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Cancer, Ophthalmology & Pediatric

Highlights

- Self-Reported Cardiovascular Risk Risk Factors among Medical Students
- Unraveling Immunological Signatures
- Comprehensive Pan-Cancer Exploration

Discovering Thoughts, Inventing Future

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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.

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

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

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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

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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

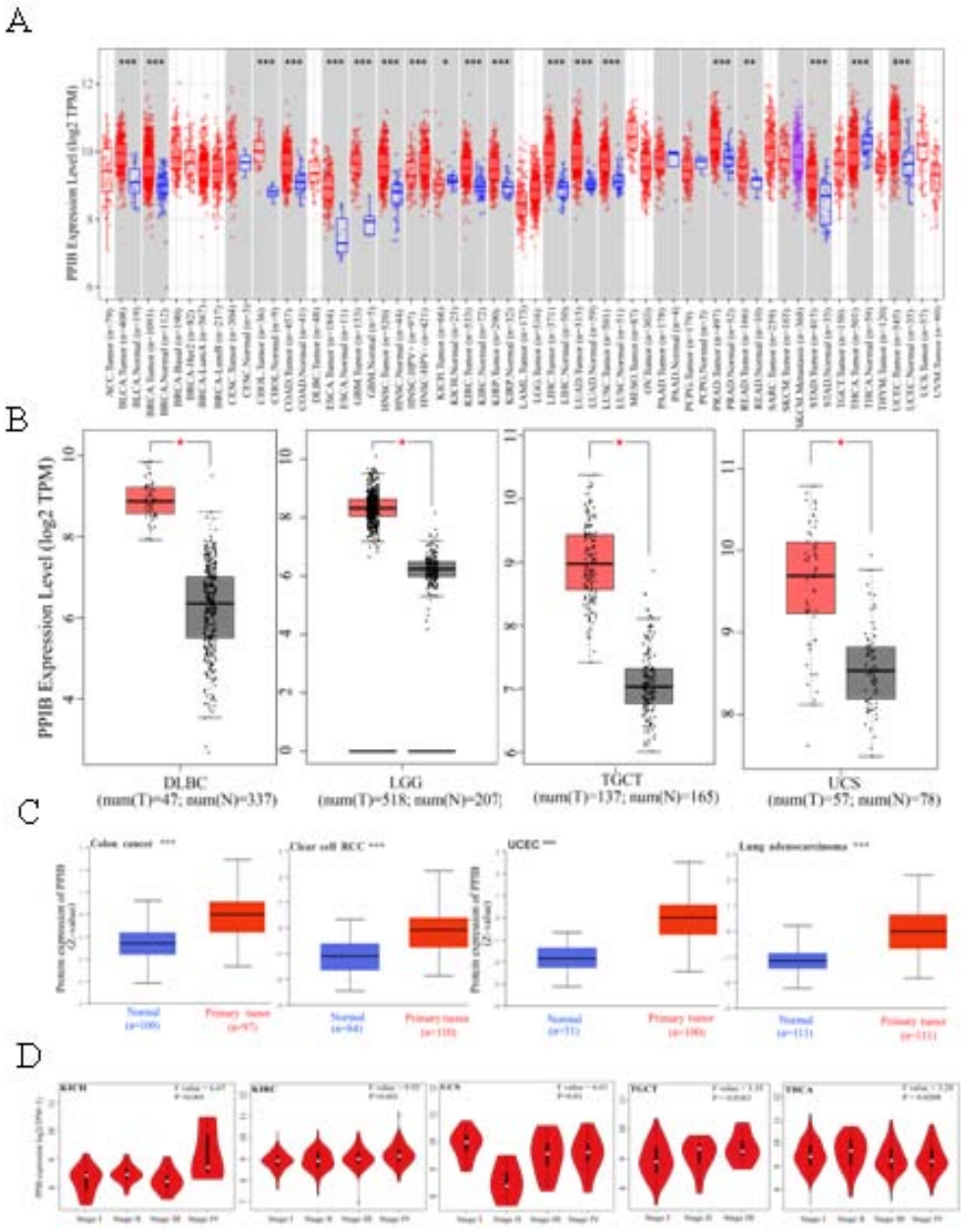

Figure 1: Expression profile of PPIB in different tumors

(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.

