle (Habero Sub-zone), Adi Berbere (Elabered Sub-zone) and Hagaz town (Hagaz-Sub zone).

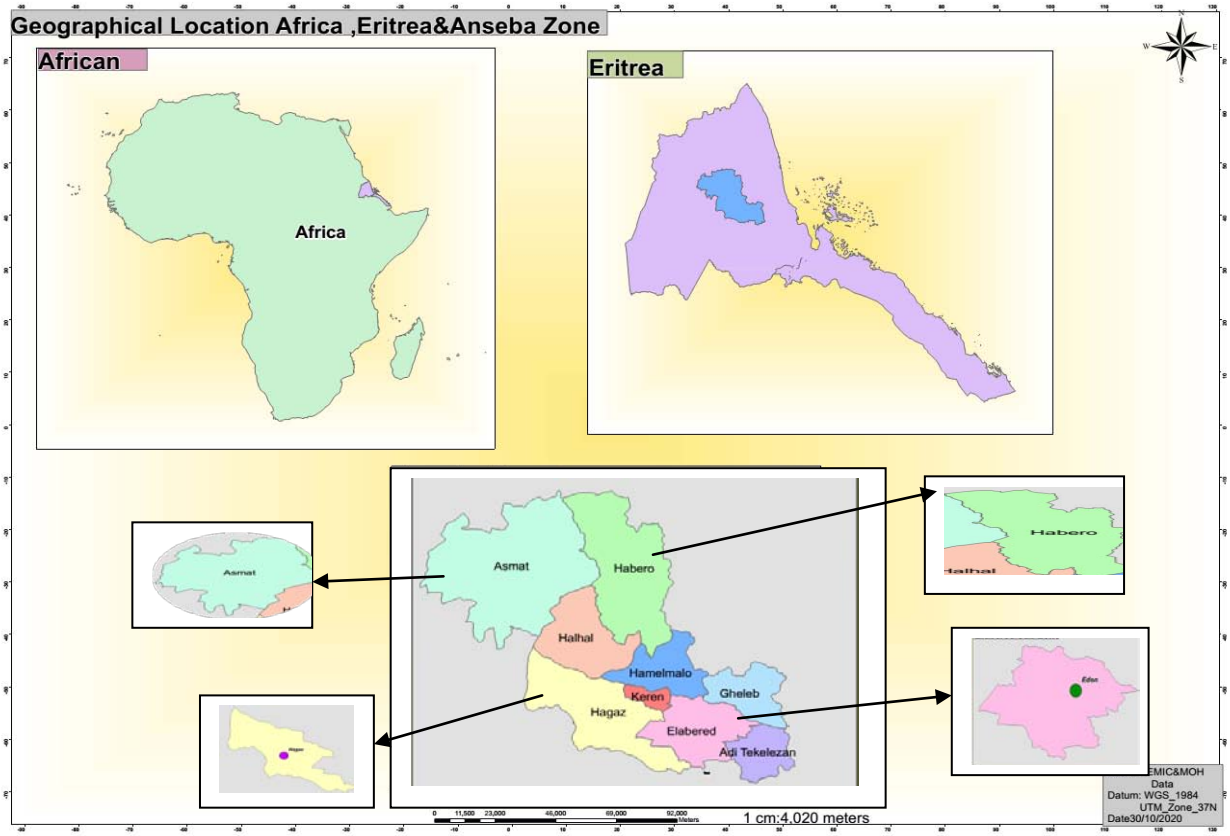



*Fig. (2.2):* Study area: Africa, Eritrea, Anseba zone, study sites, Hagaz, Asmat, Elabered & Habero sub zones.

*c) Study Population*

The study population consisted of Yorkool type long-lasting insecticidal nets (LLINs). These nets had been tested for insecticidal activity at day zero and were impregnated with deltamethrin. Nets that were given away for others to use, stolen, destroyed, used for other purposes, or were torn (with a proportionate hole index between 65-642 mm) were excluded from the study. Furthermore marked nets in which the study HH was moved from the village for different purposes.

*d) Sampling size determination*

According to different studies, a sample size of 250 nets per product was used to detect a 9%-point difference in LLIN attrition rate if the best-performing product has an attrition rate of 10%. An 8% buffer was added to the required sample size to prevent any negative impact of the mid-course withdrawal of some study participants. So, a sample size of 270 LLIN (yorkool) were retained for the study.

*e) Sampling Techniques*

In this community-based study, villages were selected using convenience sampling, while households (HHs) were chosen through simple random sampling. Villages within each sentinel site were chosen based on malaria incidence and the presence of active breeding sites. Household selection, however, employed simple random sampling. First, a complete census of all HHs in the chosen village was conducted prior to the distribution of nets. Then, from this list, ten households were randomly selected based solely on the head of household's name and provided with marked LLINs, each tested for bio-efficacy at day zero. The remaining 60 HHs per village were selected using the same method, yielding a total of 70 HHs per village to monitor attrition and fabric integrity of the nets.

*f) Data Collection*

Data was collected at the end of the malaria transmission season, just one year after mass distribution in November 2021. A standard questionnaire was used to collect data from an adult member of each household (HH) at the follow-up. Data collectors, who included public health officers, an entomologist, and insect collectors, were trained before conducting the survey to ensure understanding of the questionnaire. A pilot study was conducted by randomly selecting 10% of the sample size from a non-selected administrative area. Information collected included the status, patterns of use, and handling of each study LLIN distributed to the HH. Additionally, data on fabric integrity and the overall condition of the LLINs were collected.

*g) Data Management and Analysis*

Data were analyzed using SPSS version 23, and separately it was analyzed on the three factors attributed to durability: The number of nets in the sample, the

proportion of the indicator and 95% confidence interval was reported.

*h) Measurement of variables*

*Measurement of survivorship and attrition*

The analysis included data on all nets recorded during the exercise at each time interval.

*Survivorship:*

- Numerator: Total number of LLIN product present in surveyed HHs (and available for sleeping under) x 100
- Denominator: Total number of LLIN product distributed to surveyed HHs

*A. Attrition rate-1: for nets that have been destroyed or disposed of:*

- Numerator: Total number of LLIN product reported as lost due to wear and tear (poor condition) in surveyed HHs x 100
- Denominator: Total number of LLIN product distributed to surveyed HHs

*B. Attrition rate-2 for nets not available for sleeping under:*

- Numerator: Total number of LLIN product reported as lost for reasons other than poor fabric integrity (given away, stolen, sold or used in another location) in surveyed households x 100
- Denominator: Total number of LLIN product distributed to surveyed HHs

*C. Attrition rate-3 for nets used for other purposes:*

- Numerator: Total number of LLIN product reported as being used for another purpose in surveyed HHs x 100
- Denominator: Total number of LLIN product distributed to surveyed HHs For each LLIN product, the survivorship rate plus attrition rate-1, attrition rate-2 and attrition rate-3 will add up to 100%.

*Abbott's formula:*

$$\text{Corrected mortality} = \frac{(\%\ \text{observed mortality} - \%\ \text{control mortality})}{(100 - \%\ \text{control mortality})} \times 100$$

*i) Ethical Clearance*

First and foremost, clearance to conduct the study was obtained from the Ethical Approval Committee at the Ministry of Health, Eritrea. Upon entering a selected community, the assistance of opinion leaders was sought to obtain permission to use their communities as study sites and to inform the communities about the study objectives and methods. Informed consent was then obtained from all participants in the study who received the nets. Confidentiality was ensured by not sharing any respondent information with anyone.

*Measurement of Fabric integrity*

Fabric integrity was analyzed for all the LLINs found in the HHs (and used for sleeping under), and all the LLINs assessed for holes at each monitoring round. Two indicators were calculated at each survey time: the proportion of LLINs with holes and a hole index.

*Proportion of LLINs with any holes (with 95% confidence interval):*

- Numerator: Total number of LLINs product with at least one hole of size 1–4
- Denominator: Total number of LLINs product found and assessed in surveyed HHs

The hole index was calculated by weighting each hole by size and summing for each net. If the weight of hole sizes 1, 2, 3 and 4 was A, B, C and D, respectively, the hole index was calculated as:

*Hole index* = (A x no. of size-1 holes) + (B x no. of size-2 holes) + (C x no. of size-3 holes) + (D x no. size-4 holes).

*Measurement WHO cone bioassay:*

Standard WHO bioassays use standard susceptible 3–5 day old, non-blood fed *Anopheles* females exposed to netting under WHO cones for 5 minutes. Cones will be gently fitted on the net. Five female mosquitoes were introduced in each cone with 5 replicates per net sample (i.e. 25 mosquitoes per net).

After a 5-minute exposure time in each cone, the mosquitoes were held for 24 hours with access to a sugar solution. Knockdown was measured 60 minutes after exposure, and mortality was measured after 24 hours. A negative control, using an untreated net, was included in each round of cone bioassay testing.

If the mortality in the control was between 5% and 20%, the data was adjusted with Abbott's formula. If the mortality in the control is > 20%, all the tests were discarded for that day. Bioassays were carried out at 27 ± 2 °C and 80 ± 10% relative humidity.

## III. Result

*a) Characteristics of Respondents and Housing Condition*

Table 3.1 shows that the majority of respondents who completed the questionnaire were heads of households and parents/guardians. Among the interviewees, 57% had no formal education or only a primary level education. The housing conditions where the nets were placed revealed that 60% of the homes had concrete walls, while the rest were constructed with mud bricks or a combination of mud and wood framing.

Additionally, 74% of the floors were made of soil or sand.

*b) LLINs Utilization and Maintenance*

Table 3.2 shows that 92% of participants reported using their long-lasting insecticidal nets (LLINs) the night before the survey. Reasons for not using the nets included no reported malaria or mosquito presence. Over 95% of the LLINs found in households were reportedly used every night for sleeping, with 82% being used year-round. However, 19% of the nets were used outside the main house, such as in gardens or on farmland (Figure 3.2).

Washing frequency, materials, and drying location can impact insecticidal activity. As shown in Table 3.4, only 48% of the nets were washed one year after distribution. The primary cleaning material was local bar soap, though over 80% reported using soap or detergent powder for washing. In more than half of the cases, washed LLINs were dried in sunlight.

*Table 1:* Percentage distribution of characteristics of respondents, and housing conditions (N=101), regularity of sleeping under, LLINs displacement and maintenance

| Characteristics | | Hagaz | Elabered | Asmat | Haboro | Total |
|---|---|---|---|---|---|---|
| Interviewee | Head Of HH | 48 | 50 | 40 | 19 | 39 |
| | Guardian/Parent | 35 | 3 | 4 | 78 | 39 |
| | Other Adult | 17 | 12 | 56 | 4 | 22 |
| Educational Level | No Former Education | 9 | 15 | 4 | 33 | 15 |
| | Primary School | 52 | 3 | 32 | 44 | 42 |
| | Junior School | 17 | 19 | 12 | 19 | 17 |
| | High School | 17 | 23 | 36 | 4 | 20 |
| | Higher Level | 4 | 4 | 16 | 0 | 6 |
| Roof of the wall(Where the nets are found) | Mud Brick | 45 | 0 | 0 | 7 | 13 |
| | Mud With Wood Frame | 0 | 4 | 4 | 70 | 20 |
| | Concrete | 55 | 9 | 76 | 19 | 61 |
| | Wood | 0 | 0 | 4 | 0 | 1 |
| | Straw | 0 | 0 | 0 | 0 | 0 |
| | No Wall | 0 | 0 | 0 | 0 | 0 |
| | Other | 0 | 0 | 0 | 0 | 0 |
| Type of flooring in the Room | Soil Or Sand | 69 | 63 | 95 | 67 | 74 |
| | Wood | 0 | 0 | 0 | 15 | 4 |
| | Cement | 31 | 37 | 5 | 18 | 23 |
| | Carpet | 0 | 0 | 0 | 0 | 0 |
| How often the being used is the net being used in the last week | Every night | 100 | 92 | 96 | 96 | 96 |
| | Most nights | 0 | 0 | 0 | 4 | 1 |
| | Some nights | 0 | 8 | 0 | 0 | 2 |
| | Not used at all | 0 | 0 | 4 | 0 | 1 |
| During which period is the net used | All year round | 59 | 85 | 96 | 89 | 82.3 |
| | During transmission season | 41 | 15 | 0 | 11 | 16.7 |
| | During dry season | 0 | 0 | 0 | 0 | 0 |
| Displacement of the net from the main house | Taken to farm land | 23 | 0 | 0 | 26 | 12.3 |
| | Taken to garden | 0 | 4 | 0 | 4 | 1 |
| | Other | 0 | 4 | 8 | 7 | 4.8 |
| | Not used away | 77 | 92 | 92 | 63 | 81.9 |
| LLINs maintenance | | | | | | |
| Ever washed Nets | Yes | 50 | 36 | 47 | 61 | 48 |
| | No | 50 | 64 | 53 | 39 | 52 |
| Washing method | washed with cold water | 57 | 8 | 18 | 0 | 20 |
| | washed with a bleach/ soap | 43 | 92 | 82 | 100 | 80 |
| Those Nets Washed With Bleach / Soap | local bar soap | 17 | 0 | 22 | 6 | 9 |
| | detergent powder | 50 | 92 | 78 | 94 | 84 |
| | mix of bar and detergent | 33 | 8 | 0 | 0 | 7 |
| Drying Method | exposed to sun light | 29 | 46 | 73 | 59 | 51 |
| | dried in shaded place | 36 | 54 | 27 | 41 | 40 |
| | dried in indoor | 36 | 0 | 0 | 0 | 9 |

c) *Survivorship and attrition*

During follow-up visits at 12 months, a total of 250 LLINs marked as used and 139 marked as stored were found to be available at the households (HHs). While the text mentions a survival rate of 95.05% for used LLINs and 97.89% for stored LLINs, these percentages seem to be incorrect based on the provided data.

d) *Physical Integrity*

At 12 months of use, the percentage of nets with hole was found to be 20%. As depicted in table 4.7, it was 18.57%, 28.12%, 19.69% and 14.28% in Hagaz, Elabered, Asmat, and Haboro respectively. The average proportional hole index (pHI) of Yorkool®LN mosquito nets after 12 month of distribution was 14.31, 10.75, 3.36, 1.59 in Hagaz, Elabered, Asmat and Haboro respectively. The average of LLINs with a hole but in a good condition was 17.49%. However, the present study found that all damaged e nets could be repaired and be used further; besides, none of the nets was categorized under "to be replaced".

*Table 3.2:* Survivorship

| | Nets in the master list under use | yes use | Survivorship of nets in use | Nets in the master list under store | yes store | Survivorship of nets in store |
|---|---|---|---|---|---|---|
| Hagaz | 70 | 64 | 91.43% | 23.00 | 22 | 95.65% |
| Elabered | 66 | 62 | 93.94% | 39.00 | 37 | 94.87% |
| Asmat | 64 | 64 | 100.00% | 31.00 | 31 | 100.00% |
| Haboro | 63 | 60 | 95.24% | 49.00 | 49 | 100.00% |
| Total | 263 | 250 | 95.05% | 142.00 | 139 | 97.88% |

*Table 3.4:* Attrition

| | Nets in the master list under use | Attrition rate 1 | Attrition rate 2 | Attrition rate 3 |
|---|---|---|---|---|
| Hagaz | 70 | (0/70)*100= 0% | (6/70)*100=8.57% | (0/70)*100=0 |
| Elabered | 66 | (1/66)*100=1.52% | (3/66)*100=4.55% | (0/66)*100=0% |
| Asmat | 64 | (0/64)*100=0% | (0/64)*100=0% | (0/64)*100=0% |
| Haboro | 63 | (0/63)*100=0% | (3/63)*100=4.76% | (0/63)*100=0% |
| Total | 263 | (1/263)*100=0.38% | (12/263)*100=4.56% | (0/263)*100=0% |

*Table 3.5:* Physical integrity

| Sub zone | Nets in the master list under use | Nets with holes | Proportion of the net with holes |
|---|---|---|---|
| Hagaz | 70 | 13.00 | 0.19 |
| Elabered | 66 | 13.00 | 0.20 |
| Asmat | 64 | 18.00 | 0.28 |
| Haboro | 63 | 9.00 | 0.14 |
| Total | 263 | 53.00 | 0.20 |

*Table 3.6:* PHI Value

| | Total nets assessed | Nets with no Holes | Nets with at least one hole | | |
|---|---|---|---|---|---|
| | | | Good condition (PHI< 64) | To be repaired (65<= PHI <= 642) | To be replaced (PHI > 624) |
| Hagaz | 70 | 57(81.43%) | 10(14.28%) | 3(4.28%) | 0(0%) |
| Elabered | 66 | 53(80.30%) | 10(15.15%) | 3(4.54%) | 0(0%) |
| Asmat | 64 | 46(71.88%) | 17(26.56%) | 1(1.56%) | 0(0%) |
| Haboro | 63 | 54(85.71%) | 9(14.28%) | 0(0%) | 0(0%) |
| Total | 263 | 217(82.51%) | 46(17.49%) | 7(2.66%) | 0(0%) |

*e) Bio-Efficacy*

Table 4.7 shows that 40 nets (10 nets from each sub zone) were enrolled for WHO bio-assay test. So the mean knockdown down rate was 80. 5% and the mean mortality rate after 24 h was 71% with 95% CI of 52.3 – 81.5

*Table 3.8:* Mean KD and Mean mortality rate of the LLINs after 12 month with WHO Cone Test

| | Sub-Zone | | | |
|---|---|---|---|---|
| | Hagaz | Elabered | Haboro | Asmat |
| Total Yorkool LLINs | 10 | 10 | 10 | 10 |
| Total Mosquito Inserted (tested) | 500 | 500 | 500 | 500 |
| Mean Knockdown Rate | 89 % | 85% | 77% | 71% |
| Mean Mortality Rate After 24 Hrs. | 72% | 79% | 68% | 65% |
| 95% CI of percent's | 56 - 82 | 60 - 86 | 51 – 79 | 42- 75 |

## IV. Discussion

This study revealed that the washing frequency in the past year was less than half of what was expected for the total number of distributed nets. While washing frequency didn't seem to impact the overall survival rate of the nets, it did affect their physical integrity. This finding aligns with a study from Benin by Gnanguenon et al. (2014), which also observed a negative impact of frequent washing on the physical condition of LLINs. However, another study from Benin by Ahogni et al. (2020) found that even with holes, LLINs generally maintained a mortality rate above 50%. This highlights a crucial point: even if physically damaged, LLINs can still offer significant protection against mosquito bites, making their continued use essential.

The study results showed that, of the total observed LLINs, more than 96% was in serviceable condition after an average use of 12 months. The result also showed that almost none of the nets badly torn and removed from utilization. A study from Chad revealed, among the observed LLINs less than 30% were found to be serviceable after 14 months of utilization and nearly 40% of the total were badly torn and considered unserviceable (Allan *et al.,* 2012). As the study revealed sleeping without mattress was one of the reasons which reduced the serviceable life of the LLINs (Allan *et al.,* 2012). On the contrary a study from Benin on similar type of bed net revealed after eighteen months of use, more than two-third were found functionally survived (Ahogni *et al.,* 2020).

Our study observed a low attrition rate of only 5% after 12 months of monitoring, significantly lower than findings from Benin and Mozambique (Ahogni et al., 2020; Juliette et al., 2015). This lower rate can be attributed to the nets being primarily given away to others, unlike the other studies where misuse was reported. Interestingly, a study from Nepal (mention reference) found LLIN loss due to displacement for seasonal fieldwork, alongside instances of misuse (Ahogni *et al.,* 2020).

Nearly 80% of the study LLINs remained in good physical condition, without holes, after 12 months of use. Similar results were observed in Benin and Madagascar (Ahogni et al., 2020; Tan et al., 2016), while a higher proportion of torn nets was reported in Zambia (Kilian et al., 2011). Furthermore, the present study found that 82.5% of the Yorkool LLINs were in good working condition after 12 months of distribution. This finding is slightly lower than that reported by Ahogni et al. Additionally, the present study did not observe as high a proportion of torn nets as was observed in the study from Zambia (Craig et al., 2015). A high proportion of holes (pHI) compromises the physical protection offered by LLINs. This allows mosquitoes to feed on humans, thereby perpetuating human-vector contact and malaria transmission (Ochomo et al., 2013; Haji et al., 2013). Supporting this, another study demonstrated a direct link between the physical integrity of LLINs and human-vector contact. They found that damaged nets increased the average number of mosquito bites per person per night from zero to five (Gnanguenon et al., 2014).

