INDEXING

Crossref

Google Scholar

OPEN ACCESS

OCLC WorldCat®

DRJI Directory of Research Journal Indexing

IMEMR INDEX MEDICIUS FOR THE EASTERN MEDITERRANEAN REGION

ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

Scilit

LOCKSS

PASTIC

ASIAN Research Index

EuroPub Directory of Academic and Scientific Journals

Dimensions

ResearchGate

ASCI Asian Science Citation Index

Aims and Scope

Pakistan Biomedical Journal is an open access, peer-reviewed International journal that publishes in all fields of health, allied health and biomedical sciences for an internationally diverse authorship. Clinical studies, clinical trials, population studies, public health, discovery medicine, study of microbes, molecular and cellular biology, basic mechanisms of biology, genetics, cancer biology, molecular medicine, pharmacology, virology, chemical biology, immunobiology, chemical biology, physiological and pathological studies are within the scope of journal.

A highly-cited, multi disciplinary, international editorial board assures timely publication of manuscripts and reviews on latest advancements in biomedical sciences.

Types of Articles

- Research papers
- Short communications
- Review or mini-reviews
- Commentaries
- Perspectives, opinion
- Meta-analysis
- Case reports
- Case studies
- Case-control studies

Reviews on recent progress in biomedical sciences are commissioned by the editors. The purpose of the Pakistan Biomedical Journal is to publish scientific and technical research papers to bring attention of international researchers, scientists, academicians, health care professionals towards the recent advancements in biomedical sciences. The articles are collected in the form of reviews, original and clinical studies. It may serve as a global platform for scientists in relevant fields to connect and share ideas mutually. This journal is open to all the research professionals whose work fall within our scope. Submission are welcome and may be submitted here.

submissions@pakistanbmj.com

@JournalPakistan
@Pakistanbmj

Title
The title of the paper should provide a concise statement of the contents of the paper. A good title is very important and will attract readers and facilitate retrieval by online searches, thereby helping to maximize citations. The title should include topical keywords and allude to the interesting conclusions of the paper. A title that emphasizes the main conclusions, or poses a question, has more impact than one that just describes the nature of the study.

Running Head
Running head should be added in the header along with the page numbers.

Type of Article
Research Article/ Case Report/ Review Article/ Opinion/ Short Communication/ Mini Review/ Letter to Editor

Running Title: A short version of the paper title.
Keywords: The major keywords used in the article have to be mentioned.

Authors
List here all author names Author[1], Author[2] and Author[3]
[1]Author department, University, Country
[2]Author department, University, Country
[3]Author department, University, Country

*Corresponding Author
Author name, Affiliation, Department Name, University Name, Address, City, State, Country, E-mail:

Abstract
Abstract should include a brief content of the article. It should be structured not more than 250 words. It should include following sub headings: Objective, Methods, Results, Conclusions.

Abbreviations
If there are any abbreviations in the article they have to be mentioned.

INTRODUCTION
Provide a context or background for the study (i.e.,the nature of the problem and its significance). State the specific purpose or research objective of, or hypothesis tested by, the study or observation; the research objective is often more sharply focused when stated as a question. Both the main and secondary objectives should be made clear, and any pre-specified subgroup analyses should be described. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

METHODS
The Methods section should include only information that was available at the time the or plan of the protocol. All information gathered during the conduct of study should be included in the result section.
Study Design, Inclusion / Exclusion Criteria, Data collection procedure, Statistical analysis.

RESULTS
Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first.

Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. When data are summarized in the results section, give numeric results not only as derivatives (for example, percentages) but also as the absolute numbers from which the derivatives were calculated, and specify the statistical methods used to analyze them. Table font should be 10 and caption should be below table and figure.

Data should not be duplicated in both figures and tables. The maximum limit of tables and figures should not exceed more than 4. Mention the findings of the study in paragraph, while mentioning figure and table number in text in sequential order.

TABLE

Table should not be copy pasted or in picture form

DISCUSSION

Discuss your findings by comparing your results with other literature

REFERENCES

References should not be less than 20.
In text references should be in number style. For Example [1]
Follow the Pubmed Referencing style
Provide the DOI link

Example: Cook NR, Rosner BA, Hankinson SE, Colditz GA. Mammographic screening and risk factors for breast cancer. American Journal of Epidemiology. 2009 Dec; 170(11): 1422-32. doi: 10.1093/aje/kwp304.