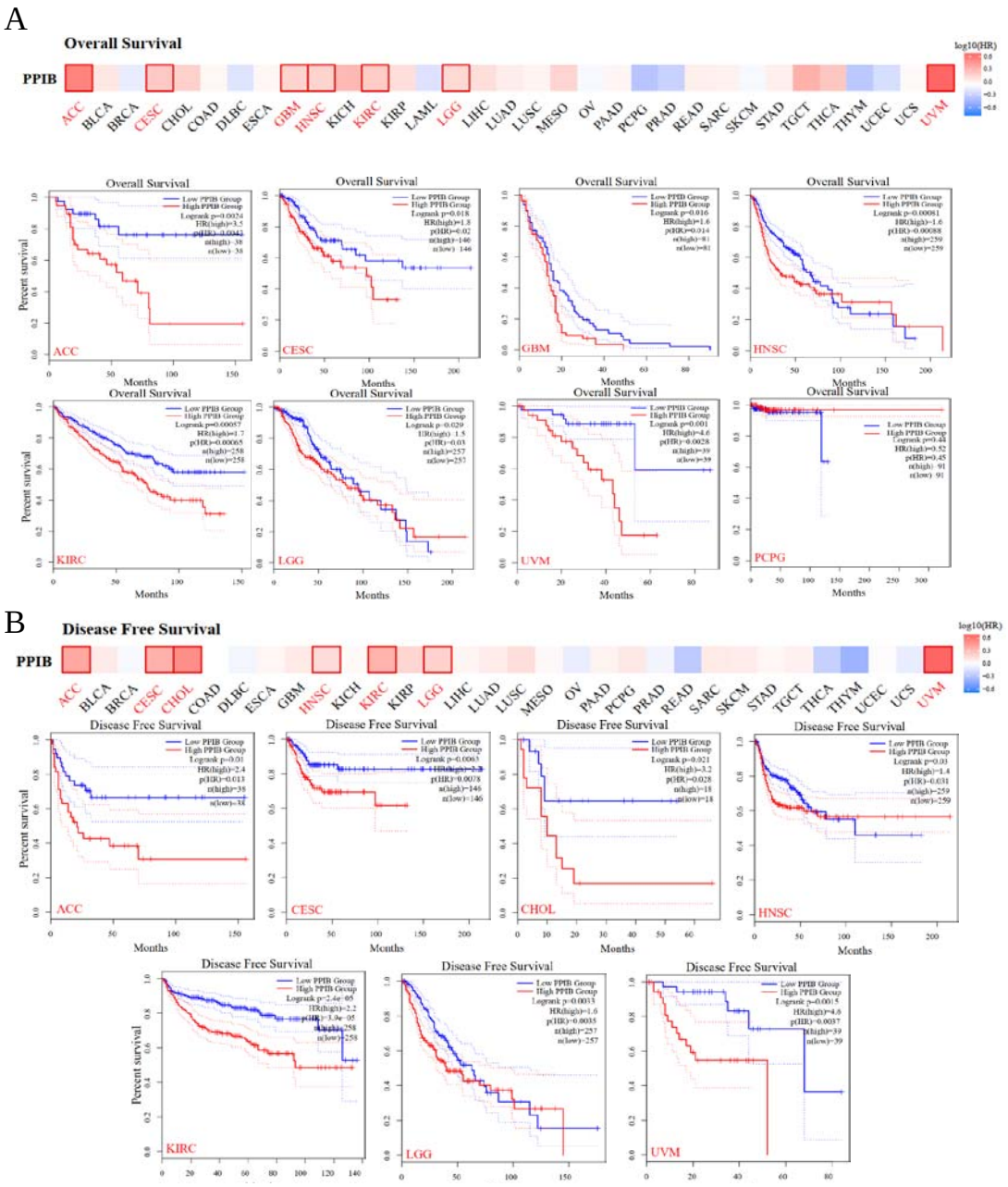

Figure 2: Survival analysis of PPIB in 33 types of tumors.
(A) Analysis of PPIB expression and overall survival in 33 types of tumors. (B)Analysis of PPIB expression and disease-free survival in 33 types of tumors.