The study measured a knock-down (KD) rate of 80.5% at 60 minutes and a 71% mortality rate after 24 hours of exposure. These findings fall short of the World Health Organization (WHO) standards. According to the WHO Pesticide Evaluation Scheme (WHOPES), an effective LLIN should retain biological efficacy for at least 3 years. This is defined as at least 80% of nets achieving either 95% knock-down or 80% mortality (WHO, 2012). Ideally, LLINs should maintain their insecticidal activity for this timeframe (Albert, 2012).

A study conducted in Madagascar on Yorkool LLINs reported similar results to the present study, with over 75% of the nets failing to meet the WHO threshold (Tan et al., 2016; Gnanguenon et al., 2014). However, Ahogni et al. (20XX) found that 58% of their study nets met the WHO quality standard for both knock-down at 60 minutes and mortality after 24 hours.

The durability and lifetime of LLINs are critical factors for program planners to determine the most cost-effective timing and distribution strategies for net replacements. However, the most crucial aspect remains the biological efficacy of LLINs. They must meet the WHO's minimum requirements for knock-down (KD) and mortality rates after 60 minutes and 24 hours, respectively.

## V. Conclusion

The newly distributed Yorkool LLINs are expected to have a lifespan of approximately 3 years, maintaining their physical integrity, survivorship, attrition resistance, and bio-efficacy. However, the present study identified holes in nearly 20% of the nets after only 12 months of use. Fortunately, none required immediate replacement.

Encouragingly, over 95% of the nets in the study remained intact despite the insecticide not meeting WHO's bio-efficacy criteria for knock-down or mortality rates. Further evaluation of net durability is recommended to determine the ongoing protective effect these nets provide to the community.

## References Références Referencias


1. Ahogni, B., Idelphonse.; Salako, S., Albert.; Akinro, Bruno.; Sovi, Arthur.; Gnanguenon, Virgile.; Azondekon, Roseric.; Dagnon, F., Jean.; Akogbeto, Pamela.; Tokponon, Filemon., and Akogbet, C., Martin., (2020). Physical integrity and survivorship of ling-lasting insecticidal nets distributed to households of the same socio-cultural community in Benin, West Africa. Malaria journal. 19: 58.
2. Ahogni, I. B.; Aïkpon, R. Y.; Ossè, R. A.; Dagnon, J. F.; Govoetchan R.; Attolou, R. H.; Agbev, A.; Azondekon, R.; Koukpo, C. Z.; Gnanguenon V.; Sagbohan, H.,; Kpanou, C.; Tokponon, F. ; Akinro, B.; Padonou, G. G., and Akogbeto M. C., (2020). Field durability of Yorkool®*LN* nets in the Benin republic. Advances in Entomology, 2020, 8, 72-92.
3. Albert, K. (2012) Roll Back Malaria: Measurement of Net Durability in the Field: Current Recommended Methodology, Presented in Lyon.
4. Allan, Richard.; O'Reilly, Laura.; Gilbos, Valery., and Kilian, Albert., (2012). An Observational Study of Material Durability of Three World Health Organization-Recommended Long-Lasting Insecticidal Nets in Eastern Chad. Am. J. Trop. Med. Hyg., 87(3), 2012, pp. 407–411.
5. Azondekon, R.; Gnanguenon, V.; Oke-Agbo, F., Houevoessa, S., Green, M., and Akogbeto, M., (2014). A tracking tool for long-lasting insecticidal (mosquito) net intervention following a 2011 national distribution in Benin. Parasites and Vectors. 14:6.
6. *Beier, J.; Keating, J.; Githure, J.; Macdonald, M.; Impoinvil, D,. (2008).* Integrated vector management for malaria control. Malaria Journal 7: doi: 10.1186/1475-2875-1187-S1181-S1184.
7. *Bhatt, S. et al., (2015).* The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature 526,* 207–211.
8. *Blanco, movilla., (2018).* Cost of malaria elimination in Kenya by means of IVM implementation. System dynamic group, Department of Geography, University of Bergen, Norway.
9. Craig, A.S., Muleba, M., Smith, S.C., Katebe-Sakala, C., Chongwe, G., Hamainza, B., Walusiku, B., Tremblay, M., Oscadal, M., Wirtz, R. and Tan, K.R. (2015). Long-Lasting Insecticidal Nets in Zambia: A Cross-Sectional Analysis of Net Integrityand Insecticide Content. Malaria Journal, 14, Article No. 239.
10. *Darriet, F. d. r.; Robert, V.; Vien, N. T., & Carnevale, P.; (1984).* Evaluation of the efficacy of permethrin impregnated intact and perforated mosquito nets against vectors of malaria. (World Health Organization, 1984).
11. *Davies, T.; Field, L.; Usher wood, P., & Williamson, M, (2007).* DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB life 59,* 151–162 (2007).
12. *Gnanguenon V, Azondekon R, Oke-Agbo F, Sovi A, Ossè R, Padonou G, Aïkpon R, Akogbeto MC* (2013): Evidence of man-vector contact in torn long lasting insecticide-treated nets. BMC Public Health, 13: 751.
13. Gnanguenon, V.; Azondekon, R.; Oke-Agbo, F.; Beach, R., and Akogbeto, M., (2014). Durability assessment results suggest a serviceable life of two, rather than three, years for the current long-lasting insecticidal (mosquito) net (LLIN) intervention in Benin. BMC Infectious Diseases. 14: 69.
14. Juliette, M., Ana Paula, A., Maria, R.P., Dulcisaria, M., Jacinta, L., Guilhermina, F., Samira, S., Adam, W., Gabriel, P.L., Adeline, C. and Jodi Vanden, E. (2015) Physical Durability of Two Types of Long-Lasting Insecticidal Nets (LLINs) Three Years after a Mass LLIN Distribution Campaign in Mozambique, 2008-2011. Journal of American Society of Tropical Medicine and Hygiene, 92, 286-293.
15. Haji, K.A., Khatib, B.O., Smith, S., Ali, A.S., Devine, G.J., Coetzee, M. and Majambere, S. (2013) Challenges for Malaria Elimination in Zanzibar: Pyrethroid Resistance in Malaria Vectors and Poor Performance of Long-Lasting Insecticide Nets. Parasites & Vectors, 6, 82. https://doi.org/10.1186/1756-3305-6-82
16. Hakizimana, E.; Cyubahiro, B.; Rukundo, A.; Kabayiza, A.; Mutabazi, A., and Beach, R., (2014). Monitoring long-lasting insecticidal net (LLIN) durability to validate net serviceable life assumptions, in Rwanda. *Malaria Journal*. 13:344.
17. Kilian, A.; Byamukama, W.; Pigeon, O.; Gimnig, J.; Atieli, F., and Koekemoer, L., (2011). Evidence for a useful life of more than three years for a polyester-based long-lasting insecticidal mosquito net in Western Uganda. Malaria Journal.10:299.
18. Koenker, H.; Kilian, A.; Hunter, G.; Acosta, A.; Scandurra, L., and Fagbemi, B., (2015). Impact of a behavior change intervention on long-lasting insecticidal net care and repair behavior and net condition in Nasarawa State, Nigeria. *Malaria Journal*. 14:18.

19. *Lengeler, C., (2004);* Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database Syst Rev 2.*
20. Massue, DJ.; Moore, SJ.; Mageni, ZD.; Moore, JD.; Bradley, J., and Pigeon, O., (2016). Durability of Olyset campaign nets distributed between 2009 and 2011 in eight districts of Tanzania. *Malaria Journal*.15: 176.
21. Mihreteab, Selam,.; Lubinda, Jailos.; Zhao, Bingxin.; Rodriguez-Morales, Alfonsa, J.; Karamehic Muratovic, Ajlina.; Giotom, Aman.; Shad, yousaf, Mehammed., and Haque,. Ubydul., (2020). Restrospective data analyses of social and environmental determinants of malaria control for elimination prospects in Eritrea. Open Access. 13: 126.
22. Ochomo, E.O., Bayoh, N.M., Walker, E.D., Abongo, B.O., Ombok, M.O., Ouma, C., Githeko, A.K., Vulule, J., Yan, G. and Gimnig, J.E. (2013) The Efficacy of Long-Lasting Nets with Declining Physical Integrity May Be Compromised in Areas with High Levels of Pyrethroid Resistance. Malaria Journal, 12, Article No. 368. https://doi.org/10.1186/1475-2875-12-368
23. Randriamaherijaona, S.; Raharinjatovo, J.; Boyer, S., (2017). Durability monitoring of long-lasting insecticidal (mosquito) nets (LLINs) in Madagascar: physical integrity and insecticidal activity. Parasite Vectors. 10:564.
24. Tan, KT.; Coleman, J.; Smith, B.; Hamainza, B.; Katebe-Sakala, C., and Kean, C., (2016). A longitudinal study of the durability of long-lasting insecticidal nets in Zambia. Malaria Journal.15: 106.
25. Vanden Eng JL, Chan A, Abílio AP, Wolkon A, Ponce de Leon G, Gimnig J, (2015) Bed Net Durability Assessments: Exploring a Composite Measure of Net Damage. PLoS ONE 10(6): e0128499. doi:10.1371/journal.pone.0128499
26. WHO, (1992). Vector resistance to pesticides fifteen report of committee n insecticides. Geneva: WHO; 1992. No: 818.
27. WHO (2011). Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions. Geneva: World Health Organization. WHO/HTM/NTD/WHOPES/2011.5.
28. WHO (2012) Global Malaria Program (a system to improve value for money in LLIN procurement through market competition based on cons par year of effective coverage).
29. WHO (2012). WHOPES Recommendations and Reports of WHOPES working Group Meetings. Geneva: World Health Organization, 2012.
30. WHO (2013). WHO guidance note for estimating the longevity of LLINs in malaria control. Global malaria program.
31. WHO (2017). Achieving and maintaining universal coverage with long-lasting insecticidal nets for malaria control. Geneva: World Health Organization, Global Malaria Programme. WHO/HTM/GMP/2017.20.
32. WHO (2020), WWW.Who.int/news-room/fact-sheet/detail/malaria
33. WHO (2020). The "world malaria report 2019" at a glance.
34. Zaim M. (1987). Malaria control in Iran-present and future. Joural America Mosquito Control Association. 3: 392-6

This page is intentionally left blank

Global Journal of Medical Research: F
Diseases
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

# Acute Effect of Photobiomodulation with (LED) Reduces Pain in Individuals with Diabetic Neuropathy: A Randomized Double-Blinded Placebo Controlled Clinical Trial

By André Timóteo Sapalo, Aline Gobbi, Gabriela de Carvalho, Elaine Caldeira de Oliveira Guirro & Rinaldo Roberto de Jesus Guirro

*University of São Paulo*

*Abstract-* *Introduction:* Neuropathic pain is one of the reasons for intervention in diabetic patients, and photobiomodulation (PBM) is a non-pharmacological therapeutic alternative, which has been increasingly used in recent years, with modulatory actions on cellular energy metabolism.

*Objective:* To evaluate the acute effect generated by photobiomodulation with light emitting diode (LED) on pain in individuals with neuropathy resulting from type 2 diabetes mellitus.

*Methods:* The study consisted of the evaluation of the signs and symptoms of neuropathic pain (Douleur Neuropathique Questionnarie – DN4) and blood flow in the posterior and dorsal tibial arteries (Doppler ultrasound) after the application of PBM for three consecutive days in 58 patients.

*Keywords:* *photobiomodulatio, neuropathic pain, diabetic polyneuropathy, painful polyneuropathy, type 2 diabetes mellitus, blood flow.*

*GJMR-F Classification:* *NLM: WB 541*


*Strictly as per the compliance and regulations of:*

# Acute Effect of Photobiomodulation with (LED) Reduces Pain in Individuals with Diabetic Neuropathy: A Randomized Double-Blinded Placebo Controlled Clinical Trial

André Timóteo Sapalo [α], Aline Gobbi [σ], Gabriela de Carvalho [ρ], Elaine Caldeira de Oliveira Guirro [ω], & Rinaldo Roberto de Jesus Guirro [¥]

*Abstract- Introduction:* Neuropathic pain is one of the reasons for intervention in diabetic patients, and photobiomodulation (PBM) is a non-pharmacological therapeutic alternative, which has been increasingly used in recent years, with modulatory actions on cellular energy metabolism.

*Objective:* To evaluate the acute effect generated by photobiomodulation with light emitting diode (LED) on pain in individuals with neuropathy resulting from type 2 diabetes mellitus.

*Methods:* The study consisted of the evaluation of the signs and symptoms of neuropathic pain (Douleur Neuropathique Questionnarie – DN4) and blood flow in the posterior and dorsal tibial arteries (Doppler ultrasound) after the application of PBM for three consecutive days in 58 patients. The procedure was carried out in 5 days, on the first day (pre-treatment), the evaluation, and from the second to the fourth day, the application of PBM directly to the skin of both legs bilaterally, using a blanket (33x42cm2) of red LEDs (620nm; 52.86 mW/cm²), infrared (940nm; 33.7 mW/cm²) or associated for 1.30 minutes (180 J per leg). On the fifth day, patients were reevaluated using the same tools as the first day.

*Results:* The signs and symptoms of neuropathic pain showed a reduction ($p<0.05$) between 4.7 and 5.5 points in DN4 in all groups irradiated with LEDs, with a clinical effect. No change in arterial blood flow of the legs was observed.

*Conclusion:* Photobiomodulation therapy was effective in reducing the signs and symptoms of neuropathic pain in the lower limbs of type 2 diabetic patients.

*Keywords: photobiomodulatio, neuropathic pain, diabetic polyneuropathy, painful polyneuropathy, type 2 diabetes mellitus, blood flow.*

*Corresponding Author α: Postgraduate Program in Functional Rehabilitation and Performance, Department of Health Science, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto – SP, Brazil. e-mail: sapalo@usp.br*

*Author σ ρ ω ¥: Postgraduate Program in Functional Rehabilitation and Performance, Department of Health Science, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto – SP, Brazil.*

## I. Introduction

The causes of the onset of pain in diabetic patients are diverse and include mainly the metabolic deficit of the neuronal cell and reduced vascular supply, currently, these two factors are identified as the main reasons for early hospitalization and responsible for 50% of amputations of lower members globally [1-3].

The repercussion of diabetes mellitus (DM) in face of vascular impairment is centered on the damage at the level of peripheral nerve cells, thus the micro-vascular deficit and the constant increase in free radicals generate impairment in the non-myelinated fibers of the sympathetic system and in the cholinergic-type vasodilator nerve endings, reducing the release of acetylcholine (ACh) and limiting the production of nitric oxide (ON), by means of the enzyme nitric oxide synthase (eONs) [4].

Nervous impairment resulting from diabetes mellitus not only affects the sensorimotor nerve fibers but, at an advanced stage, can have repercussions for the peripheral autonomic fibers [5], thus the diabetic distal polyneuropathy is the most frequent of the neuropathies, affecting mainly the lower extremities [6-8].

The sensory axons are the most affected because they have a larger mitochondrial population, leaving them in a condition more vulnerable to oxidative stress [9,10]. Diabetic polyneuropathy is identified as the main factor for the appearance of clinical signs and symptoms in these patients, since 20% of patients may experience neuropathic pain [11].

The choice of a non-pharmacological therapeutic line, which involves the relief of neuropathic pain in the clinical environment, still deserves further investigation. Clinical and experimental evidence suggests that changes in cell function, resulting in oxidative stress, act as a major factor in the development and progression of pain diabetic neuropathies [12].

The great effects resulting from photobiomodulation (laser or LED) are atributed to the heme cofactors existing in erythrocytes and

mitochondria. Thus, half of the pho-tons generated by the irradiation are attenuated by the enzyme cytochrome C oxidase of the respiratory chain, although hemoglobin, myoglobin, and melanin may also present a high attenuation coefficient for photons, mainly in the red light spectrum [13,14].

Today, in clinical practice, two resources are used for photobiomodulation (Laser and LED), although the laser emits collimated light, different of LED light, both have the same therapeutic effect [15], since this difference ends when the light reaches the biological tissues, due to its dispersion .

Scientific evidence shows that some biological tissues have a high coefficient of therapeutic light attenuation (photobiomodulation), such as the skin (higher melanin concentration) [16], the erythrocyte [17,18], nervous tissue [19-21], and muscle tissue [22,23]. Thus, when the light is absorbed by photoreceptors, photonic energy is trans-formed into chemical energy, making photobiomodulation a non-pharmacological alter-native that can generate an increase in energy metabolism [24,25], vasodilation, in-creased blood flow, angiogenesis, increased inflammatory response, accelerated healing process [26,27], increased oxygen affinity hemoglobin [18], and reduction of neuro-pathic symptoms through cytokine stimulation and release mechanisms [28].

Given the above, the purpose of this study was to show that photobiomodulation with light emitting diode (LED) can improve the signs and symptoms of pain generated by the metabolic disorder of lower limbs in diabetic patients with polyneuropathy (Painful polyneuropathy), from the effects of metabolic increase and favoring biophysical photobiomodulation responses.

## II. Materials and Methods

### a) *Ethical aspects*

This study is a randomized double-blinded placebo-controlled clinical trial, it was approved by the Ethics Committee on Experimentation with Human Beings of the Clinical Hospital - FMRP/USP (process no. 3.805.967) and registered as a clinical trial on ClinicalTrials.gov. (NCT03369834). Patients who agreed to participate in the study were informed about the objectives and procedures and signed a free and informed consent form. The study was developed from April 2018 to March 2020.

### b) *Sample and Randomization*

The sample calculation was performed using the Ene® software (version 3.0, Barcelona, Spain). The sample size was calculated based on the study by Lorne et al. (2004) [29], which evaluated pain in diabetic polyneuropathy using laser as a therapeutic tool. We considered the average values of the Visual Analog Pain Scale (VAS), based on the four evaluation periods. The calculation was based on the detection of comparison between the groups, with a mean of the reference group of 9 and the experimental group 9. Considering the statistical power of 90% and alpha 0.05, the number of 10 patients per group was estimated. Considering a sample loss of 10%, 12 patients were recruited per group.

Seventy patients with type 2 diabetes mellitus of both sexes with more than 5 years of diagnosis at the age of 45 to 70 years old were recruited at the Hospital das Clínicas de HC/USP. 58 of whom had neuropathic pain ranging from 4 to 6 points (DN4), classified as moderate to severe on the scale of diabetic distal polyneuropathy (DSDDP) in the lower limbs. The exclusion criteria involved neurological lesions that that impeded the proposed exams, chronic renal failure, and patients who have had part of the saphenous vein removed for myocardial revascularization. Some of the volun-teers took medication to control blood pressure, among them ACE antidiuretics and beta-blockers. All were instructed not to take analgesics or anti-inflammatory drugs during the week of the study.

Randomization was performed using a table of random numbers in the Excel software, which was placed in sealed opaque paper envelopes, opened only in the presence of the patient. Patients were randomly divided into the following groups: Control (C, n = 11), Sham (S, n = 12), Red LED (R, n = 11), Infrared LED (IR, n = 13), Red+Infrared LED (R+IR, n = 11).

Researchers 2 and 3 and the patients themselves were blinded regarding the distribution of groups and the interventions applied. The blinding of the patient in relation to the groups, with the exception of the control, was accomplished with the use of a blindfold. As shown in the flowchart in Figure 1.