If there are more than six authors, write *et al*. after the first six names.

CONCLUSION(S)

Conclusion should elucidate how the results communicate to the theory presented as the basis of the study and provide a concise explanation of the allegation of the findings.

ACKNOWLEDGEMENT

Provide the list of individuals who contributed in the work and grant details where applicable

Plagiarism policy

Similarity index should be less than 19 and less than 5 from individual sources.

Authorship Letter

Signed authorship letter by all authors including there current department, University, City, Country, Email.

Declaration Form

Signed declaration form submit by corresponding author.

The submission of article should include: manuscript according to journal guidelines, authorship letter, declaration form. It should be submitted to the following email id:

submissions@pakistanbmj.com

EDITORIAL TEAM

VOLUME 06 ISSUE 07

Published by:
CrossLinks International Publishers
www.clip.com.pk
isni 0000000503896516

EDITORIAL BOARD

VOLUME 06 ISSUE 07

Advisory Board

Prof. Dr. Shagufta Naz, Ph.D.
Lahore College for Women University, Lahore, Pakistan

Prof. Dr. Farkhanda Manzoor, Ph.D.
Lahore College for Women University, Lahore, Pakistan

Prof. Dr. Nadeem Sheikh ,Ph.D.
University of the Punjab, Lahore, Pakistan

Prof. Dr. Muhammad Saleem Rana, Ph.D.
The University of Lahore, Lahore, Pakistan

National Members

Dr. Munir Bhinder, Ph.D.
Associate Professor
University of Health Sciences, Lahore, Pakistan

Dr. Fareeha Hameed , Ph.D.
Associate Professor
Forman Christian College, Lahore, Pakistan

Dr. Maham Akhlaq, MBBS, M.Phil, DipRCpath, Ph.D.
Assistant Professor
University of Health Sciences, Lahore, Pakistan

Dr. HA Raza, Ph.D.
University of Agriculture, Faisalabad, Pakistan

Dr. Sami Ullah Mumtaz, MBBS, FCPS
Assistant Professor
Mayo Hospital, Lahore, Pakistan

Dr. Kulsoom Rahim, Ph.D.
Assistant Professor
University of Engineering and Technology, Texila, Pakistan

Dr. Shafqat Ali, Ph.D.
Assistant Professor
Ghulam Ishaq Khan University, Swabi, Pakistan

International Members

Dr. Diki, M.ED., Ph.D.
University Terbuka, Indonesia

Dr. Beatrice Paradiso, MD, Double Ph.D.
Dolo Hospital, Venice, Italy

Dr. Rizwan Ullah Khan, MBBS, FCPS
Associate Professor
King Fahad Specialist Hospital, Jeddah, Saudi Arabia

Published by:
CrossLinks International Publishers
www.clip.com.pk
isni 0000000503896516

TABLE OF

CONTENTS

VOLUME 06 ISSUE 07

Published by:
CrossLinks International Publishers
www.clip.com.pk
isni 0000000503896516

DOI: https://doi.org/10.54393/pbmj.v6i07.909

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 7 (July 2023)

Telemedicine and Telehealth Solutions

Ahmed Alwazzan[1*]

[1]Division of Gynecology Oncology, Faculty of Medicine, King Abdul Aziz University, Jeddah, Saudi Arabia

ab_wazzan@yahoo.com

ARTICLE INFO

How to Cite:

Alwazzan, A. . (2023). Telemedicine and Telehealth Solutions: Telemedicine and Telehealth Solutions. Pakistan BioMedical Journal, 6(07). https://doi.org/10.54393/pbmj.v6i07.909