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.

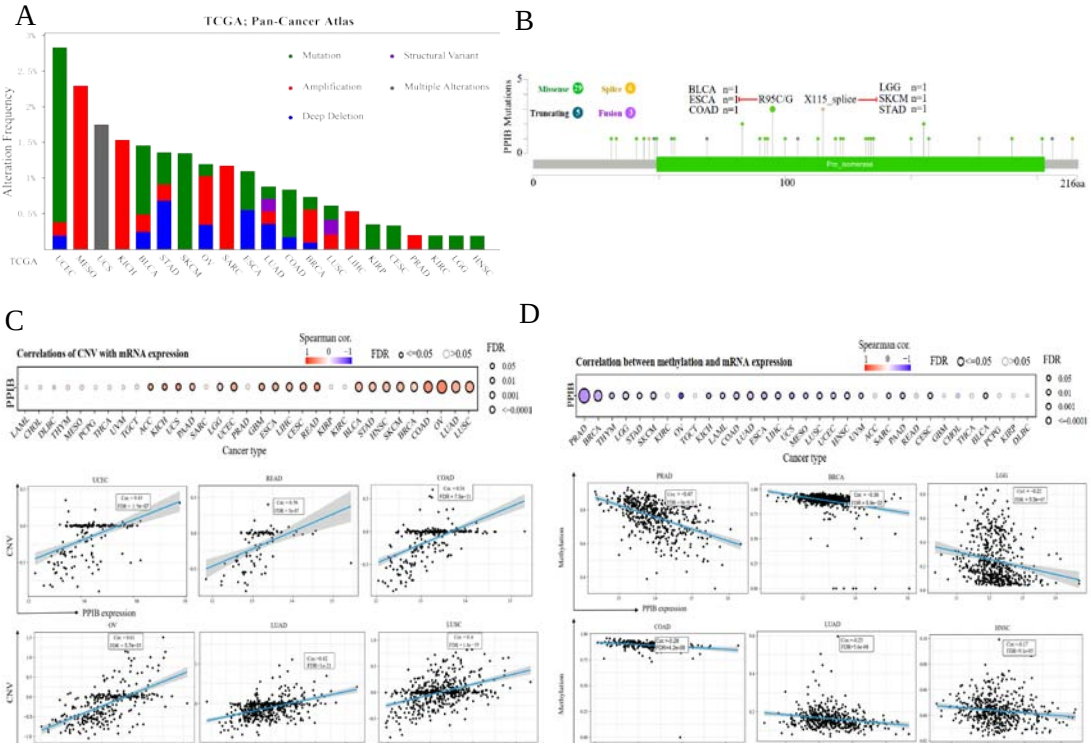

Figure 3: Genetic variation analysis of PPIB in tumors.

(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.