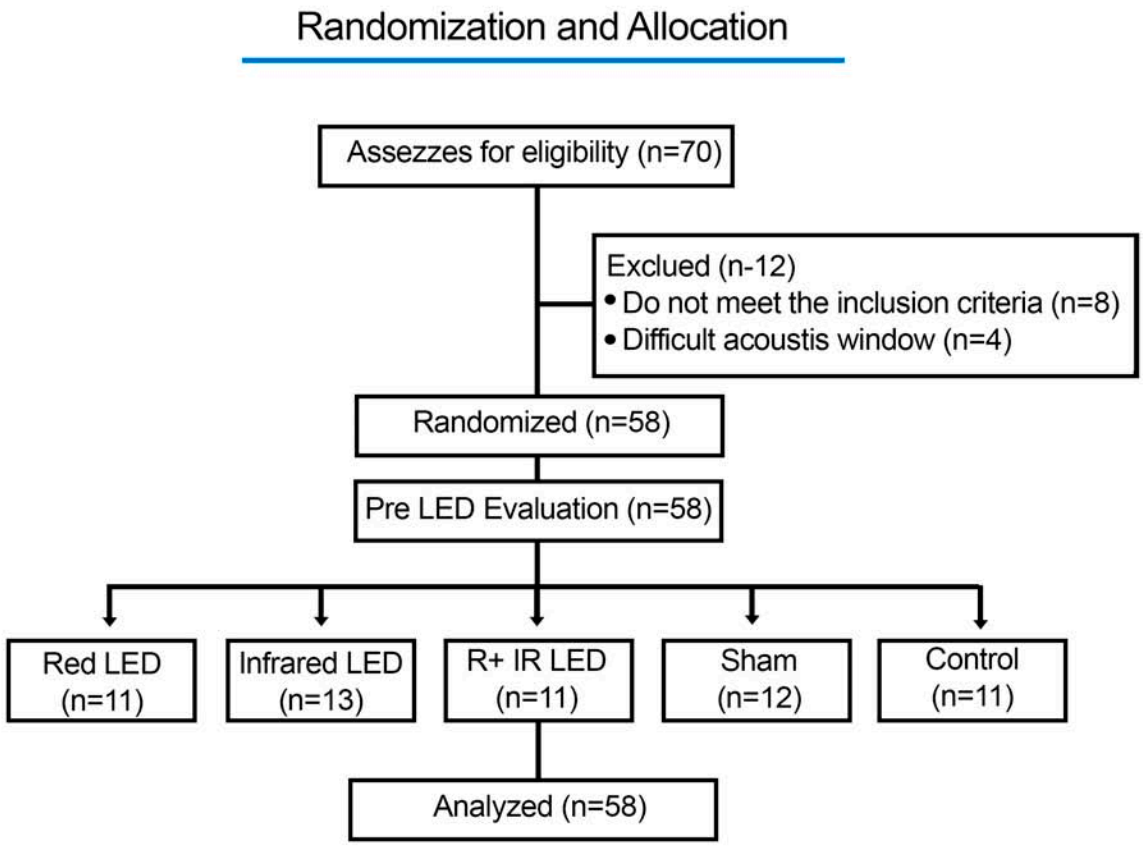



*Fig. 1:* Flowchart of the study design

*c) Procedure*

The study procedures were performed in 5 days. On the first day, the researchers collected the anthropometric and clinical data, applied the diagnostic scale for distal diabetic polyneuropathy (DSDDP) and the neuropathic pain questionnaire (DN4), and evaluated the blood flow in the posterior and dorsal tibial arteries through Doppler ultrasound. From the second to the fourth day, the patients received irradiation (treatment group) and the simulation of application (Sham group) of photobiomodulation with light emitting diode (LED) for one minute and 37 seconds, on average, in each lower limb, totaling 3 minutes per patient, respecting the 24-hour interval between irradiations. On the fifth day, reassessments were carried out using the same assessment tools of the first day (Figure 2).

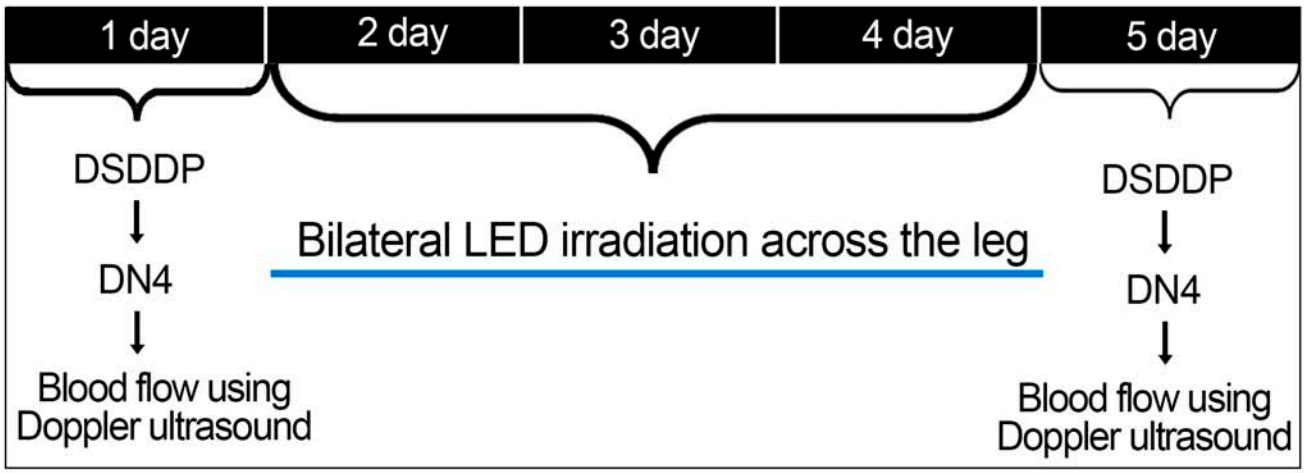



*Figure 2:* Description of the evaluation, intervention and reassessment during the proposed 5 days.

*d) Assessment of neuropathy*

i. *Diagnostic scale for diabetic distal polyneuropathy*

To quantify the degree of polyneuropathy, the Diagnostic Scale for Diabetic Distal Polyneuropathy (DSDDP) was used, which evaluates neuropathic symptoms with six questions and neuropathic impairment with the Aquileu reflex tests and the hallux vibratory, painful and thermal sensitivity [30]. Thus, patients should have a score equal to or greater than 5 for symptoms (ESN), associated with a score equal to or greater than 3 for signs (ECN). To characterize neuropathic pain, the patient should have a score equal to or greater than 4 on the Neuropathic Pain Diagnosis questionnaire (DN4).

ii. *Aquileu reflex test*

The Aquileu reflex test was performed using Buck's neurological hammer to as-sess motor changes in alpha-thick myelinated A-fibers [30]. With the patient seated, the foot hanging and in a neutral position, percussion was performed with the reflex ham-mer on the Achilles tendon of both lower limbs. As a positive answer, the volunteer is expected to do reflex plantar flexion, as a consequence of percussion, and the absent or diminished reflex response means an altered result. Therefore, a second percussion was performed to confirm the altered result [31,32].

iii. *Vibratory sensitivity test*

For the vibration sensitivity test, a 128 Hertz frequency tuning fork was used to test changes in beta-thick myelinated A-fibers [30]. With the patient in the supine position, the vibrating tuning fork was applied with the end of the nail, perpendicular and with constant pressure, on the dorsal portion of the distal phalanx of the hallux and medial malleolus or tibial tuberosity, as an alternative, if the volunteer could not feel it in the first region tested. Two applications were made, alternating with a false application, in which the tuning fork was not vibrating. Because of this, the result should be negative, that is, the absence of protective sensitivity, with two incorrect answers from the three applications. The test was performed on both lower limbs [31,32]. According to Perkins et al. [33], the vibration test has a sensitivity of 53% and specificity of 99% for peripheral neuropathy.

iv. *Pain and thermal sensitivity test*

Buck's neurological hammer needle was used for the painful sensitivity test, to evaluate sensory changes in C-thin unmyelinated fibers[30]. With the volunteer in the supine position, the tip of the reflex hammer was applied to the back of the hallux, bilat-erally, with sufficient pressure to deform the skin. An altered result was the inability to feel the pressure exerted on the hallux [31,32]. In the assessment of thermal sensitivity, the test was carried out with a test tube containing ice water to assess the sensitive alterations of delta-thin myelinated A-fibers [30]. With the volunteer in the supine position, a test tube containing ice water was applied to the dorsal region of the feet of both lower limbs, in which the result was considered altered when the patient did not report the cold sensation at the test site [33].

v. *DN4 Questionnaire for diagnosis of neuropathic pain*

For the qualification of neuropathic pain, the DouleurNeuropathique 4 (DN4) questionnaire was used, it has seven questions and three sensory exams, which are able to discriminate neuropathic pain from nociceptive pain. It was translated to Portuguese and validated, with 100% sensitivity and 93.2% specificity [34].

For scoring, DN4 assigns 1 when it is positive and 0 when it is negative (total score range varies from 0 to 10). The cut-off value for the diagnosis of neuropathic pain is a total score equal to or greater than 4

*Assessment of arterial blood flow*

A portable continuous-wave Doppler device with spectral analysis (Nicolet Vascular Versalab SE, San Carlos, CA, USA) was used, coupled to a notebook. To capture and process the signals, Care Fusion 7.0 software (Nicolet Vascular Versalab SE, San Carlos, CA, USA) was used, which allows the quantification of blood flow, including the peak systolic velocity and resistivity index, as well as its qualification in an interval of time.

Blood flow was assessed after 10 minutes of rest in the supine position. Collections were performed in the posterior and dorsal tibial arteries, with frequencies of 4 and 8 MHz, respectively, in both lower limbs.

*Photobiomodulation*

Irradiation was performed individually in 58 patients with moderate to severe diabetic distal polyneuropathy. Thus, 116 legs were irradiated during the second, third, and fourth days of therapy. The light was irradiated by a 33x42cm2 LED mat, using SMD5050 diodes fed with 12-volt voltage and fixed in an ethylene-vinyl-acetate (EVA) plate with equidistant distribution (1 cm) between the LEDs [35]. The energy applied to each leg was 180 J, on the anterior and posterior regions, bilaterally. The blanket was malleable to adapt to the contour of the leg, so the application of photobiomodulation was directly on the skin involving the entire leg of the patients (Figure 3).

*Figure 3:* Application of the LED mat directly on the skin involving the whole leg bilaterally.

All LEDs were previously checked at the Photobiophysics Laboratory of the Faculty of Philosophy, Sciences, and Letters of RibeirãoPreto, University of São Paulo, where the wavelengths, power, and power density were evaluated. 285 LEDs were used in both blankets (red, infrared), and 320 LEDs for the mixed blanket, 160 LEDs in the red spectrum (620 nm), and 160 in the infrared spectrum (940 nm) (Table 1).

*Table 1:* Physical parameters of light-emitting diodes (LED) and photobiomodulation therapy protocol.

| Variables | Red | Infrared | Red+Infrared |
|---|---|---|---|
| Wave-length | 620±10nm | 940±10nm | |
| Number of diodes | 285 | 285 | 320 |
| Diode diameter | 0.125 $cm^2$ | 0.178 $cm^2$ | |
| Power Density | 52.86 $mW/cm^2$ | 33.7 $mW/cm^2$ | |
| Diode power | 0.0066 W | 0.006 W | |
| Total blanket power | 1.88 W | 1.71 W | 2.01 W |
| Application time | 96 s | 106 s | 90 s |
| Total energy per leg | 180 J | 180J | 180 J |

*mW: milliwatt; s: Second; j: joules; W: watt*

*e) Statistical analysis*

Statistical analysis was performed using SPSS for Windows (version 20; SPSS Inc., Chicago, IL). The mean differences (initial minus final value) of the groups and the 95% confidence intervals (CI) were calculated using mixed linear models, with the baseline as a covariate. The Bonferroni correction test was used to compare the groups, with a p value of 0.05.

To determine the size of the clinical effect of the proposed therapies, Cohen's d was used, with interpretation of the values based on the classification established by Cohen (1988): less than 0.2, small effect; around 0.5, moderate effect; and above 0.8, great effect.

## III. Results

Of the 70 recruited patients, 58 were included and randomized. To characterize the homogeneity of the sample, anthropometric assessments and some clinical routes are available in table 2.

*Table 2:* Anthropometric and clinical data of patients with type 2 diabetes mellitus, distributed in the experimental groups.

| | | | C n=11 | S n=12 | R n=11 | IR n=13 | R+IR n= 11 |
|---|---|---|---|---|---|---|---|
| Age | | | 63.66 (4.18) | 61 (5.37) | 61.38 (4.44) | 60.08 (7.31) | 61.3 (6.99) |
| Sex | Male | | 10 | 5 | 9 | 5 | 4 |
| | Female | | 3 | 7 | 4 | 8 | 7 |
| Weight | | | 80.15 (13.78) | 80.5 (18.46) | 89.36 (21.78) | 86.38 (13.42) | 97.54 (19.65) |
| BMI | | | 30 (4.50) | 30.25 (7.72) | 32.49 (5.72) | 32.39 (4.78) | 36.73 (4.97) |
| Time of diagnosis (years) | | | 17.23 (6.67) | 14.91 (9.17) | 13.92 (6.34) | 14.08 (6.76) | 16.27 (8.79) |
| Arterial hypertension | | | 9 | 10 | 8 | 10 | 9 |
| DSDDP | DM2 | Symptoms | 6.46±1.89 | 6.76±2.16 | 7.8±1.72 | 6.25±2.22 | 7.00±1.63 |
| | | Signals | 4.61±1.12 | 4.07±0.75 | 4.54±1.21 | 4.00±0.73 | 4.23±0.83 |
| DN4 (pain initial) | | | 6.3 (1.94) | 5.6 (1.42) | 6 (2.30) | 6.9 (3.66) | 6.7(1.42) |

*BMI: body mass index; DM2: type 2 diabetes mellitus; DSDDP: scale for the diagnosis of diabetic distal polyneuropathy; DN4:* Douleur Neuropathique 4 Questionnaire**p<0.05.R: red group, IR: infrared group, R+IR: red+infrared group, S: sham group, C: control group.*

As for the assessment of neuropathic pain by the DN4 questionnaire, after irradiation with LED, there was a significant difference in the comparison between the groups that received the treatment (LED) with the control and sham groups. Specifically, when comparing LED red groups with control and sham, the difference ($p < 0.001$) between the means (lower limit and upper limit) was -4.18 (-5.95 -2.40) and -4.08 (-5.81 -2.35), respectively; when comparing infrared LED with control and sham there were also differences ($p < 0.001$) with values of -4.21 (-5.91 -2.50) and -4.11 (-5.77 -2.45) respectively; as well as LED red+infrared compared to control and sham with differences ($p<0.001$) of -4.58 (-6.37 -2.80) and -4.49 (-6.23 - 2.74), respectively. In the comparisons between the different groups irradiated with LED, there was no significant difference, as well as between control and sham. The size of the clinical effect of photobiomodulation therapy in the different groups, using Cohen's d, had a great effect on relieving neuropathic pain for all groups irradiated with LED (Table 3).

*Table 3:* Values of differences between means, 95% confidence interval, upper and lower limits, and assessment of effect size of different groups, considering signs and symptoms of neuropathic pain from the neuropathic Douleur 4 questionnaires (DN4).

| Comparison between groups | Difference of means | *95% confidence intervals* Inferior limit | Upper limit | Effect size *Cohen's d* |
|---|---|---|---|---|
| R x IR | 0.03 | -1.66 | 1.73 | -0.02 |
| R x R+IR | 0.40 | -1.36 | 2.18 | -0.08 |
| R x C | -4.18* | -5.95 | -2.40 | -2.08 |
| R x S | -4.08* | -5.81 | -2.35 | -1.92 |
| IR x R+IR | 0.37 | -1.33 | 2.08 | 0.05 |
| IR x C | -4.21* | -5.91 | -2.50 | -2.15 |
| IR x S | -4.11* | -5.77 | -2.45 | -1.61 |
| R+IR x C | -4.58* | -6.37 | -2.80 | -2.18 |
| R+IR x S | -4.49* | -6.23 | -2.74 | -1.07 |
| C x S | -0.09 | -1.82 | 1.63 | -0.01 |

** p<0.05. R: red group, IR: infrared group, R+IR: red+infrared group, S: sham group, C: control group.*

As for the qualitative assessment of the signs and symptoms of DN4, there was a reduction from 75% to 100% for responses to pain qualification after the application of LED and from 50% to 85% in responses to physical examination of pain. Considering the groups, the table shows that the reduction in pain, with regard to their qualification, occurred in the three groups irradiated in an equitable way, unlike the groups sham and control that did not present any changes. For the answers to the exams, it is observed that only in red+infrared there was pain reduction (Table 4).

*Table 4:* Number of patients who present the characteristics/symptoms of pain before and after application of LED, in the Douleur neuropathic 4 questionnaire (DN4).

| DN4 | R | | IR | | R+IR | | S | | C | |
|---|---|---|---|---|---|---|---|---|---|---|
| Features / Symptoms | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| Burning | 8 | 0 | 8 | 1 | 8 | 2 | 6 | 7 | 7 | 7 |
| Painful cold sensation | 7 | 0 | 5 | 0 | 6 | 1 | 5 | 5 | 4 | 4 |
| Electric shock | 8 | 1 | 8 | 1 | 7 | 0 | 5 | 5 | 7 | 7 |
| Tingling | 7 | 1 | 11 | 1 | 8 | 1 | 8 | 8 | 10 | 10 |
| Pinned and needled | 8 | 0 | 11 | 1 | 7 | 1 | 6 | 6 | 6 | 6 |
| Fall asleep | 7 | 1 | 10 | 1 | 6 | 0 | 7 | 7 | 8 | 7 |
| Itching | 5 | 1 | 8 | 2 | 4 | 0 | 3 | 4 | 3 | 3 |
| Exams | | | | | | | | | | |
| Hypoesthesia to the touch | 2 | 3 | 1 | 1 | 7 | 1 | 5 | 5 | 6 | 6 |
| Needle prick hypoesthesia | 2 | 3 | 1 | 1 | 8 | 3 | 5 | 5 | 6 | 6 |
| Brushing | 6 | 3 | 6 | 6 | 6 | 3 | 5 | 5 | 6 | 6 |

*R: red group, IR: infrared group, R+IR: red+infrared group, S: sham group, C: control group.*

Considering that neuropathic pain could be associated with circulatory restriction, we opted for the Doppler flow assessment of the lower limb arteries, distally. The results did not show significant changes ($p > 0.050$) for the PVS, IR, IP, and HR parameters for any of the groups, despite presenting a clinical effect size between mild to moderate for the groups irradiated with LED (Table 5).

*Table 5:* Values of the difference in means and the confidence interval of blood flow variables and do Effect Size of the Doppler ultrasound variables for the tibial and dorsal arteries of the lower limbs.

| | Variáveis | R x IR | R x R+IR | R x S | R x C | IR x R+IR | IR x S | IR x C | R+IR x S | R+IR x C | S x C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dorsal R | PVS | 4,21 (-10.07 18.50) | 2.56 (-12.40 17.53) | 3.36 (-10.94 17.66) | -1.38 (-16.67 13.91) | -1.65 (-15.65 12.34) | -0.85 (-14.12 12.41) | -5.59 (-20.02 8.82) | 0.80 (-13.15 14.76) | -3.94 (-18.82 10.94) | -4.74 (-19.00 9.51) |
| | *ES* | 0.34 | 0.00 | 0.08 | -0.48 | -0.32 | -0.80 | -0.28 | 0.07 | -0.46 | 0.57 |
| | IR | 0.09 (-0.03 0.22) | 0.03 (-0.10 0.17) | -0.02 (-0.15 0.11) | -0.00 (-0.14 0.13) | -0.05 (-0.18 0.07) | -0.11 (-0.24 0.00) | -0.09 (-0.23 0.03) | -0.06 (-0.19 0.07) | -0.04 (-0.18 0.09) | 0.01 (-0.11 0.15) |
| | *ES* | 0.74 | 0.42 | -0.22 | 0.00 | -0.40 | -0.98 | -0.73 | -0.67 | -0.40 | -0.22 |
| Tibial R | PVS | 1.71 (-16.15 19.59) | -1.82 (-20.81 17.17) | -2.66 (20.55 15.22) | -12.89 (-31.34 5.54) | -3.54 (-22.32 15.24) | -4.38 (-22.06 13.30) | -14.61 (-32.82 3.59) | -0.84 (-19.64 17.95) | -11.07 (-30.39 8.24) | -10.23 (-28.46 7.99) |
| | *ES* | 0.11 | -0.07 | -0.16 | -0.78 | -0.22 | -0.27 | -0.89 | -0.09 | -0.79 | 0.73 |
| | IR | 0.05 (-0.06 0.18) | 0.00 (-0.13 0.13) | -0.03 (-0.16 0.08) | -0.02 (-0.15 0.10) | -0.05 (-0.19 0.07) | -0.09 (-0.22 0.02) | -0.08 (-0.21 0.04) | -0.04 (-0.17 0.09) | -0.02 (-0.16 0.10) | 0.01 (-0.11 0.14) |
| | *ES* | 0.59 | 0.00 | -0.46 | -0.29 | -0.61 | -1.05 | -0.82 | -0.50 | -0.31 | -0.11 |
| Dorsal L | PVS | -0.40 (-17.88 17.08) | -8.11 (-26.71 10.49) | -15.77 (-33.26 1.71) | -9.59 (-27.87 8.68) | -7.70 (-25.94 10.52) | -15.37 (-32.48 1.73) | -9.18 (-27.10 8.72) | -7.66 (-25.91 10.59) | -1.47 (-20.47 17.51) | 6.18 (-11.73 24.10) |
| | *ES* | -0.03 | -0.54 | -1.02 | -0.54 | -0.63 | -1.17 | -0.63 | -1.17 | -0.09 | -0.39 |
| | IR | -0.01 (-0.15 0.12) | -0.02 (-0.16 0.12) | -0.08 (-0.21 0.05) | -0.04 (-0.19 0.09) | -0.00 (-0.14 0.13) | -0.06 (-0.19 0.06) | -0.03 (-0.17 0.10) | -0.05 (-0.19 0.08) | -0.02 (-0.17 0.12) | 0.03 (-0.10 0.17) |
| | *ES* | -0.08 | -0.15 | -0.62 | -0.34 | -0.09 | -0.70 | -0.34 | -0.53 | -0.20 | -0.43 |
| Tibial L | PVS | 4.39 (-15.34 24.12) | 4.79 (-17.58 27.18) | -1.68 (-22.12 18.76) | -10.81 (-31.83 10.19) | 0.40 (-21.25 22.06) | -6.07 (-25.71 13.56) | -15.20 (-35.45 5.03) | -6.47 (-28.76 15.80) | -15.61 (-38.44 7.21) | -9.13 (-30.08 11.81) |
| | *ES* | 0.29 | 0.38 | -0.18 | -0.63 | 0.04 | -0.83 | -0.42 | -0.48 | -0.89 | 0.42 |
| | IR | 0.04 (-0.11 0.20) | 0.04 (-0.13 0.22) | -0.02 (-0.19 0.13) | -0.04 (-0.20 0.12) | 0.00 (-0.17 0.17) | -0.08 (-0.23 0.07) | -0.08 (-0.25 0.07) | -0.08 (-0.26 0.09) | -0.08 (-0.27 0.09) | 0.00 (-0.16 0.17) |
| | *ES* | 0.30 | 0.34 | -0.35 | -0.45 | 0.00 | -0.69 | -0.82 | -0.65 | -0.76 | 0.09 |

*Dorsal (R, L): right and left dorsal artery, Tibial (R, L): right and left posterior tibial artery, PVS: peak systolic velocity, IR: resistivity index.R: red group, IR: infrared group, R+IR: red+infrared group, S: sham group, C: control group, ES: Effect Size*

## IV. Discussion

The hypothesis that photobiomodulation with LED could be effective in reducing the signs and symptoms of neuropathic pain of the lower limbs was confirmed, being an effective therapy in short-term intervention, with a great clinical effect for all groups irradiated with LED.

According to Kallenborn-Gerhardt et al. (2013) [36], the main causes of pain in peripheral neuropathy still need further studies. In diabetic patients, the emergence of neuropathy is believed to be due to multiple factors, including the increased production of reactive oxygen species at the mitochondrial level, reduced antioxidant capacities such as superoxide dismutase catalase and glutathione in enzymatic and non-enzymatic cells [37], and vascular impairment generated by chronic hyperglycemia [38,39]. These factors are identified as the main causes of reverse neuroinflammation, which promotes an imbalance in the production of ATP and the induction of apoptosis in nerve cells. Thus, the results of this study open a window so that photobiomodulation can be used as a non-pharmacological alternative, contributing to the reduction of pain generated by painful neuropathy.

In the light of the evidence, the results of Cg et al. (2015) [40] corroborated the results found in this study, based on the hypothesis that the biophysical responses generated by photobiomodulation may control neurological pain in diabetic patients with polyneuropathy. Thus, it is highlighted that the three LED irradiations in the leg were sufficient to reduce pain in the lower limbs. Gobbi et al. (2020) [41] report that PBMT applied for a short period does not bring important gains for the muscular performance and functionality of diabetic individuals.

As it is a subjective experience, pain has some limitations regarding its measurement. The use of a standardized and validated questionnaire that involves other aspects than just its intensity is important since it contemplates the subjective experience, involving the affective responses associated with pain. Thus, the application of DN4 sought to expand this analysis.

The quantification of pain through DN4 showed a reduction between 4.7 and 5.5 points, on a 10 point scale, in the LED groups, so it is believed that this response is due to the effects of metabolic increment and favoring biophysical responses generated by photobiomodulation, as proposed by Janzadeh et al. (2016) [42] in an animal model, where they emphasized that photobiomodulation can generate an increase in the levels of antioxidants, such as superoxide dismutase catalase and glutathione, improve mitochondrial function, safeguard the survival of neural cells, and improve symptoms of pain.

It speculates that the affective responses associated with pain reduction in the red+infrared LED group are caused by the proportional increase in the metabolic effect generated by the combined irradiation of the two light spectra. Since the two lengths tend to improve electrochemical activity and increase ATP re-synthesis[14]. In addition, irradiation with LED in the infrared spectrum promotes an increase in the concentration of oxyhemoglobin and total oxymyhemoglobin, which increases the availability of oxygen [18] that may favor aerobic metabolism.

Although the main factor for the emergence of neuropathic pain is assigned to metabolic stresses (ROS)[43], it is also emphasized that the vascular impairment, generated by reduced flow and hypoperfusion due to hyperglycemia, can lead to the failure of several tissues and contribute to the onset of neuropathic pain early [44].

Although there were no significant changes in blood flow, the clinical effect was also in favor of the irradiated groups.

Considering that oxidative stress plays a central role in the development of microvascular complications of diabetes [45], as well as a secondary variable, the blood flow of the posterior and dorsal tibial arteries was measured in order to support the context of blood circulation in the appearance of neuropathic pain. Although there was no significant improvement in the quantitative assessments of Doppler ultrasound, it is believed that photobiomodulation tends to improve blood flow and reduce arterial resistance, since there was an improvement from mild to moderate in the effect size in the irradiated groups, for the peak systolic velocity variables and the resistivity index in the three therapeutic intervention groups. The mechanisms proposed to explain the vascular changes induced by glucose and lipids in diabetes include the accelerated formation of advanced glycation end products (AGEs), activation of protein C kinase, inflammatory signaling, and oxidative stress [46]. Accordingly, the deficits in the bioavailability of nitric oxide and the large concentrations of ROS are the main responsible for vasculopathy in diabetic patients, [47,48] responses that can be reversed with PBM.

According to our searches, in order to confront the findings of the influence of LED light on blood flow, there was an absence of studies that analyzed vascular resistance (IR) and improved blood flow (PVS) in patients with pain secondary neuropathic diabetes mellitus, what leads to a limitation in the discussion of the results presented.

In view of the results obtained, we believe that the irradiation time of three days is one of the factors that can be extended, allowing a longer response time of the tissues in face of photobiomodulation, allowing an effect that can generate changes that can be reflected

at the level of macrocirculation. In addition, using different means of evaluation, such as microcirculation, tissue perfusion of the lower limbs, biomarkers of neuronal dysfunction, and systemic inflammation, could complement the analysis of neuropathic pain and vascular c condition, secondary to diabetes mellitus.

Thus, despite the positive limitations faced due to lack of equipment to better assess the painful polyneuropathy caused by type 2 diabetes mellitus, this study opens a new window for photobiomodulation by light-emitting diode (LED) in the red, infrared, or associated spectrum is now filled as a non-pharmacological therapeutic line for reducing the signs and symptoms of neuropathic pain in patients with type 2 diabetes with polyneuropathy of the lower limbs.

## Acknowledgements

This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES)

*Conflict of Interest*

The authors declare no conflicts of interest.

*Funding*

This study was financed in parts by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001

*Ethics approval*

Comitê de Éticaem Experimentação com Seres Humanos do Hospital das Clínicas – FMRP/USP (no. 3.805.967)

## References Références Referencias

1. Ulbrecht JS, Cavanagh PR, Caputo GM. Foot problems in diabetes: an overview. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America.* Aug 1 2004; 39 Suppl2: S73-82.
2. Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. *The Lancet.* 2005; 366(9498): 1719-1724.
3. Schreml S, Berneburg M. The global burden of diabetic wounds. *The British journal of dermatology.* Apr 2017; 176(4): 845-846.
4. Bello A, Biliaminu S, Wahab K, Sanya E. Distal symmetrical polyneuropathy and cardiovascular autonomic neuropathy among diabetic patients in Ilorin: Prevalence and predictors. *The Nigerian postgraduate medical journal.* Apr-Jun 2019; 26(2): 123-128.
5. Chan AC, Wilder-Smith EP. Small fiber neuropathy: Getting bigger! *Muscle & nerve.* May 2016; 53(5): 671-682.
6. Gwathmey KG, Pearson KT. Diagnosis and management of sensory polyneuropathy. *Bmj.* May 8 2019; 365: l1108.
7. Callaghan BC, Cheng HT, Stables CL, Smith AL, Feldman EL. Diabetic neuropathy: clinical manifestations and current treatments. *The lancet NEUROLOGY.* 2012; 11(6): 521-534.
8. Leahy JL. Pathogenesis of type 2 diabetes mellitus. *Archives of medical research.* May-Jun 2005; 36(3): 197-209.
9. Lotfy M, Adeghate J, Kalasz H, Singh J, Adeghate E. Chronic Complications of Diabetes Mellitus: A Mini Review. *Current diabetes reviews.* 2017; 13(1): 3-10.
10. Marfella R, Nappo F, De Angelis L, Paolisso G, Tagliamonte MR, Giugliano D. Hemodynamic effects of acute hyperglycemia in type 2 diabetic patients. *Diabetes care.* May 2000; 23(5): 658-663.
11. Avetisov SE, Chernenkova NA, Surnina ZV. [Clinical features and diagnosis of diabetic polyneuropathy]. *Vestnikoftalmologii.* 2017; 133(5): 98-102.
12. Babizhayev MA, Strokov IA, Nosikov VV, et al. The Role of Oxidative Stress in Diabetic Neuropathy: Generation of Free Radical Species in the Glycation Reaction and Gene Polymorphisms Encoding Antioxidant Enzymes to Genetic Susceptibility to Diabetic Neuropathy in Population of Type I Diabetic Patients. *Cell biochemistry and biophysics.* Apr 2015; 71(3): 1425-1443.
13. Pastore D, Greco M, Passarella S. Specific helium-neon laser sensitivity of the purified cytochrome c oxidase. *International journal of radiation biology.* Jun 2000; 76(6): 863-870.
14. Hamblin MR. Mechanisms and Mitochondrial Redox Signaling in Photobiomodulation. *Photochemistry and photobiology.* Mar 2018; 94(2): 199-212.
15. Heiskanen V, Hamblin MR. Photobiomodulation: lasers vs. light emitting diodes? *Photochemical & photobiological sciences: Official journal of the European Photochemistry Association and the European Society for Photobiology.* Aug 8 2018; 17(8): 1003-1017.
16. Brondon P, Stadler I, Lanzafame RJ. Melanin density affects photobiomodulation outcomes in cell culture. *Photomedicine and laser surgery.* Jun 2007; 25(3): 144-149.
17. Wajih N, Basu S, Ucer KB, et al. Erythrocyticbioactivation of nitrite and its potentiation by far-red light. *Redox biology.* Jan 2019; 20: 442-450.
18. Linares SN, Beltrame T, Ferraresi C, Galdino GAM, Catai AM. Photobiomodulation effect on local hemoglobin concentration assessed by near-infrared spectroscopy in humans. *Lasers in medical science.* Apr 2020; 35(3): 641-649.
19. Rosso MPO, Buchaim DV, Kawano N, Furlanette G,Pomini KT, Buchaim RL. Photobiomodulation Therapy (PBMT) in Peripheral Nerve Regeneration: A Systematic Review. *Bioengineering.* Jun 9 2018; 5(2).

20. Shen CC, Yang YC, Huang TB, Chan SC, Liu BS. Low-Level Laser-Accelerated Peripheral Nerve Regeneration within a Reinforced Nerve Conduit across a Large Gap of the Transected Sciatic Nerve in Rats. *Evidence-based complementary and alternative medicine: eCAM.* 2013; 2013: 175629.
21. Hennessy M, Hamblin MR. Photobiomodulation and the brain: a new paradigm. *Journal of optics.* Jan 2017; 19(1): 013003.
22. Dos Santos SA, Serra AJ, Stancker TG, et al. Effects of Photobiomodulation Therapy on Oxidative Stress in Muscle Injury Animal Models: A Systematic Review. *Oxidative medicine and cellular longevity.* 2017; 2017: 5273403.
23. Ferraresi C, Bertucci D, Schiavinato J, et al. Effects of Light-Emitting Diode Therapy on Muscle Hypertrophy, Gene Expression, Performance, Damage, and Delayed-Onset Muscle Soreness: Case-control Study with a Pair of Identical Twins. *American journal of physical medicine & rehabilitation.* Oct 2016; 95(10): 746-757.
24. Miranda EF, de Oliveira LV, Antonialli FC, Vanin AA, de CarvalhoPde T, Leal-Junior EC. Phototherapy with combination of super-pulsed laser and light-emitting diodes is beneficial in improvement of muscular performance (strength and muscular endurance), dyspnea, and fatigue sensation in patients with chronic obstructive pulmonary disease. *Lasers in medical science.* Jan 2015; 30(1): 437-443.
25. Nampo FK, Cavalheri V, Dos Santos Soares F, de Paula Ramos S, Camargo EA. Low-level phototherapy to improve exercise capacity and muscle performance: a systematic review and meta-analysis. *Lasers in medical science.* Dec 2016; 31(9): 1957-1970.
26. Desmet KD, Paz DA, Corry JJ, et al. Clinical and experimental applications of NIR-LED photobiomodulation. *Photomedicine and laser surgery.* Apr 2006; 24(2):121-128.
27. Eells JT, Wong-Riley MT, VerHoeve J, et al. Mitochondrial signal transduction in accelerated wound and retinal healing by near-infrared light therapy. *Mitochondrion.* Sep 2004; 4(5-6): 559-567.
28. Wong-Riley MT, Liang HL, Eells JT, et al. Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome c oxidase. *The Journal of biological chemistry.* Feb 11 2005; 280(6): 4761-4771.
29. Zinman LH, Ngo M, Ng ET, Nwe KT, Gogov S, Bril V. Low-intensity laser therapy for painful symptoms of diabetic sensorimotor polyneuropathy: a controlled trial. *Diabetes care.* Apr 2004; 27(4): 921-924.
30. Moreira RO, Castro AP, Papelbaum M, et al. Tradução para o português e avaliação da confiabilidade de umaescala para diagnóstico da polineuropatia distal diabética. *Arquivos Brasileiros de Endocrinologia & Metabologia.* 2005; 49: 944-950.
31. Milech A, Angelucci AP, Golbert A, Matheus A, Carrilho AJF, Ramalho A. Diretrizes da sociedadebrasileira de diabetes (2015-2016). *São Paulo: AC Farmacêutica.* 2016:13.
32. Boulton AJ, Armstrong DG, Albert SF, et al. Comprehensive foot examination and risk assessment. *Endocrine practice: official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists.* Jul-Aug 2008; 14(5): 576-583.
33. Perkins BA, Olaleye D, Zinman B, Bril V. Simple screening tests for peripheral neuropathy in the diabetes clinic. *Diabetes care.* Feb 2001; 24(2): 250-256.
34. Van Seventer R, Vos C, Meerding W, et al. Linguistic validation of the DN4 for use in international studies. *European journal of pain.* Jan 2010; 14(1): 58-63.
35. de Jesus Guirro RR, de Carvalho G, Gobbi A, de Oliveira Assuncao FF, de Souza Borges NC, Bachmann L. Measurement of Physical Parameters and Development of a Light Emitting Diodes Device for Therapeutic Use. *Journal of medical systems.* Mar 12 2020; 44(4): 88.
36. Kallenborn-Gerhardt W, Schroder K, Geisslinger G, Schmidtko A. NOXious signaling in pain processing. *Pharmacology & therapeutics.* Mar 2013; 137(3): 309-317.
37. Dongre UJ, Meshram VG, Pitale S. Oxidative Stress and 3243 A/G Mitochondrial Dna Mutation In Maternally Inherited Type 2 Diabetes Mellitus.
38. Sandireddy R, Yerra VG, Areti A, Komirishetty P, Kumar A. Neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. *International journal of endocrinology.* 2014; 2014: 674987.
39. Said G. Diabetic neuropathy--a review. *Nature clinical practice. Neurology.* Jun 2007; 3(6): 331-340.
40. Cg SK, Maiya AG, Hande HM, Vidyasagar S, Rao K, Rajagopal KV. Efficacy of low level laser therapy on painful diabetic peripheral neuropathy. *Laser therapy.* Oct 2 2015; 24(3): 195-200.
41. Gobbi A, de Carvalho G, Sapalo AT, de Jesus Guirro RR. Acute application of photobiomodulation does not bring important gains for the muscular performance and functionality of diabetic individuals. *Lasers in medical science.* Jul 2021; 36(5): 995-1002.
42. Janzadeh A, Nasirinezhad F, Masoumipoor M, Jameie SB, Hayat P. Photobiomodulation therapy reduces apoptotic factors and increases glutathione levels in a neuropathic pain model. *Lasers in medical science.* Dec 2016; 31(9): 1863-1869.
43. Volpe CMO, Villar-Delfino PH, Dos Anjos PMF, Nogueira-Machado JA. Cellular death, reactive

oxygen species (ROS) and diabetic complications. *Cell death & disease.* Jan 25 2018; 9(2): 119.
44. Kim SE, Park KM, Park J, et al. Vascular factors and neuropathy in lower limb of diabetic patients. *Journal of clinical neuroscience: official journal of the Neurosurgical Society of Australasia.* Jan 2019; 59: 130-135.
45. Domingueti CP, Dusse LM, Carvalho M, de Sousa LP, Gomes KB, Fernandes AP. Diabetes mellitus: The linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. *Journal of diabetes and its complications.* May-Jun 2016; 30(4): 738-745.
46. Potenza MA, Gagliardi S, Nacci C, Carratu MR, Montagnani M. Endothelial dysfunction in diabetes: from mechanisms to therapeutic targets. *Current medicinal chemistry.* 2009; 16(1): 94-112.
47. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circulation research.* Oct 29 2010; 107(9): 1058-1070.
48. Hink U, Li H, Mollnau H, et al. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circulation research.* Feb 2 2001; 88(2): E14-22.