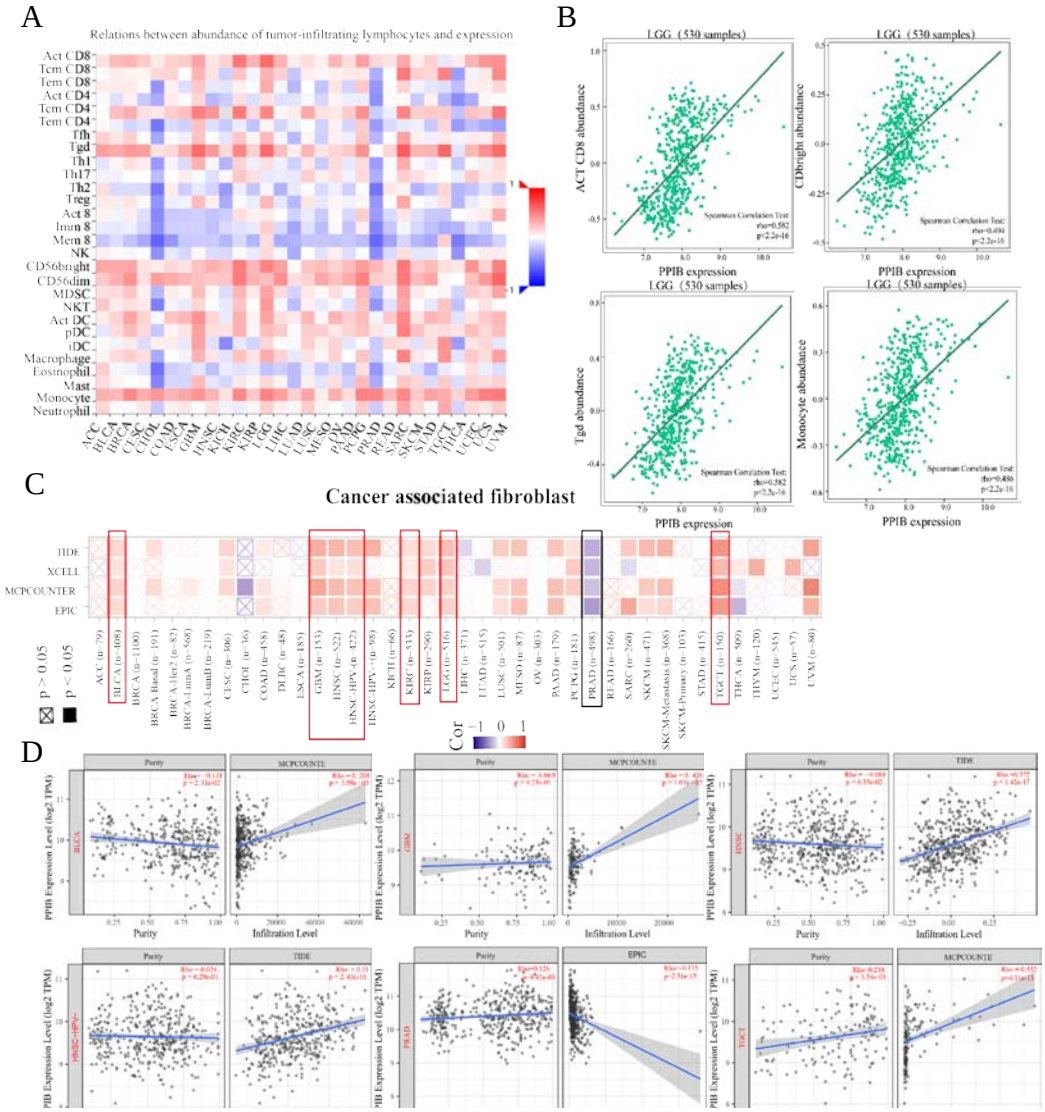

Figure 4: Analysis of PPIB in immune microenvironment.

(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).