Global Journal of Medical Research: F
Diseases
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

# Exploring Biomarkers for Early Prognosis of COVID-19-Induced Acute Kidney Injury: A Comprehensive Systematic Review

By Bruna Morais de Souza Neves, Venine Prado Saêta, Thaís Reis Oliveira, Lilian Carla Carneiro & Nadya da Silva Castro Ragagnin

*Federal University of Goiás*

*Abstract-* Acute kidney injury (AKI) is one of the main secondary manifestations developed in patients with COVID-19. Based on this aspect, the present study aimed to identify the main biomarkers used to predict acute kidney injury and prognosis in COVID-19 and gather information about the biomarkers studied. Articles published in the Virtual Health Library, between January 2020 and February 2022, in English, Portuguese and Spanish were evaluated, with the guiding question "What is the scientific evidence on new biomarkers for diagnosis and determination of the prognosis of induced acute kidney injury?" in COVID-19?". Initially, more than 75 thousand articles were identified, however, with the attribution of inclusion and exclusion criteria, 8 articles were selected and analyzed during the present study. The findings of the present review emphasize that early diagnosis has a strong influence on the prognosis of AKI and that new biomarkers are useful for detecting and predicting the prognosis of the injury early. The potential of these assays with new biomarkers proved to be significant and could allow their use in future research.

*Keywords:* *acute kidney injury, biomarkers, COVID-19, diagnosis, glomerular filtration, performance, prognosis, secondary manifestations, serum creatinine.*

*GJMR-F Classification:* *NLM Code: WJ 300-378*


*Strictly as per the compliance and regulations of:*

# Exploring Biomarkers for Early Prognosis of COVID-19-Induced Acute Kidney Injury: A Comprehensive Systematic Review

Bruna Morais de Souza Neves [α], Venine Prado Saêta [σ], Thaís Reis Oliveira [ρ], Lilian Carla Carneiro [ω] & Nadya da Silva Castro Ragagnin [¥]

*Abstract-* **Acute kidney injury (AKI) is one of the main secondary manifestations developed in patients with COVID-19. Based on this aspect, the present study aimed to identify the main biomarkers used to predict acute kidney injury and prognosis in COVID-19 and gather information about the biomarkers studied. Articles published in the Virtual Health Library, between January 2020 and February 2022, in English, Portuguese and Spanish were evaluated, with the guiding question "What is the scientific evidence on new biomarkers for diagnosis and determination of the prognosis of induced acute kidney injury?" in COVID-19?". Initially, more than 75 thousand articles were identified, however, with the attribution of inclusion and exclusion criteria, 8 articles were selected and analyzed during the present study. The findings of the present review emphasize that early diagnosis has a strong influence on the prognosis of AKI and that new biomarkers are useful for detecting and predicting the prognosis of the injury early. The potential of these assays with new biomarkers proved to be significant and could allow their use in future research.**

*Keywords: acute kidney injury, biomarkers, COVID-19, diagnosis, glomerular filtration, performance, prognosis, secondary manifestations, serum creatinine.*

## I. Introduction

During the COVID-19 pandemic, part of the individuals infected with SARS-CoV-2 manifested a mild to moderate respiratory illness, however, many elderly people and people with other comorbidities evolved to severe conditions[1]. Patients in the intensive care unit (ICU) developed secondary complications, such as liver damage, venous thromboembolism, acute kidney injury (AKI), and others[2]. AKI in this case is correlated with poor prognosis and a higher risk of patient mortality[3].

Currently, the definition and staging of acute kidney injury are based on the KIDGO (Kidney Disease Improving Global Outcomes) criteria, which unifies the RIFLE (Risk, Injury, Failure, Loss and End-Stage) and AKIN (Acute Kidney Injury Network) criteria[4]. Thus, KDIGO uses changes in serum creatinine and urinary output, requiring at least two serum creatinine values obtained in 48 hours[5].

Serum creatinine levels increase as a 50% drop in renal function occurs, that is, it is not directly correlated with the decrease in glomerular filtration rate (GFR), which makes early diagnosis impossible[6]. Given this, in recent years, studies with new biomarkers for AKI have gained emphasis in different clinical settings. Among the new biomarkers tested are: Cystatin C, Lipocalin Associated with Human Neutrophil Gelatinase (NGAL), N-acetyl -B-D-glucosaminidase (NAG), Kidney Injury Molecule-1 (KIM-1), Interleukin-18 (IL -18), Netrin-1 and others[7,8].

Given the difficulty of diagnosing AKI using serum creatinine and the inefficient prognosis in COVID-19, the use of biomarkers such as Cystatin C and interleukin-18 is suggested[9]. Cystatin C is a cysteine proteinase inhibitor protein, which is related to several pathological processes. It is freely filtered in the glomeruli, as it has a low molecular weight, but is almost completely reabsorbed in the proximal tubules[10]. Interleukin-18 is a pro-inflammatory cytokine that induces the release of inflammatory cytokines and TNF, thus acting as a mediator in the immune system[6].

The present work is a systematic review of the comprehensive literature, with the main objective of exploring biomarkers for the early diagnosis of AKI induced by COVID-19. Predicting and/or determining the patient's prognosis, as this is of clinical importance and is extremely necessary to apply as a laboratory practice.

## II. Methods

*a) Instrument for selection of studies and inclusion criteria*

The instrument for selecting the studies was the Relevance Test (TR), based on Pereira's model (2006)[11]. This consists of forms containing four selection steps, to include or exclude the articles found with the search strategy.

Initially, TR1 was applied to the references to select studies that meet the following inclusion criteria:

- Publications made from January 2020 to February 2022;
- Published in Portuguese, English and Spanish.

*Author α ¥: University Federal of Jataí, Goiás, Brazil.*
*Author σ ρ: Graduate Program in Health Sciences, Federal University of Goiás, Goiás, Brazil. e-mail: veninesaeta@hotmail.com*
*Author ω: Institute of Tropical Pathology and Public Health, Federal University of Goiás, Goiás, Brazil.*

TR2 was applied in the title and abstract of the studies, with the help of the search platform. At this stage, the defined inclusion criteria were: primary and complete article; study with humans; work with biomarkers; study with COVID-19 or SARS-CoV-2.

TR3 was applied only in the abstract of articles containing the following inclusion criteria: studies that address AKI in people diagnosed with COVID-19; and studies using renal biomarkers.

TR4 is the final step of the procedure, which was applied to the entire article and intended to answer the following questions defined as inclusion criteria: Was the kidney disease due to COVID-19? Were there specific biomarkers for AKI? Was there a correlation between changes in biomarkers and prognosis for AKI-COVID?

As exclusion criteria, those who did not meet the criteria described above were considered.

*b) Definition of descriptors and search in the literature*

A literature search was performed by crossing the following descriptors in Health Sciences/Medical Subject Headings (DeCS/MeSH) available on the VHL network: Acute Kidney Injury and COVID-19 "*OR*" SARS-CoV- two. The articles were searched using the advanced method, using the term "title/abstract/subject" with the combination of descriptors and the Boolean operators "*AND*" and "*OR*".

*c) Data extraction and analysis*

Taking into account the methodological rigor of a systematic literature review (SLR), a form was prepared to extract the following information from the included studies: bibliographic reference, type of study, research objectives, methodology, and results obtained[12]. To synthesize as much information as possible about the biomarkers for the prognosis of AKI, the following data were collected: characteristics and clinical conditions of the patients, criteria for diagnosing AKI, biomarkers used, the value of the biomarker and serum creatinine on admission and whether there was any change in the glomerular filtration rate (GFR), biomarker performance for AKI prognosis and clinical outcomes.

## III. Results

Bearing in mind that new articles are inserted in the databases every day, April 24, 2022, was chosen to carry out the bibliographic research of this RSL. A total of 75,631 articles were obtained, of which TR1 was applied. After applying the first test, a total of 13,531 articles were included, in which the TR2 was applied in the titles and abstracts with the help of the search platform, 12,492 studies were excluded and 1,039 were included. In the next step, TR3 was applied to the abstracts of previously selected articles, which resulted in several 30 selected. All 30 articles were accessed in full, so TR4 was applied to these by reading the full article, which resulted in the exclusion of 22 articles and the inclusion of 08 articles for this review. Finally, the 08 articles were submitted for analysis and data extraction, as shown in Figure 1.

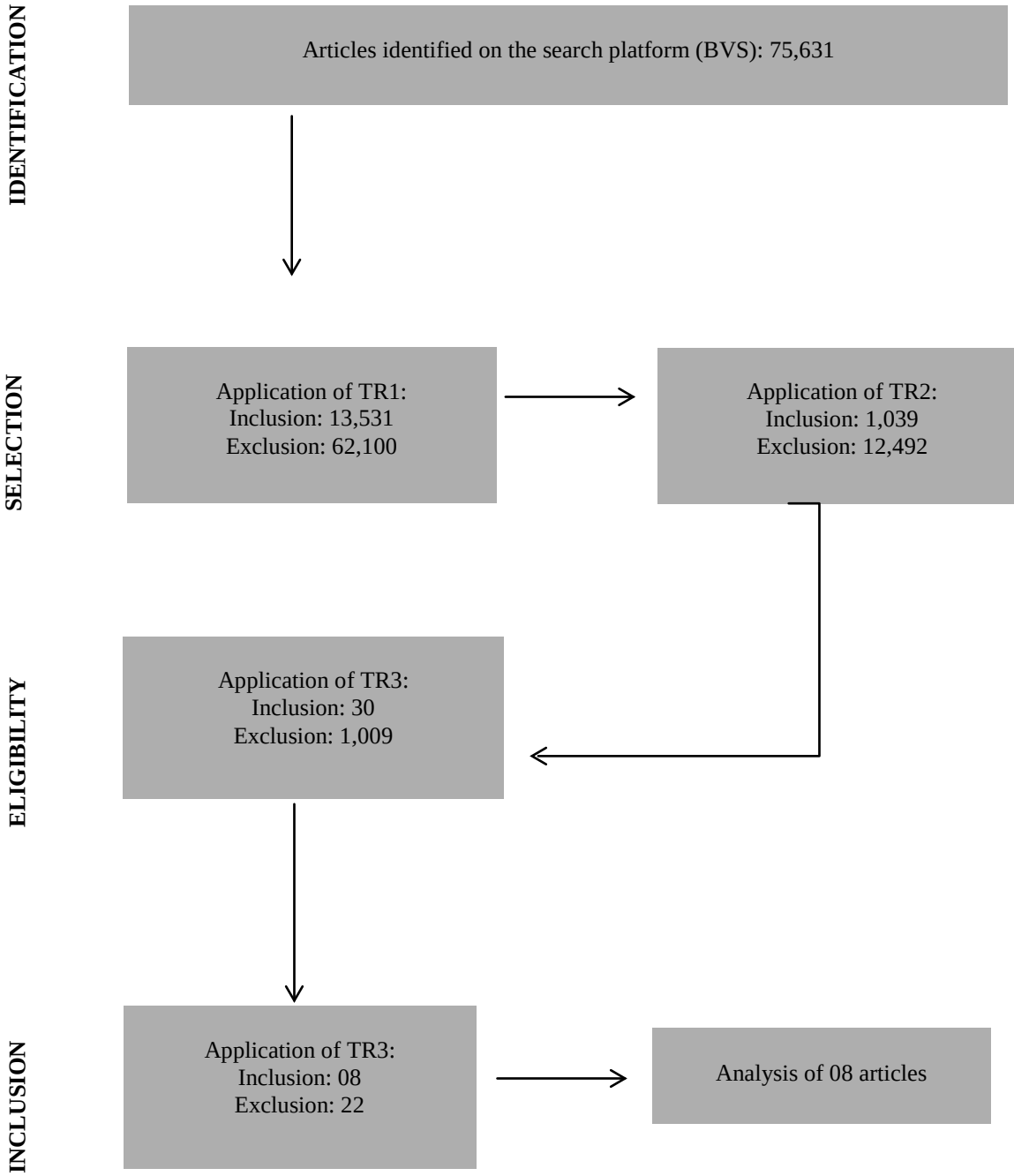



*Figure 1:* Methodological approach used in the Systematic Review of the Literature on biomarkers for the prognosis of acute kidney injury induced by COVID-19 (BVS, 2022).

After analyzing the eight studies selected for this SLR, a summary of the general characteristics was prepared and made available in synoptic tables. In Frame 1, information and characteristics of the articles analyzed during the study are presented.

*Frame 1:* Information and characteristics of the articles selected during the study

| Reference | Study design | Selection of participants |
|---|---|---|
| Temiz *et al*. (2022) | A prospective pilot study | Participants were selected from the urology and ICU departments suspected, suspected of COVID-19 and with specific findings on tomography |
| Shakked *et al.* (2022) | Prospective observational investigation | Adults (≥18 years) and patients who test positive for Covid-19 |
| Husain-Syed *et al*. (2021) | Prospective, observational, single-centre study | Patients were included if they consented to the linkage with administrative data for longterm follow-up |
| Gradin *et al*. (2021) | Study is a sub-study of a larger prospective observational | Adult patients with COVID-19 admitted to the ICU and with informed consent, in addition to urinary samples included in the study |
| Vogel *et al.* (2021) | Cohort of COVID-19 patients in this prospective observational clinical trial | Patients presenting with acute symptoms of respiratory infection. The secondary outcome was a composite of acute kidney injury, ICU admission, and death |
| Indirli *et al.* (2022) | A single-centre, observational, retrospective, case–control study | Data were retrospectively extracted from the COVID-19 Network registry |
| Fukao *et al*. (2021) | Retrospective study | Used data from all patients with COVID-19 seen |
| Wang *et al*. (2021) | Prospective observational investigation | Patients admitted to for COVID- 19, were eligible for this study |

Biomarker predictors of poor prognosis such as ICU stay, renal replacement therapy (RRT), or death were observed for patients with AKI in COVID-19, as shown in Frame 2.

*Frame 2:* Prognostic predictor biomarkers for AKI in COVID-19

| Reference | Biomarker | Prognosis |
|---|---|---|
| Temiz *et al*. (2022) | KIM-1/creatinine and NGAL/creatinine | Death |
| Shakked *et al.* (2022) | Serum creatinine<br>Serum cystatin C and NGAL | AKI<br>AKI aggravation<br>RRT needs<br>Admission in ICU |
| Husain-Syed *et al*. (2021) | Urinary DKK33 | AKI Chronification |
| Gradin *et al*. (2021) | Serum creatinine<br>Urinary KIM-1<br>Urinary NGAL | Correlation with urinary cytokines<br>AKI aggravation |
| Vogel *et al.* (2021) | Serum creatinine<br>urinary KIM-1<br>urinary NAG | AKI, ICU e Death |
| Indirli *et al.* (2022) | MR – Serum ProADM<br>serum copeptin | Sepsis and AKI;<br>Death, ICU or<br>Hospital complications. |

*DKK33* Dickkopf-related protein 3*, KIM-1* Kidney Injury Molecule 1*, AKI* Acute Kidney Injury*, NAG* N-acetyl-β-D-glucosaminidase*, NGAL* Neutrophil gelatinase-associated lipocalin*, MR – ProADM* Mid-regional pro-adrenomedullin*, TRS* Therapy Renal replacement*, ICU* Intensive care unit.

Two studies evaluated biomarkers that were useful for early diagnosis, these markers are shown in Frame 3.

*Frame 3:* Biomarkers for early diagnosis

| Reference | Biomarker |
|---|---|
| FUKAO *et al*. (2021) | L-FABP and<br>urinary β2MG |
| WANG *et al*. (2021) | Serum procalcitonin (PCT) |

*β2MG β2-microglobulin, L-FABP Liver-type fatty acid binding protein.*

When analyzing the characteristics of the clinical conditions of the populations of the eight studies, it was observed that among the eight studies, the size of the population with COVID-19 ranged from 18 to 389 patients, with three studies presenting, in addition to the COVID-19 cohort, a control group, such as can be observed in Table 1.

*Table 1:* Characteristics and clinical conditions of patients with COVID and AKI

| Reference | Sample No. | AKI (%) | Age (average or median + IQ) | Comorbidities |
|---|---|---|---|---|
| Temiz *et al.* (2022) | 75 COVID-19 11 Control | 16 | 55,77 ± 17,47 | Hypertension |
| Shakked *et al.* (2022) | 52 | 42,3 | 66 | Heart failure, hypertension, chronic kidney disease, cerebrovascular disease |
| Husain-Syed *et al.* (2021) | 55 | 10,45 | 54 | Hypertension, diabetes, chronic kidney disease, coronary artery disease |
| Gradin *et al.* (2021) | 29 COVID-19 9 Control | 66 | 57 ± 3 | Hypertension, chronic lung disease, and diabetes mellitus |
| Vogel *et al* (2021) | 54 COVID-19 26 Control | 14,8 | 56,8 | Hypertension, chronic lung disease, and diabetes mellitus |
| Indirli *et al*. (2022) | 116 | 3 | 66 | Hypertension, diabetes, obesity, and coronary artery disease |
| Fukao *et al.* (2021) | 18 | 11 | 64,0 (44,0–74,5) | Hypertension, diabetes mellitus, and coronary heart disease |
| Wang *et al.* (2021) | 389 | 7,8 | 66 (15) | Hypertension diabetes mellitus, liver disease |

In Frames 4 and 5, the general characteristics of six out of eight studies are presented in detail. It was observed that among the eight selected studies, four are prospective and two are retrospective. These studies were carried out in Turkey (1), the United States (1), Germany (2), Sweden (1), Italy (1), Japan (1), and China (1), published among the years 2021 and 2022.

*Frame 4:* General characteristics of studies with biomarkers for prognosis

| Reference | Type of study | Objectives | Methodology | Conclusions |
|---|---|---|---|---|
| Temiz *et al.* (2022). | Prospective pilot | Investigate whether there is kidney damage during COVID-19; Identify the predictive value of renal biomarkers and estimate survival. | KIM-1/creatinine and NGAL/ creatinine ratios were compared among 36 patients with COVID-19 and 11 controls. Mortality rates were determined using the Kaplan-Meier method. | Urine KIM-1/creatinine ratio associated AKI with COVID-19-specific death. In clinical practice, serum Cystatin C (sCysC) and urine KIM-1/creatinine are associated with survival. |
| Shakked *et al*. (2022). | Prospective, observational | To evaluate the usefulness of serum CysC (sCysC) and serum NGAL (sNGAL). | Demographic data of 52 patients were retrieved from medical records. sCysC, serum creatinine, and serum and urine NGAL were analyzed. | sCysC was an excellent early predictor of AKI and the need for RRT in patients with COVID-19, but it did not outperform serum creatinine. While sNGAL showed good performance for the diagnosis of AKI. |
| Husain-Syed *et al*. (2021). | Prospective, observational | Evaluate the role of renal biomarkers to monitor the progression of COVID-19. | Spot urine samples were collected from 55 patients daily and for analysis of uDKK3 and IL-6 it was collected three times a week from hospital admission until the day of discharge. | Biphasic patterns of urinary uDKK3 and IL-6 in patients with a greater decrease in eGFR are suggestive of a chronification of AKI and commonly used urinary markers may be less suitable. |

*CysC Cystatin C, eGFR Estimated Glomerular Filtration Rate, IL-6 Interleukin 6, KIM-1 Kidney Injury Molecule 1, AKI Acute kidney injury. NGAL Neutrophil gelatinase-associated lipocalin, RRT Renal replacement therapy, uDKK3 Urinary Dickkopf-3.*

*Frame 5:* General characteristics of studies for early diagnosis of AKI

| Reference | Type of study | Objectives | Methodology | Conclusions |
|---|---|---|---|---|
| Fukao *et al*. (2021). | Prospective observational clinical trial | Investigate relationships of tubular injury, COVID-19 severity, and markers of inflammation<br>To address cytokine-mediated mechanisms in the development of AKI. | Analysis of markers and respiratory status was performed in 18 patients with COVID-19.<br>Correlation analysis among levels of tubular and laboratory markers. | Urinary markers L-FABP and uβ2MG were significantly associated with IL-6 levels even in patients without overt AKI.<br>It is suggested that L-FABP and urinary uβ2MG are useful as early diagnostic biomarkers. |
| Wang *et al.* (2021). | Exploratory | To assess the value of PCT in predicting AKI during COVID-19.<br>Build a risk classification score. | The biomarker concentrations of 28 patients with COVID-19 were analyzed.<br>A multivariate risk score was created. | Single PCT value is a valuable predictive marker of AKI in patients with COVID-19.<br>The risk score can help assess the possibility of developing AKI. |

*β2MG β2-microglobulin, IL-6 Interleucina 6, L-FABP Liver-type fatty acid binding protein, AKI Acute kidney injury, PCT Procalcitonin.*

After analyzing the studies, it is observed that the classification used for the detection of AKI was KDIGO (2012), having been used in seven of the eight studies found, and only one study did not present this information. Biomarkers were analyzed in blood and urine samples, with analysis of serum biomarkers being the most prevalent among the eight studies. The dosage of these markers was carried out from the moment of consultation in the emergency department or hospital admission. In summary, this information is available in Frames 6 and 7.

*Frame 6:* Biomarkers for AKI prognosis in patients with COVID-19.

| Reference | Biomarker | Serum or urinary | Biomarker value on admission (median ± IQR) | Biomarker performance for early diagnosis and prognosis |
|---|---|---|---|---|
| Temiz *et al*. (2022) | Creatinine | Serum | 0,75 +/- 0,39 (mg/dL) | Elevated KIM-1/creatinine: death from COVID-19. |
| | Cystatin C | Serum | 0,96+/- 0,59 (mg/L) | Higher elevation of urinary KIM-1/creatinine and NGAL/creatinine levels in the ICU.<br>Increased levels: death from COVID-19. |
| | KIM-1 | Urinary | 12,95 +/- 5,82 (ng/mL) | Increased uKIM-1/creatinine: death from COVID-19. |
| | NGAL | Urinary | 61,26 +/- 75,35 (ng/mL) | Altered uNGAL/creatinine levels: death from COVID-19. |
| | KIM-1/ Creatinine | Urinary | | Increased levels: death from COVID-19. |
| | | | Decrease in GFR | |
| Shakked *et al.* (2022) | Creatinine | Serum | 0,76 (0,71–0,92) (mg/dL) | It was increasingly correlated with the increase in the severity of AKI.<br>AUC: 0.86 to predict AKI and AUC: 0.94 to need RRT. |
| | Cystatin C | Serum | 0,82 (0,74–0,99) (mg/L) | It correlated increasingly with AKI severity.<br>Excellent for predicting AKI (AUC: 0.87) and RRT requirement (AUC: 0.94).<br>Moderate performance in predicting the need for ICU admission. |
| | NGAL | Serum | 57,6 (49,1-95) (ng/mL) | Moderate performance for predicting ARL (AUC: 0.81) and TRS (AUC: 0.87).<br>Significantly elevated levels among patients with severe AKI requiring RRT.<br>Moderate performance in predicting the need for ICU admission. |

| | | | | |
|---|---|---|---|---|
| | | | Decrease in GFR | |
| Husain-Syed *et al.* (2021) | Creatinine | Serum | Média + IQR 1,23 (1,04–1,45) mg/Dl | There was no correlation. |
| | α1MGCR/ Creatinine | Urinary | 82,7 (50,2–136,2) mg/g | There was no correlation. |
| | uDKK33/ Creatinine | Urinary | 3781 (1402–10192) pg/g | Biphasic uDKK33 patterns in patients with greater eGFR decline were suggestive of AKI-CKD transition. |
| | Cystatin C | Serum | 1,62 (1,35–1,94) mg/L<br>TFGe ≥5 mL/min/1,73 m | There was no correlation. |
| Gradin *et al.* (2021) | Creatinine | Serum | 68 (62-81) mmol/L | Maximum creatinine correlated significantly with 19 cytokines. |
| | KIM-1<br>NGAL | Urinary<br>Urinary | 5,3 (2,7 - 10,6) ng/g UCr<br>33 (13 - 130) U/g UCr | Correlation with many urinary cytokines of incidence and worsening of AKI. |
| | Cytokines | Urinary | | 31 cytokines were associated with the maximum stage of AKI, ie, the need for RRT. |
| | | | Decrease in GFR | |
| Vogel *et al.* (2021) | Creatinine | Serum | 0,95 (0,77–1,26) mg/Dl | Significant increase in patients who achieved the composite outcome of AKI, ICU, and death. |
| | KIM-1 | Urinary | 1316(485–2316) ng/g UCr | Elevated levels in patients who achieved the composite endpoint of acute kidney injury, ICU, and death. For predicting ICU admission, AUC was obtained: 0.76. |
| | NAG | Urinary | 4,75 (1,54–10,7) U/g UCr | Elevated in COVID-19 patients who have suffered from AKI. |
| | | | Decrease in GFR | |
| Indirli *et al.* (2022) | Creatinine | Serum | 0,9 (0,7–1,1) mg/Dl | Significant increase in patients achieving the composite endpoint. |
| | MR-proADM | Serum | 0,9 (0,6–1,3) nmol/L | The value on admission was useful for predicting sepsis and AKI during hospitalization. |
| | Copeptin | Serum | 13,2 (6,3–30,8) pmol/L | The value on admission was useful for predicting sepsis and AKI during hospitalization.<br>It was useful for predicting the composite outcome of death, ICU, or hospital complications.<br>Copeptin was associated with length of stay and a more complicated clinical picture. |
| | | | Decrease in GFR | |

*AUC* Area under the curve, *α1MGCR* α1-microglobulin-creatinine ratios, *CKD* Chronic kidney disease, *eGFR* Estimated glomerular filtration rate, *KIM-1* Kidney Injury Molecule 1, *MR – ProADM* Mid-regional pro-adrenomedullin, *NAG* N-acetil-β-D-glicosaminidase, *NGAL* Neutrophil gelatinase-associated lipocalin, *AKI* Acute kidney injury, *GFR* Glomerular filtration rate, *RRT* Renal replacement therapy, *uα1MG* Urinary α1 microglobulin, u*DKK3* Urinary Dickkopf-3, *ICU* Intensive care unit.

*Frame 7:* Biomarkers for early diagnosis of AKI in patients with COVID-19

| Reference | Biomarker | Serum or urinary | Biomarker value on admission (median ± IQR) | Biomarker performance for early diagnosis and/or prognosis |
|---|---|---|---|---|
| Fukao *et al.* (2021) | NAG | Urinary | 32,5 U/L | There was no correlation. |
| | β2MG | Urinary | 10.516 μg/L | Elevations in L-FABP and uβ2MG |

| | | | | |
|---|---|---|---|---|
| | α1MG | Urinary | 65,8 mg/L | reflected early tubular injury. |
| | L-FABP | Urinary | 47,9 μg/gCr | |
| | Decrease in GFR | | | |
| Wang *et al.* (2021) | Creatinine | Serum | 160 (65–293) umol/L | PCT correlated with AKI in patients with COVID-19. |
| | Procalcitonin | Serum | 0,440 (0,133–2,433) ng/ml | |
| | Urea nitrogen | Serum | 15,40 (6,50–30,65) mmol/L | |
| | Uric acid | Serum | 408 (235-670) (umol/L) | |
| | Decrease in GFR | | | |

*α1MG* α1 microglobulin, *β2MG* β2-microglobulin, *L-FABP* Liver-type fatty acid binding protein *AKI* Acute kidney injury, *NAG* N-acetil-β-D-glicosaminidase, *PCT* Procalcitonin, *GFR* Glomerular filtration rate.

Regarding the results for prognosis, it was seen that the biomarkers used were predictors of poor prognosis. KIM-1/creatinine and NGAL/creatinine ratios were predictors of death from COVID-19[13]. Serum creatinine and cystatin C were indicators of AKI, worsening of the lesion, and need for RRT. The NAG marker was significant for predicting AKI in patients with severe AKI who required RRT[14]. Elevation in the urinary biomarker uDKK33 at six months from hospital admission was suggestive of AKI chronicity by COVID-19[15]. Serum creatinine and urinary biomarkers KIM-1 and NGAL correlated with urinary cytokines of incidence and worsening of AKI[16]. Serum creatinine and KIM-1 were significantly elevated in patients who achieved the composite endpoint (acute kidney injury, ICU, and death) and the NAG marker was significantly elevated in patients who suffered from AKI[17].

In Frame 7, the results of two studies with biomarkers for the early diagnosis of AKI in COVID-19 are available.

## IV. Discussion

The electronic survey involved studies carried out during the COVID-19 pandemic, published between 2021 and 2022. Given the pandemic context, one can observe agility in carrying out the surveys. In addition, according to the analyzed studies, it is observed that the search for new AKI laboratory biomarkers for the identification and progression of AKI continues to advance.

Despite the size of the sample (18 to 389 participants), no influence was verified on the characteristics and clinical conditions of the patients. It was observed that among the eight studies, the patients who developed AKI were over 50 years old, and were hypertensive or diabetic. In addition, the KDIGO diagnostic criteria, GFR calculation using the CKD-EPI formula, and biomarker analysis (ELISA) were also similar among studies. In addition to this, nephelometric tests were also used, mainly to analyze Cystatin C, which helps in the comparison between them. Although unusual, Cystatin C is considered an early marker of AKI and its dosage has been performed with nephelometric tests, as they are considered quite stable[18].

Among the biomarkers, urinary KIM-1 stood out as the most promising for the diagnosis and prognosis of AKI. Significant elevations were found in the KIM-1/urinary creatinine ratio in patients with AKI and specific deaths from COVID-19[13]. Consistently, a significant increase in KIM-1 levels between 24h and 48h after admission to the ICU in patients with septic AKI due to other diseases who did not survive was also verified[19]. In a study by [16], a correlation of urinary KIM-1 with several urinary cytokines related to the incidence and worsening of AKI in COVID-19 was observed. The results of [17] showed elevations in urinary KIM-1 levels in COVID-19-positive patients with a composite outcome of AKI, ICU admission, and death. Similarly, in a study carried out with patients hospitalized with AKI in other diseases, the increase in urinary KIM-1 and NAG levels at the time of consultation with a nephrologist were predictors of the composite outcome of RRT or in-hospital death[20].

The second prominent biomarker was NGAL, both for serum concentrations and urinary levels. Serum NGAL was useful for predicting AKI, need for RRT, ICU stay, and expressly highs in severe AKI requiring RRT[14]. NGAL/creatinine was useful as a good predictor of mortality in patients with COVID-19 and AKI[13]. Similar results were found in a study with critically ill patients due to other diseases, where 40% more cases of AKI were detected with the evaluation of NGAL and creatinine than when using creatinine alone, and in these patients, the risk of ICU admission, need for TRS and death was higher[21,22].

Another biomarker with prognostic results for AKI was sCysC, whose elevation exceeded the urinary KIM-1/creatinine and NGAL/creatinine ratio in ICU patients, but was not considered a predictor of specific mortality from COVID-19[13]. Serum and urine creatinine were used in all eight studies, as it is the standard AKI diagnostic marker. On admission, serum creatinine was similar to sCysC for predicting AKI and the need for

RRT[14]. However, the elevation of sCysC compared to serum creatinine may have been influenced by high doses of corticosteroids[15].

In the analysis of urinary dickkopf-3 (uDKK3), a new biomarker of CKD progression. uDKK3 levels remained high 6 months after hospital admission for COVID-19 in patients with AKI and a greater decline in glomerular filtration. The existence of a secondary AKI over an unresolved AKI has been suggested, which may contribute to the transition from AKI to CKD at 6 months post-discharge15. These findings are similar to what is in the literature, since in a study with post-cardiac surgery patients, urinary DKK3 was associated with a risk of severe loss of glomerular filtration after the transition from AKI to CKD during the patients' follow-up period[23].

As for biomarkers for early diagnosis of AKI, the highlights were β2MG and L-FABP, which were elevated in urine samples as a reflection of the onset of AKI[24]. L-FABP is a protein present in the liver that plays an important role in regulating the metabolism of fatty acids. In addition, it has a high affinity for lipid peroxidation products, which promotes its elimination in the urine[25].

[26] Evaluated common biomarkers, but included serum PCT dosage in their study, which in turn was useful for early detection of AKI in patients with COVID-19[27].

Although the cost of testing may still be a limiting factor, the results have shown promise for assessing the incidence of AKI with new biomarkers[16,28,14,17], predict AKI severity, help identify patients who need RRT[16,14] or ICU[28,14,17], predict death[28,17,13] and perform early detection of AKI in COVID-19[24,26], thus minimizing the cost of the test.

With the use of new biomarkers detected before serum creatinine, it was possible to detail the AKI caused by COVID-19 for the first time in the literature[13]. The evaluation of biomarkers such as KIM-1 and Cystatin C at admission can provide diagnostic data and help to outline the prognosis of AKI, which can influence medical decisions and thus increase the chances of better outcomes[17,14]. In addition, it is considered important to evaluate the renal system in COVID-19 with AKI biomarkers, as well as to consider their continuous evaluation during hospitalization[13].

The application of new sensitive biomarkers for AKI proved to be important for the diagnosis and prognosis of the lesion in the COVID-19 scenario.

Different biomarkers were able to predict AKI severity, need for RRT, hospitalization, and death. The poor prognosis of AKI in COVID-19 observed in the analyzed studies highlights the importance of identifying new biomarkers and applying them in laboratory practice and different clinical scenarios.

Although the use of some of these biomarkers is limited due to the high cost of the necessary reagents, the potential of these assays with new biomarkers has proved to be significant and may enable their use in future assays and research. In addition, to improve the clinical evaluation, aiming to favor clinical outcomes, it is suggested to measure these biomarkers in the first contact with the patient, that is, since the positive test result for SARS-CoV-2.

As observed in the RSL, the prominent marker to predict different outcomes of patients with AKI was KIM-1. Thus, in the future, the use of this biomarker, combined with serum creatinine and other markers, may make the diagnosis of AKI more assertive and identify the risks of worsening AKI early.

*Disclosure statement*

No potential conflict of interest was reported by the author(s).

*Funding*

This research was funded by the Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG).

## References Références Referencias

1. World Health Organization (WHO). WHO-convened global study of origins of SARS-CoV-2: China Part. Genebra, 2021. Available in: https://www.who.int/publications/i/item/who-convened-global-study-of-origins-of-sars-cov-2-china-part.
2. Nicholas JB, Tom EF, Robert F. Doença do coronavírus 2019 (COVID-19). BMJ Best Practice, 2021. Available in: https://bestpractice.bmj.com/topics/pt-br/3000201.
3. Sociedade Brasileira de Nefrologia (SBN) e Associação de Medicina Intensiva Brasileira. Nota técnica e orientações sobre a injúria renal aguda (IRA) em pacientes com COVID-19. São Paulo: 2020. 11 p. Available in: https://www.sbn.org.br/fileadmin/user_upload/sbn/2020/04/15/Nota_tecnica_e_orientacoes_sobre_a_injuria_renal_aguda__IRA__em_pacientes_com_COVID-19.pdf.
4. Levi TM, Souza SP de, Magalhães JG de, Carvalho MS de, Cunha ALB, Dantas JGAO et al. Comparação dos critérios RIFLE, AKIN e KDIGO para predizer mortalidade em pacientes críticos. Revista Brasileira de Terapia Intensiva. 2013; 25(4): 290-6. http://dx.doi.org/10.5935/0103-507x.20130050.
5. Lopes JA, Jorge S. The RIFLE and AKIN classifications for acute kidney injury: a critical and comprehensive review. Clinical Kidney Journal. 2013;6(1):8-14. https://doi.org/10.1093/ckj/sfs160.
6. Macedo E. Biomarcadores na injuria renal aguda. In: Abensur, Hugo (ed.). Biomarcadores na nefrologia. São Paulo: Roche. 2011:1-114. Available in: https://arquivos.sbn.org.br/pdf/biomarcadores.pdf.
7. Peres LAB, Júnior ADC, Schäfer AJ, Silva AL de, Gaspar AD, Scarpari DF et al. Biomarcadores da

injúria renal aguda. Jornal Brasileiro de Nefrologia. 2013; 35(3): 229-36. https://doi.org/10.5935/0101-2800.20130036.
8. Kashani K, Cheungpasitporn W, Ronco C. Biomarkers of acute kidney injury: the pathway from discovery to clinical adoption. Clinical Chemistry and Laboratory Medicine (CCLM). 2017; 55(8): 1074-89. https://doi.org/10.1515/cclm-2016-0973.
9. Koza, Y. Acute kidney injury: current concepts and new insights. Journal of Injury And Violence Research. Journal of Injury and Violence Research. 2016; 8(1): 58-62. http://dx.doi.org/10.5249/jivr.v8i1.610.
10. Okay TS. Cistatina C: Um novo marcador de função renal em crianças. Revista da Associação Médica Brasileira. 2002; 48(2): 93-117. https://doi.org/10.1590/S0104-42302002000200025.
11. Pereira AL. Revisão sistemática da literatura sobre produtos usados no tratamento de feridas. Dissertation (Master's) - Faculty of Nursing, Federal University of Goiás, Goiânia, 2006, 129 p.
12. Pedroso, LA. Utilização de biomarcadores no diagnóstico precoce de lesão renal aguda em pacientes criticamente enfermos: revisão sistemática da literatura. Dissertação (Mestrado) - Curso de Farmácia, Pós-Graduação, Universidade Federal de Minas Gerais, Belo Horizonte, 2017. Cap. 5. Available in: https://repositorio.ufmg.br/bitstream/1843/BUOS-B32JG9/1/disserta__o_luana_pronta_ppgmaf_.pdf.
13. Temiz MZ, Hacibey I, Yazar RO, Sevdi MS, Kucuk SH, Alkurt G et al. Altered kidney function induced by SARS-CoV-2 infection and acute kidney damage markers predict survival outcomes of COVID-19 patients: a prospective pilot study. Renal Failure. 2022; 44(1): 233-40. https://doi.org/10.1080/0886022X.2022.2032743.
14. Shakked NP, Oliveira MHS de, Cheruiyot I, Benoit JL, Plebani M, Lippi G. Early prediction of COVID-19-associated acute kidney injury: Are serum NGAL and serum Cystatin C levels better than serum creatinine? Clinical Biochemistry. 2022; 102:1-8. Available in: https://doi.org/10.1016/j.clinbiochem.2022.01.006.
15. Husain-Syed F, Villa G, Wilhelm J, Samoni S, Matt U, Vadász I et al. Renal markers for monitoring acute kidney injury transition to chronic kidney disease after COVID-19. Nephrology Dialysis Transplantation. 2021; 36(11): 2143-47. https://doi.org/10.1093/ndt/gfab235.
16. Gradin A, Andersson H, Luther T, Anderberg SB, Rubertsson S, Lipcsey M et al. Urinary cytokines correlate with acute kidney injury in critically ill COVID-19 patients. Cytokine. 2021; 146: 155589. https://doi.org/10.1016/j.cyto.2021.155589.
17. Vogel MJ, Mustroph J, Staudner ST, Leininger SB, Hubauer U, Wallner S et al. Kidney injury molecule-1: a potential biomarker of acute kidney injury and disease severity in patients with COVID-19. Journal of Nephrology. 2021; 34(4): 1007-18. https://doi.org/10.1007/s40620-021-01079-x.
18. Neto OAF. Avaliação da Cistatina C como marcador precoce de lesão renal guda em pós-operatório de cirurgia cardíaca pediátrica com uso de circulação extracorpórea. Dissertação (Mestrado) - Universidade Federal de Mato Grosso do Sul, Campo Grande, 2013. Available in: https://repositorio.ufms.br/bitstream/123456789/1912/1/Oreste.pdf.
19. Tu Y, Wang H, Sun R, Ni Y, Ma L, Xv F et al. Urinary netrin-1 and KIM-1 as early biomarkers for septic acute kidney injury. Renal Failure. 2014; 36(10): 1559–63. https://doi.org/10.3109/0886022X.2014.949764.
20. Liangos O, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H et al. Urinary N-Acetyl-β-(D)-Glucosaminidase Activity and Kidney Injury Molecule-1 Level Are Associated with Adverse Outcomes in Acute Renal Failure. Journal of the American Society of Nephrology. 2007; 18(3): 904-12. https://doi.org/10.1681/ASN.2006030221.
21. Haase M, Devarajan P, Haase-Fielitz A, Bellomo R, Cruz DR, Wagener G et al. The Outcome of Neutrophil Gelatinase-Associated Lipocalin-Positive Subclinical Acute Kidney Injury. Journal of the American College of Cardiology. 2011; 57(17): 1752-61. https://doi.org/10.1016/j.jacc.2010.11.051.
22. Krishnasamy S, Sinha A, Bagga A. Management of Acute Kidney Injury in Critically Ill Children. Indian Journal of Pediatrics. 2023; 90: 481-91. https://doi.org/ 10.1007/s12098-023-04483-2.
23. Schunk SJ, Speer T, Petrakis I, Fliser D. Dickkopf 3—a novel biomarker of the "kidney injury continuum". Nephrology Dialysis Transplantation. 2020; 36(5): 761-7. https://doi.org/10.1093/ndt/gfaa003.
24. Fukao Y, Nagasawa H, Nihei Y, Hiki M, Naito T, Kihara M et al. COVID-19-induced acute renal tubular injury associated with elevation of serum inflammatory cytokine. Clinical and Experimental Nephrology. 2021; 25(11): 1240–6. https://doi.org/10.1007/s10157-021-02101-z.
25. Silva S da. O papel dos biomarcadores TIMP-2, IGFBP7 e L-FABP no diagnóstico da lesão renal aguda. Trabalho de Conclusão do Curso (Graduação) – Curso de Farmácia, Universidade Federal de Santa Catarina, Florianópolis. 2020. Available in: https://repositorio.ufsc.br/bitstream/handle/123456789/218407/TCC%202%20Suzana%20-%20Vers%c3%a3o%20BU%20%281%29.pdf?sequence=1&isAllowed=y.
26. Wang RR, He M, Kang, Y. A risk score based on procalcitonin for predicting acute kidney injury in COVID-19 patients. Journal of Clinical Laboratory

Analysis. 2021; 35(6): e23805. https://doi.org/10.1002/jcla.23805.
27. Castro MC de, SILVA MR da. Procalcitonina no diagnóstico de doenças infecciosas. Rev Bras Clin Med. 2012; 10(6): 521-8. Available in: http://files.bvs.br/upload/S/1679-1010/2012/v10n6/a3188.pdf.
28. Indirli R, Bandera A,Valenti L, Ceriotti F, Modugno AD, Tettamanti M et al. Prognostic value of copeptin and mid-regional proadrenomedullin in COVID-19-hospitalized patients. European Journal of Clinical Investigation. 2022; 52(5): e13753. https://doi.org/10.1111/eci.13753.