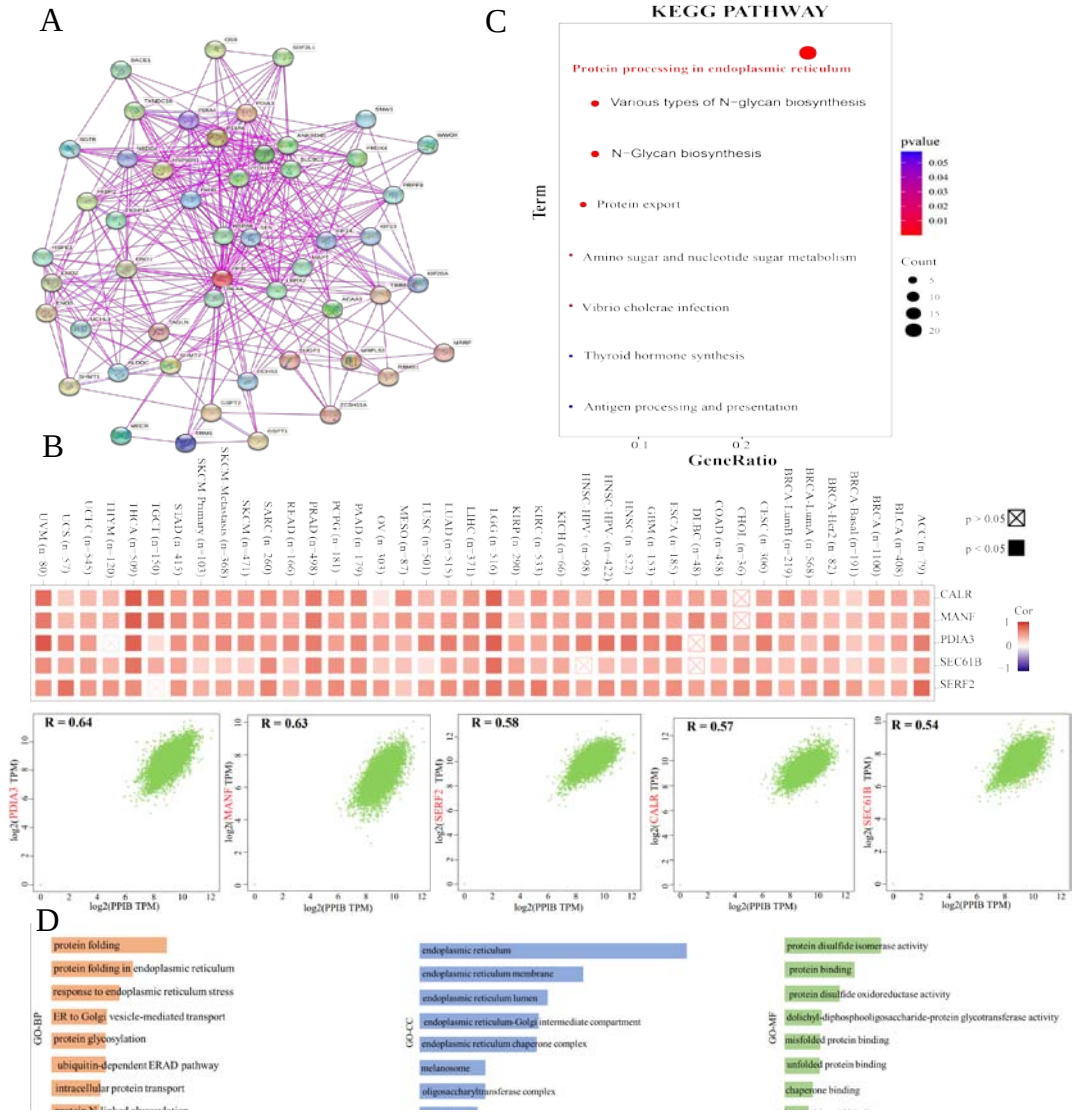

Figure 5: Analysis of molecular mechanism of PPIB in tumorigenesis.

(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.

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Authors' contributions

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All authors read and approved the final manuscript.

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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.

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Self-Reported Cardiovascular Risk Factors among Medical Students

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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],
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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.

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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.

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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.

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Keywords

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

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

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

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

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

Numerical Methods

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

Abbreviations

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

Formulas and equations

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

Tables, Figures, and Figure Legends

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

Figures

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

Preparation of Eletronic Figures for Publication

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

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

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

Tips for writing a good quality Medical Research Paper

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final points:

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

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

The discussion section:

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

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

General style:

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

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

Mistakes to avoid:

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

Title page:

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

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

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

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

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

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

Approach:

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

Introduction:

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

The following approach can create a valuable beginning:

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

Approach:

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

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

Procedures (methods and materials):

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

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

Materials:

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

Methods:

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

Approach:

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

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

What to keep away from:

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

Results:

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

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

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

Content:

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

What to stay away from:

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

Approach:

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

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

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

Figures and tables:

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

Discussion:

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

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

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

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

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

Approach:

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

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

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

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

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

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

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

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

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

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