This page is intentionally left blank

Global Journal of Medical Research: F
Diseases
Volume 24 Issue 1 Version 1.0 Year 2024
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

# The Impact of Smoking on Systemic Arterial Hypertension in the Latin American Population

By Pérez Vázquez Sarahi Irasema, Ladewig Bernaldez Guillermo Ivan, Mondragón Morales Josué, Ruiz Espinosa José David & Bautista de Anda Rocío

*Abstract-* Systemic Arterial Hypertension (SAH) is the most important risk factor for the development of cardiovascular diseases being one of the most prevalent diseases worldwide. Tobacco use has been linked with the development of systemic arterial hypertension or with a difficult control of it, However, it has not yet been universally accepted or demonstrated whether this relationship is directly proportional to vascular damage in people who consume it. The purpose of the study is to compare the effect of smoking on hypertensive smokers vs non-smoking hypertensive patients.

*Keywords:* *systemic arterial hypertension, smoking, blood pressure, smoker, latin american, antihypertensive, cigarette.*

*GJMR-F Classification:* *NLM: WG 300*


*Strictly as per the compliance and regulations of:*

# The Impact of Smoking on Systemic Arterial Hypertension in the Latin American Population

Pérez Vázquez Sarahi Irasema [α], Ladewig Bernaldez Guillermo Ivan [σ], Mondragón Morales Josué [ρ], Ruiz Espinosa José David [ω] & Bautista de Anda Rocío [¥]

*Abstract-* Systemic Arterial Hypertension (SAH) is the most important risk factor for the development of cardiovascular diseases being one of the most prevalent diseases worldwide. Tobacco use has been linked with the development of systemic arterial hypertension or with a difficult control of it, However, it has not yet been universally accepted or demonstrated whether this relationship is directly proportional to vascular damage in people who consume it. The purpose of the study is to compare the effect of smoking on hypertensive smokers vs non-smoking hypertensive patients.

*Results:* 409 participants were obtained, 55% were men, the mean age was between 55 and 60 years, the most frequent academic grade in smokers and former smokers was high school with 25.4% and 31.3% respectively. Most people had less than ten years of diagnosis in smokers with 53.2% of the population and a minority had a diagnosis under 30 years. The statistical analysis did not show a relationship between smoking history and the number of antihypertensive agents for disease control (p= 0.736, 95% CI), as well as the number of years of diagnosis of hypertension and history of smoking (p = 0.160, 95% CI).

*Conclusions:* Patients who have been smoking for more than 30 years tend to have a higher smoking rate and therefore consume more antihypertensive drugs so, the more tobacco use and longer being hypertensive, the greater the pulmonary complications, In addition, patients who have stopped smoking or never smoked have a better control of blood pressure, since we found that, the lower the year of diagnosis of arterial hypertension, fewer drugs are needed for blood pressure control.

*Keywords: systemic arterial hypertension, smoking, blood pressure, smoker, latin american, antihypertensive, cigarette.*

## I. Introduction

Systemic Arterial Hypertension (SAH) is the most common risk factor for the development of cardiovascular diseases, it is diagnosed when blood pressure (BP) is ≥140/90 mmHg after repeated examination. HAS has been divided into primary and secondary HAS, with primary (essential) multifactorial HAS, with direct effects on cardiovascular and renal structure and function, while secondary HAS occurred as consequence of one or several diseases, half of hypertensive people do not have adequate BP control and of these, only half are controlled. Worldwide, the prevalence of resistant hypertension is 10.3% (Guirguis-Blake et al. 2021). The main risk factors for developing HAS are advanced age (Campos-Nonato et al. 2021), sex, obesity, family history of HAS (being 2 times more common in subjects who have 1 or 2 hypertensive parents), black population, reduced number of nephrons, low socioeconomic level, high intake of sodium >3 g/day, excessive alcohol intake, physical inactivity, diabetes mellitus, family history of premature brain vascular disease in 1st degree family, it has also been found that exposure to tobacco is related to a difficult control of blood pressure levels requiring treatment with 2 or more antihypertensive drugs (Guirguis-Blake et al. 2021). According to a study by Kaplan et.al., smoking >5 packs of cigarettes per year increases the risk of hypertension up to 30% not being proportional the percentage of cigarettes with a higher risk of suffering hypertension or a difficult hypertension control (Kaplan et al. 2021). In addition, smoking has been directly linked to ischemic strokes in young adults, this is directly proportional to the number of cigarettes smoked during their lifetime (Markidan et al. 2018), the effects of electronic cigarettes are currently under investigation, however, more research is needed(Shi et al. 2023). It has also been related that the increased risk of hypertension could be attributed to low levels of vitamin D induced by exposure to tobacco, this has been demonstrated by measuring cotinine in the body, which is a chemical substance formed by the breakdown of nicotine in the body, used to measure active and passive smoking, having vitamin D deficiency increases the concentration of cotinine in the body, which increases the risk of hypertension (Wu et al. 2022).

Several studies have evaluated the acute effect of cigarette smoke on blood pressure (BP) and have shown consistent results that cigarette smoking temporarily increases BP. This could be explained by the effect of nicotine on the activation of the sympathetic nervous system; however, chronic smoking should not be overlooked as this behavior leads to several harmful effects on the cardiovascular system (Chen et al. 2022a).

Environmental factors, air pollution and green spaces have been associated with hypertension in the field of environmental epidemiology (Chen et al. 2022b).

*Author: e-mail: dra.sarahi.irasema.pv@gmail.com*

The increase in arterial stiffness occurs with old age, with the development of chronic conditions such as high blood pressure and the presence of cardiovascular risk factors such as smoking, it has been shown that these two factors make a marked increase in arterial stiffness (Scallan et al. 2010). Levenson et.al. decided to analyze the different independent cumulative effects of hypertension and smoking, they shown that hypertension and smoking affect blood flow properties and arterial wall behavior, these changes are characterized by several abnormalities such as increased blood and plasma viscosity, increased hematocrit, and blood proteins(Levenson et al. 1987).

Smoking undoubtedly confers a significant cardiovascular risk and therefore smoking cessation is one of the best cost-effective measures in the field of medicine. Certain observations have documented that smokers who remove smoking before middle age typically have a life expectancy like non-smoking population (Lu et al. 2018).According to research by Tamotsu Nagao et. al. concluded that by reducing alcohol intake and smoking, the risk of hypertension is considerably reduced (Nagao et al. 2021). As mentioned above, chronic smoking has a direct influence on the development of vascular resistance, producing systemic arterial hypertension regardless of age, sex, and environmental factors, that's why it is of great relevance to know how the increase of blood pressure develops in smoker patients. Smoking has been linked to the use of 2 or more medications for the proper control of arterial hypertension (Kaplan et al. 2021), which leads us to a public health problem and health expenses.

## II. Methods

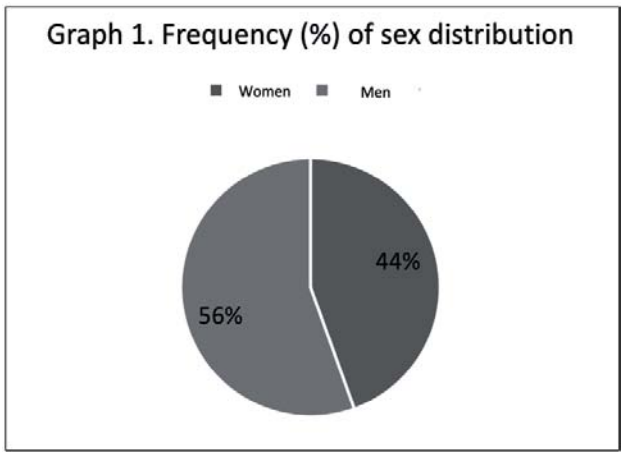
Our study is an observational, cross-sectional, prospective, projective, descriptive, and comparative study, the research was carried out in the General Hospital of Zone Number 58 and in the General Hospital "La Villa" Mexico. We interviewed 409 patients aged 40 to 60 years with a diagnosis of SAH through a google forms questionnaire. These patients were divided into two groups depending on smoking history. Pregnant patients, patients with comorbidities, such as chronic kidney disease, pheochromocytoma, vascular or renal anatomical abnormalities were not included, and incomplete questionnaires were removed. The protocol was reviewed and approved by the Research Ethics Committee and the Local Health Research Committee of the Mexican Social Security Institute.

Data analysis was performed using the SPSS and STATA v.14.2 program. The normality test of the variables was calculated using the form measures of bias and curtosis, as well as the Shapiro-Wilks test. The frequency measure to estimate smoking prevalence was obtained by the ratio of people who currently smoke divided by the total sample, multiplied by 100 for its interpretation in percentages. The statistical significance value was established at $p < 0.05$, with a 95% Confidence Interval(CI).

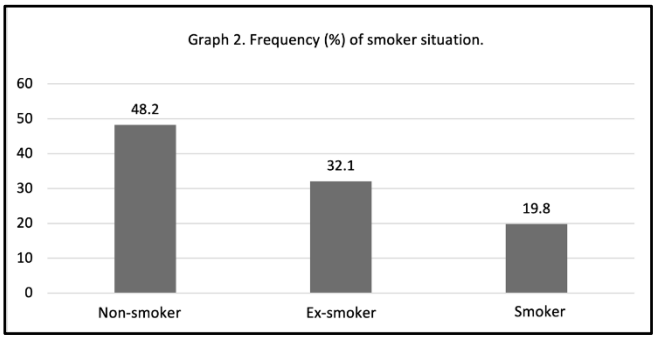
## III. Results

1. Characterization of the study population according to sex

Of a total of 409 patients, 55.5% were men and 44.5% were women, 63.7% were aged between 55 and 60 years and the most frequent school level was primary school (42.8%), followed by high school (45.8%), and University (20.3%)) (Table 1) (Graph 1).




Of the 409 patients, 48.2% have never smoked, 32.1% are former smokers and 19.8% are active smokers (Graph 2).




Almost half of the patients (44.6%) used one antihypertensive drug to control their BP, followed by those who used two (37.8%), three (15%) and four drugs (2.5%).

*Table 1:* Study population characterization (n=409).

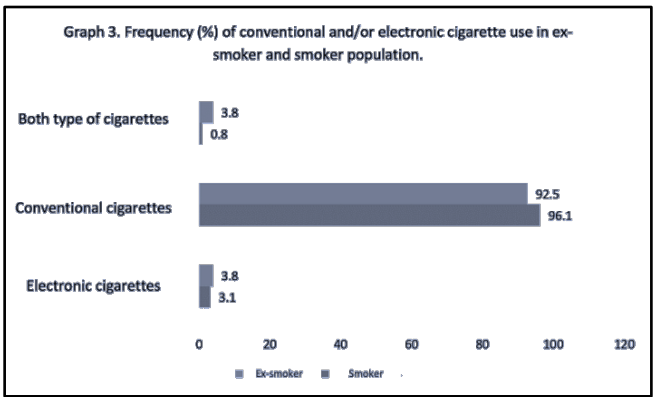
| Variables. | Total (n=409) (%) |
|---|---|
| **Sex** | |
| Women | 182 (44.5) |
| Men | 227 (55.5) |
| **Age (n=408)** | |
| 40 to 50 years old | 69 (16.9) |
| 50 to 55 years old | 79 (19.4) |
| 55 to 60 years old | 260 (63.7) |
| **Schooling (n=408)** | |
| I don't write and read | 8 (2.0) |
| I only know how to write and read | 13 (3.2) |
| Primary school | 101 (24.8) |
| High school 1 | 91 (22.3) |
| High School 2 | 96 (23.5) |
| University | 83 (20.3) |
| Master's degree | 13 (3.2) |
| PhD | 3 (0.7) |
| **Smoking (n=405)** | |
| Never | 195 (48.2) |
| Ex smoker | 130 (32.1) |
| Smoker | 80 (19.8) |
| **SAH evolution (years) (n=401)** | |
| <10 years | 197 (49.1) |
| 10-20 years | 122 (30.4) |
| 20-30 years | 50 (12.5) |
| >30 years | 32 (8.0) |
| **Number of antihypertensive drugs (n=399)** | |
| 1 | 178 (44.6) |
| 2 | 151 (37.8) |
| 3 | 60 (15.0) |
| 4 | 10 (2.5) |

2. Characterization of the study population according to smoking history

Among former smokers, 59.2% were men, a similar proportion in those current smokers (61.2%), being more frequent to find women without a history of smoking (71.8%) (Table 2).

In the three categories according to smoking history, more than half of the population was aged between 55 and 60 years. Regarding schooling, among non-smokers it was more common to find people with the primary school level (28.7%) followed by high school (21.5%). In former smokers and smokers, the highest proportion was found in those with high school education (25.4% and 31.3%, respectively).

The type of cigarette most used among smokers and ex-smokers was conventional cigarette (96.1% and 92.5%, respectively), only 3% used electronic cigarettes and among ex-smokers, 0.8% employed both, and in the case of smokers it was 3.8% (p value< 0.000)(Graph 3).




The World Health Organization (WHO) classifies smoking severity according to cigarettes consumed per day, mild (less than 5 cigarettes per day), moderate (6 to 15 cigarettes per day) and severe (more than 16 cigarettes per day)(Londoño Pérez et al. 2011; Chang et al. 2021). In our study, most smokers were classified as

mild smokers (62.8% and 58.2%), followed by moderate smokers (27.7% and 35.4%), and 15.5% of former smokers were classified as severe smokers, compared to 6.3% of smokers.

A higher prevalence of cases with less than 10 years of diagnosis of SAH was observed in the categories of former smokers (50%), smokers (53.2%) and non-smokers (47.9%), while the minority of patients had a diagnosis over 30 years of evolution.

Finally, with respect to the number of antihypertensives used to treat SAH, a similar proportion was found among the categories of former smokers, smokers, and non-smokers, being more than 40% those who use a single antihypertensive, followed by two antihypertensives (greater than 30%), three antihypertensives (greater than 10%), and finally, less than 4% employed four or more antihypertensives (Graph 4).

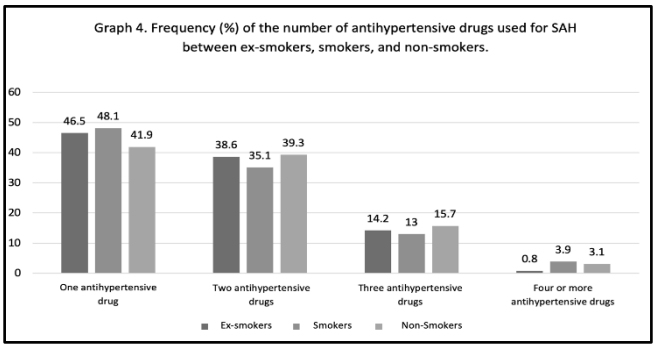



*Table 2:* Population characterization according to smoking history.

| Variables | Ex-smoker (n=130) | Smoker (n=80) | No smoker (n=195) | P Value (95% CI) |
|---|---|---|---|---|
| **Sex** | | | | |
| Women | 53 (40.8) | 31 (38.8) | 140 (71.8) | 0.000 |
| Men | 77 (59.2) | 49 (61.2) | 55 (28.2) | |
| **Age (n=408)** | | | | |
| 40 to 50 years old | 15 (11.6) | 21 (26.3) | 33 (16.9) | 0.072 |
| 50 to 55 years old | 27 (20.9) | 17 (21.3) | 35 (18.0) | |
| 55 to 60 years old | 87 (67.4) | 42 (52.5) | 127 (65.1) | |
| **Schooling (n=408)** | | | | |
| I don't write and read | 2 (1.5) | - | 6 (3.1) | 0.416 |
| I only know how to write and read | 2 (1.5) | 2 (2.5) | 8 (4.1) | |
| Primary school | 30 (23.1) | 14 (17.5) | 56 (28.7) | |
| High school 1 | 31 (23.9) | 17 (21.3) | 42 (21.5) | |
| High School 2 | 33 (25.4) | 25 (31.3) | 38 (19.5) | |
| University | 29 (22.3) | 17 (21.3) | 37 (19.0) | |
| Master degree | 2 (1.5) | 4 (5.0) | 7 (3.6) | |
| PhD | 1 (0.8) | 1 (1.3) | 1 (0.5) | |
| **Type of cigarette** | | | | |
| Conventional | 124 (96.1) | 74 (92.5) | - | 0.000 |
| Electronic | 4 (3.1) | 3 (3.8) | - | |
| Both | 1 (0.8) | 3 (3.8) | - | |
| **Smoker severity (n=208)** | | | | |
| Mild | 81 (62.8) | 46 (58.2) | - | 0.03 |
| Moderate | 28 (27.7) | 28 (35.4) | - | |
| Severe | 20 (15.5) | 5 (6.3) | - | |
| **Smoking Index** | | | | |
| Mild risk (<10) | 96 (73.9) | 62 (77.5) | - | 0.422 |

| | | | | |
|---|---|---|---|---|
| Moderate risk (10-20) | 17 (13.1) | 14 (17.5) | - | |
| Intense risk (21-40) | 8 (6.2) | 1 (1.3) | - | |
| High risk (>40) | 9 (6.9) | 1 (1.3) | - | |
| **SAH evolution in years (n=401)** | | | | |
| <10 years | 65 (50.0) | 42 (53.2) | 90 (47.9) | 0.160 |
| 10-20 years | 36 (27.7) | 30 (38.0) | 53 (28.2) | |
| 20-30 years | 19 (14.6) | 4 (5.1) | 27 (14.4) | |
| >30 years | 10 (7.7) | 3 (3.8) | 18 (9.6) | |
| **Number of antihypertensive drugs (n=399)** | | | | |
| 1 | 59 (46.5) | 37 (48.1) | 80 (41.9) | 0.736 |
| 2 | 49 (38.6) | 27 (35.1) | 75 (39.3) | |
| 3 | 18 (14.2) | 10 (13.0) | 30 (15.7) | |
| 4 | 1 (0.8) | 3 (3.9) | 6 (3.1) | |

3. Relationship between the diagnosis of arterial hypertension and smoking history

Through the Pearson correlation, a weak negative relationship of-0.10 was observed between the years of diagnosis of arterial hypertension and the smoking index a lower year of diagnosis of arterial hypertension, lower the smoking index ($p = 0.8850$).

In addition, a positive relationship of 0.3672 was observed between the years of diagnosis of SAH and the number of antihypertensives used, that is, to older years of diagnosis of arterial hypertension, greater need to employ more antihypertensive (p value $< 0.000$).

## IV. Discussion

In our research we observed that mostsmoker patients are men, for that reason we can associate it with sex since the majority of patients with lung diseases or even with Chronic Obstructive Pulmonary Disease (COPD) are mostly men, so also that men smoke more than women has a socio-background cultural since the Latin American idiosyncrasy urges the male to a greater consumption of substances(Kuntz et al. 2018; Chang et al. 2021), also the majority of our population is between 55 to 60 years of age (63.7%), which gives us to understand that the older group smoked or smoked more than the younger people (16.9% are 40 to 45 years old and 19.4% are 50 to 55 years old)(Agustí and Hogg 2019).

Subsequently, if we analyze the time taken by each patient with the diagnosis of SAH, most of patients have less than 10 years of evolution (about 49.1%) and only 8% had more than 30 years of evolution, therefore we observe that in several cases of our population group the onset of SAH occurs in people who are adults or elderly and this is not directly related to smoking but to the aging process. Although active smoking poses a significant cardiovascular risk, the effect of passive smoking on hypertension is rarely studied. According to Zhang et. al. passive smoking is significantly associated with an increased prevalence of hypertension, as well as a lower rate of disease control(Zhang et al. 2021).In our study passive smoking was not valued and the final results did not coincide with the results of Zhang, we considered that race and ethnicity were decisive for the results obtained.

Furthermore, it was observed that about half of the patients was treated with a single antihypertensive drug(about 44.6%), while only 37.8% use two, 15% employed three, and 2.5% used four antihypertensives drugs, for this reason we perceive that most patients having little time being hypertensive require fewer doses of drugs to achieve their antihypertensive treatment goals, however, there is also a minority that requires four or more drugs, we linked this relationship with a greater number of years of SAH evolution, which coincides with the recent literature. Regarding the patient's schooling, we found a significant relationship with smoking, since a large number of our patients reported smoking during teenage, specifically during high school, at 25.4% and 31.3% respectively, whereas non-smoking patients were people with 28.7% of primary school attendance, which reflects that during the process of teenage and the search for identity is yielded to social pressure for the use of harmful substances, among them tobacco, so smoking as psychosocial factors play an important role in the development of hypertension(Wu et al. 2016; Herrera et al. 2017).

In our population the use of electronic cigarettes was studied, however, the relationship with hypertension was not statistically significant, we consider that the variable and the size of the population consuming electronic cigarettes was not sufficient. The relationship between these two variables is currently being studied (Ruokolainen et al. 2021; Falk et al. 2022). According to the WHO classification we can observed that the correlation of tobacco in patients with SAH does not

reflect a significant statistical relevance since our patients are mostly mild smokers and therefore due to the inequality in the number of participants corresponding to each degree of smoking index would need to perform more studies to obtain evidence focused on moderate and severe smoking groups. Currently there are no studies that relate the smoking index with SAH, so it cannot be assert that such a relationship exists, and in turn we believe that more research is needed (Zhao et al. 2023). After reviewing the data we can associate the years suffering from hypertension and consumption of antihypertensive drugs since the greater number of years SAH evolution, the greater amount of consumption of antihypertensives drugs, in the matter of SAH and smokers we can say that with less years being hypertensive decreases the value of smoking index of our population, which shows us that if there is influence of tobacco consumption in the hypertensive population.

## V. Conclusions

For years the relationship between hypertension and tobacco consumption has been the subject of discussion among the scientific community. In multiple scientific articles it has been described that there is no relevant relationship between these two factors while in other publications it is mentioned that if a relationship exists, the relationship between smoking and patients who have been diagnosed with high BP for years opens up a series of interesting proposals to carry out more in-depth and detailed research in the Latino American population, we conclude that patients who have been smoking for more than 30 years tend to have a higher smoking index and therefore consume more antihypertensive drugs so, the more tobacco consumption and longer being hypertensive, greater are the pulmonary complications. In addition, the patients who have stopped smoking or never smoked have a better control of BP, since we found that, to the lesser year of diagnosis of SAH, fewer antihypertensive drugs used in BP control.

*List of Abbreviations:*

- Systemic Arterial Hypertension (SAH)
- Blood Pressure (BP)
- World Health Organization (WHO)
- Chronic Obstructive Pulmonary Disease (COPD)
- Confidence Interval (CI)

*Declaration Statements*

*Ethical Approval:* The protocol was reviewed and approved by the Research Ethics Committee and the Local Health Research Committee of the Mexican Institute of Social Security; this study did not involve animals.

*Consent for Publication:* No personal data, images or videos were collected from patients.

*Availability of Data and Materials:* Data collection was conducted with a questionnaire conducted at Google Forms, while data analysis was performed using the SPSS and STATA v.14.2 program.

*Conflict of Interest:* The authors declare that they have no conflict of interest.

*Financing:* None

*Authors' Contributions:* SP performed data collection, first version of the manuscript, graphs and tables, GL performed statistical analysis, JM collaborated with the final version of the manuscript and manuscript translation, JR participated in data collection, all authors read and approved the final manuscript, RB was dedicated to coordinating the research project.

## References Références Referencias

1. Agustí A, Hogg JC (2019) Update on the Pathogenesis of Chronic Obstructive Pulmonary Disease. New England Journal of Medicine 381: 1248–1256. https://doi.org/10.1056/NEJMRA1900475/SUPPL_FILE/NEJMRA1900475_DISCLOSURES.PDF
2. Campos-Nonato I, Hernández-Barrera L, Oviedo-Solís C, Ramírez-Villalobos D, Hernández B, Barquera S (2021) Epidemiología de la hipertensión arterial en adultos mexicanos: diagnóstico, control y tendencias. Ensanut 2020. Salud Publica Mex 63:692–704. https://doi.org/10.21149/12851
3. Chang JT, Anic GM, Rostron BL, Tanwar M, Chang CM (2021) Cigarette Smoking Reduction and Health Risks: A Systematic Review and Meta-analysis. Nicotine Tob Res 23: 635–642. https://doi.org/10.1093/NTR/NTAA156
4. Chen H, Liang X, Chen L, Zuo L, Chen K, Wei Y, Chen S, Hao G (2022a) Associations Between Household Pesticide Exposure, Smoking and Hypertension. Front Public Health 10. https://doi.org/10.3389/FPUBH.2022.754643
5. Chen Q, Ma X, Geng Y, Liao J, Ma L (2022b) Association between smoking and hypertension under different PM2.5 and green space exposure: A nationwide cross-sectional study. Front Public Health 10. https://doi.org/10.3389/FPUBH.2022.1026648
6. Falk GE, Hayrettin Okut, Ph.D., Mohinder R. Vindhyal, M.D., M.Ed., Elizabeth Ablah, Ph.D., MPH (2022) Hypertension and Cardiovascular Diseases among Electronic and Combustible Cigarette Users. Kans J Med 15: 226–230. https://doi.org/10.17161/KJM.VOL15.16752
7. Guirguis-Blake JM, Evans C V., Webber EM, Coppola EL, Perdue LA, Weyrich MS (2021) Screening for Hypertension in Adults: Updated

Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA 325:1657–1669. https://doi.org/10.1001/JAMA.2020.21669
8. Herrera AM, Corvalán MP, María A, María H, Corvalán P (2017) Adolescents and smoking. Rev Chil Pediatr 88:697–698. https://doi.org/10.4067/S0370-41062017000600697
9. Kaplan RC, Baldoni PL, Strizich GM, Pérez-Stable EJ, Saccone NL, Peralta CA, Perreira KM, Gellman MD, Williams-Nguyen JS, Rodriguez CJ, Lee DJ, Daviglus M, Talavera GA, Lash JP, Cai J, Franceschini N (2021) Current Smoking Raises Risk of Incident Hypertension: Hispanic Community Health Study-Study of Latinos. Am J Hypertens 34:190–197. https://doi.org/10.1093/AJH/HPAA152
10. Kuntz B, Kroll LE, Hoebel J, Schumann M, Zeiher J, Starker A, Lampert T (2018) [Time trends of occupational differences in smoking behaviour of employed men and women in Germany : Results of the 1999-2013 microcensus]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 61: 1388–1398. https://doi.org/10.1007/S00103-018-2818-8
11. Levenson J, Simon AC, Cambien FA, Beretti C (1987) Cigarette smoking and hypertension. Factors independently associated with blood hyperviscosity and arterial rigidity. Arteriosclerosis 7:572–577. https://doi.org/10.1161/01.ATV.7.6.572
12. Londoño Pérez C, Rodríguez Rodríguez I, Andrés C, Díaz G (2011) Cuestionario para la clasificación de consumidores de cigarrillo (C4) para jóvenes. Diversitas: Perspectivas en Psicología 7:281–291
13. Lu Q, Gottlieb E, Rounds S (2018) Effects of cigarette smoke on pulmonary endothelial cells. Am J Physiol Lung Cell Mol Physiol 314: L743–L756. https://doi.org/10.1152/AJPLUNG.00373.2017
14. Markidan J, Cole JW, Cronin CA, Merino JG, Phipps MS, Wozniak MA, Kittner SJ (2018) Smoking and risk of ischemic stroke in young men. Stroke 49: 1276–1278. https://doi.org/10.1161/STROKEAHA.117.018859
15. Nagao T, Nogawa K, Sakata K, Morimoto H, Morita K, Watanabe Y, Suwazono Y (2021) Effects of Alcohol Consumption and Smoking on the Onset of Hypertension in a Long-Term Longitudinal Study in a Male Workers' Cohort. Int J Environ Res Public Health 18. https://doi.org/10.3390/IJERPH182211781
16. Ruokolainen O, Härkänen T, Lahti J, Haukkala A, Heliövaara M, Rahkonen O (2021) Association between educational level and smoking cessation in an 11-year follow-up study of a national health survey. https://doi.org/101177/1403494821993721 49: 951–960. https://doi.org/10.1177/1403494821993721
17. Scallan C, Doonan RJ, Daskalopoulou SS (2010) The combined effect of hypertension and smoking on arterial stiffness. Clin Exp Hypertens 32:319–328. https://doi.org/10.3109/10641960903443558
18. Shi H, Leventhal AM, Wen Q, Ossip DJ, Li D (2023) Sex Differences in the Association of E-cigarette and Cigarette Use and Dual Use With Self-Reported Hypertension Incidence in US Adults. Nicotine Tob Res 25: 478–485. https://doi.org/10.1093/NTR/NTAC170
19. Wu L, Yang S, He Y, Liu M, Wang Y, Wang J, Jiang B (2016) Association between passive smoking and hypertension in Chinese non-smoking elderly women. Hypertension Research 2017 40:4 40:399–404. https://doi.org/10.1038/hr.2016.162
20. Wu Z, Wu Y, Rao J, Hu H, Wang C, Wu J, Shi Y, Fu Y, Cheng X, Li P (2022) Associations among vitamin D, tobacco smoke, and hypertension: A cross-sectional study of the NHANES 2001–2016. Hypertension Research 2022 45:12 45:1986–1996. https://doi.org/10.1038/s41440-022-01023-x
21. Zhang Y, Feng Y, Chen S, Liang S, Wang S, Xu K, Ning D, Yuan X, Zhu H, Pan H, Shan G (2021) Relationship between the duration of smoking and blood pressure in Han and ethnic minority populations: a cross-sectional study in China. BMC Public Health 21:1–12. https://doi.org/10.1186/S12889-020-09975-W/TABLES/5
22. Zhao B, Li J, Zhang L, Liu J, Feng D, Hao X, Li Y, Li X, Ding J, Li L, Li L, Yin X, Cui W (2023) Prevalence of smoking among nurses and its association with blood pressure: A cross-sectional study in 11 cities of China. Int J Nurs Pract 29:e13118. https://doi.org/10.1111/IJN.13118

# Global Journals Guidelines Handbook 2024

www.GlobalJournals.org

# Memberships

## Fellows/Associates of Medical Research Council

### FMRC/AMRC Memberships

## Introduction

FMRC/AMRC is the most prestigious membership of Global Journals accredited by Open Association of Research Society, U.S.A (OARS). The credentials of Fellow and Associate designations signify that the researcher has gained the knowledge of the fundamental and high-level concepts, and is a subject matter expert, proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice. The credentials are designated only to the researchers, scientists, and professionals that have been selected by a rigorous process by our Editorial Board and Management Board.

Associates of FMRC/AMRC are scientists and researchers from around the world are working on projects/researches that have huge potentials. Members support Global Journals' mission to advance technology for humanity and the profession.

## FMRC

### Fellow of Medical Research Council

FELLOW OF MEDICAL RESEARCH COUNCIL is the most prestigious membership of Global Journals. It is an award and membership granted 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. Fellows 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 Fellow Members.

## To the institution

### Get letter of appreciation

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

## Exclusive Network

### Get access to a closed network

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


## Certificate

### Certificate, LoR and Laser-Momento

Fellows receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member's university.

Career | Credibility | Exclusive | Reputation


## Designation

### Get honored title of membership

Fellows can use the honored title of membership. The “FMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., FMRC or William Walldroff, M.S., FMRC.

Career | Credibility | Exclusive | Reputation

## Recognition on the Platform

### Better visibility and citation

All the Fellow members of FMRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation. All fellows get a dedicated page on the website with their biography.

Career | Credibility | Reputation

## Future Work

### Get discounts on the future publications

Fellows receive discounts on the future publications with Global Journals up to 60%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

Career | Financial

## GJ Internal Account

### Unlimited forward of Emails

Fellows get secure and fast GJ work emails with unlimited storage of emails that they may use them as their primary email. For example,
john [AT] globaljournals [DOT] org.

Career | Credibility | Reputation

## Premium Tools

### Access to all the premium tools

To take future researches to the zenith, fellows receive access to all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

Financial

## Conferences & Events

### Organize seminar/conference

Fellows are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

Career | Credibility | Financial

## Early Invitations

### Early invitations to all the symposiums, seminars, conferences

All fellows receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.

Exclusive


## Publishing Articles & Books

### Earn 60% of sales proceeds

Fellows can publish articles (limited) without any fees. Also, they can earn up to 70% of sales proceeds from the sale of reference/review books/literature/publishing of research paper. The FMRC member can decide its price and we can help in making the right decision.

Exclusive | Financial

## Reviewers

### Get a remuneration of 15% of author fees

Fellow members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

## Access to Editorial Board

### Become a member of the Editorial Board

Fellows and Associates may join as a member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer.

Career | Credibility | Exclusive | Reputation

## And Much More

### Get access to scientific museums and observatories across the globe

All members get access to 5 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 10 GB free secure cloud access for storing research files.

## ASSOCIATE OF MEDICAL RESEARCH COUNCIL

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.

## To the institution

### Get letter of appreciation

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

## Exclusive Network

### Get access to a closed network

A AMRC member gets access to a closed network of Tier 2 researchers and scientists with direct communication channel through our website. Associates can reach out to other members or researchers directly. They should also be open to reaching out by other.

Career | Credibility | Exclusive | Reputation


## Certificate

### Certificate, LoR and Laser-Momento

Associates receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member's university.

Career | Credibility | Exclusive | Reputation


## Designation

### Get honored title of membership

Associates can use the honored title of membership. The “AMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., AMRC or William Walldroff, M.S., AMRC.

Career | Credibility | Exclusive | Reputation

## Recognition on the Platform

### Better visibility and citation

All the Associate members of AMRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation.

Career | Credibility | Reputation

## Future Work

### Get discounts on the future publications

Associates receive discounts on future publications with Global Journals up to 30%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

Career | Financial

## GJ Account

### Unlimited forward of Emails

Associates get secure and fast GJ work emails with 5GB forward of emails that they may use them as their primary email. For example,
john [AT] globaljournals [DOT] org.

Career | Credibility | Reputation

## Premium Tools

### Access to all the premium tools

To take future researches to the zenith, fellows receive access to almost all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

Financial

## Conferences & Events

### Organize seminar/conference

Associates are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

Career | Credibility | Financial

## Early Invitations

### Early invitations to all the symposiums, seminars, conferences

All associates receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.

Exclusive


## Publishing Articles & Books

### Earn 60% of sales proceeds

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

## Reviewers

### Get a remuneration of 15% of author fees

Associate members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

## And Much More

### Get access to scientific museums and observatories across the globe

All members get access to 2 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 5 GB free secure cloud access for storing research files.

| Associate | Fellow | Research Group | Basic |
|---|---|---|---|
| $4800<br>**lifetime designation** | $6800<br>**lifetime designation** | $12500.00<br>**organizational** | APC<br>**per article** |
| **Certificate,** LoR and Momento<br>**2** discounted publishing/year<br>**Gradation** of Research<br>**10** research contacts/day<br>**1 GB** Cloud Storage<br>**GJ** Community Access | **Certificate,** LoR and Momento<br>**Unlimited** discounted publishing/year<br>**Gradation** of Research<br>**Unlimited** research contacts/day<br>**5 GB** Cloud Storage<br>**Online Presense** Assistance<br>**GJ** Community Access | **Certificates,** LoRs and Momentos<br>**Unlimited** free publishing/year<br>**Gradation** of Research<br>**Unlimited** research contacts/day<br>**Unlimited** Cloud Storage<br>**Online Presense** Assistance<br>**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.

## Before and during Submission

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.
6. Proper permissions must be acquired for the use of any copyrighted material.
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.

## Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

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

## Authorship Policies

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

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.

**Copyright**

During submission of the manuscript, the author is confirming an exclusive license agreement with Global Journals which gives Global Journals the authority to reproduce, reuse, and republish authors' research. We also believe in flexible copyright terms where copyright may remain with authors/employers/institutions as well. Contact your editor after acceptance to choose your copyright policy. You may follow this form for copyright transfers.

**Appealing Decisions**

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

**Acknowledgments**

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

**Declaration of funding sources**

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

## Preparing your Manuscript

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

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

***Manuscript Style Instruction (Optional)***

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

***Structure and Format of Manuscript***

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

A research paper must include:

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

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

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

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

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

## Format Structure

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

All manuscripts submitted to Global Journals should include:

**Title**

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

**Author details**

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

**Abstract**

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

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

**Keywords**

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

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

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

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

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

**Numerical Methods**

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

**Abbreviations**

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

**Formulas and equations**

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

**Tables, Figures, and Figure Legends**

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

**Figures**

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

## Preparation of Eletronic Figures for Publication

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

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

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

## Tips for writing a good quality Medical Research Paper

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## Informal Guidelines of Research Paper Writing

***Key points to remember:***

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

**Final points:**

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

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

**The discussion section:**

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

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

**General style:**

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

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

*Mistakes to avoid:*

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

**Title page:**

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

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

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

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

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

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

**Approach:**

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

**Introduction:**

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

*The following approach can create a valuable beginning:*

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

**Approach:**

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

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

**Procedures (methods and materials):**

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

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

**Materials:**

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

**Methods:**

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

**Approach:**

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

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

**What to keep away from:**

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

**Results:**

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

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

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

**Content:**

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

**What to stay away from:**

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

**Approach:**

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

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

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

**Figures and tables:**

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

**Discussion:**

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

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

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

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

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

**Approach:**

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

Describe generally acknowledged facts and main beliefs in present tense.

## The Administration Rules

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

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

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

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

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

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

| Topics | Grades | | |
|---|---|---|---|
| | **A-B** | **C-D** | **E-F** |
| ***Abstract*** | Clear and concise with appropriate content, Correct format. 200 words or below | Unclear summary and no specific data, Incorrect form Above 200 words | No specific data with ambiguous information<br>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 |

# INDEX

save our planet

# Global Journal of Medical Research

Visit us on the Web at www.GlobalJournals.org | www.MedicalResearchJournal.org
or email us at helpdesk@globaljournals.org

ISSN 9755896