Telemedicine and telehealth solutions are witnessing a dramatic shift in the global healthcare sector at a time of unprecedented technological advancements. These innovative approaches are changing the way healthcare is delivered, increasing accessibility, efficacy, and patient-centeredness. Telemedicine has evolved as a powerful technique for overcoming the geographic limits that have long prohibited patients from obtaining high-quality medical treatment. Telemedicine allows patients and healthcare providers to connect without being physically separated, whether they are in crowded cities or remote rural regions. This technical breakthrough has improved patient outcomes while also allowing for the provision of emergency treatment during times of crisis and disaster. Patients may rapidly schedule online consultations, obtain medical advice, and receive tailored treatment plans. This degree of ease encourages patients to actively manage their health, strengthening the trust- and cooperation-based doctor-patient relationship. Telehealth technologies play a critical role in improving healthcare accessible in areas with inadequate healthcare infrastructure. Medical expertise may be extended to underserved areas using telemedicine platforms, allowing professionals to consult, diagnose, and treat patients who would otherwise face major hurdles to timely medical care. Furthermore, telemedicine programs have shown to be critical in delivering mental health care, particularly in areas where mental health resources are few. The combination of telemedicine and telehealth technology has transformed the delivery of healthcare services. Remote patient monitoring gadgets and wearable technology have enabled healthcare practitioners to continually monitor their patients' health state, allowing them to discover possible issues early and respond proactively. Furthermore, telemedicine has simplified medical record administration, improved care coordination, and reduced medical mistakes. While the advantages of telemedicine solutions are obvious, difficulties remain. These include worries about data security, privacy, and the need for regulatory frameworks to properly control telemedicine operations. Addressing these issues would need the collaboration of governments, healthcare institutions, and technology suppliers. We can establish a safe atmosphere for telehealth solutions to thrive by creating strong rules and guidelines. Telemedicine and telehealth have emerged as viable options for addressing Pakistan's healthcare concerns. With a rising population and restricted access to medical facilities, these revolutionary technologies are reshaping the country's healthcare scene. Telemedicine connects patients from rural and underprivileged locations with healthcare specialists via virtual consultations, allowing for quick diagnosis and treatment suggestions [1]. Furthermore, telehealth efforts enable people to take an active role in their health management by putting medical information and services at their fingertips. Despite certain infrastructure and regulatory difficulties, the use of telemedicine and telehealth in Pakistan has enormous potential to improve healthcare access and patient outcomes across the country.

REFERENCES

[1] Malik AZ. Telemedicine country report-Pakistan. 2007 9th International Conference on e-Health Networking, Application and Services. 2007 Jun: 90-94.

DOI: https://doi.org/10.54393/pbmj.v6i07.905

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 7 (July 2023)

Review Article

Irisin and its Effects on the Metabolic Diseases

Mirza Fahad Baig[1], Muhammad Khalil Ahmad Khan[1*], Mahnoor[1], Munazza Perveen[1], Muhammad Atif [1], Usman Younas[1] and Sadia Sharif[1]

[1]Department of Zoology, University of Okara, Okara, Pakistan

ARTICLE INFO

Key Words:

Fibronectin, NAFLD, FNDC5, Diabetes, Lipid Metabolism, Heart Disease, Myokine

How to Cite:

Baig, M. F. ., Ahmad Khan, M. K. ., Mahnoor, ., Perveen, M. ., Atif , M. ., Younas, U. ., & Sharif, S. . (2023). Irisin and its Effects on the Metabolic Diseases: Irisin and its Effects on the Metabolic Diseases. Pakistan BioMedical Journal, 6(07). https://doi.org/10.54393/pbmj.v6i07.905

***Corresponding Author:**

Muhammad Khalil Ahmad Khan

Department of Zoology, University of Okara, Okara, Pakistan

dr.khalil@uo.edu.pk

Received Date: 29th June, 2023

Acceptance Date: 20th July, 2023

Published Date: 31st July, 2023

ABSTRACT

Irisin, also known as Fibronectin type III, is a hormone that is secreted by muscle cells and was first discovered in the muscles of a mouse in 2012. Irisin has a molecular weight of 23,231 KDa and belongs to the domain containing 5 (FNDC5) family. It has been shown to have some very beneficial effects in humans, such as thermoregulation and weight loss, and it is also secreted by the muscles of humans when they exercise or work out. The gene symbol for irisin is FNDC5, which represents the precursor of irisin. At the protein level, both FNDC5 and irisin have characteristics that are similar, but FNDC5 is not appropriate in some situations. It is released during physical activity and is linked to a variety of metabolic diseases such as obesity, type 2 diabetes, lipid metabolism, heart disease, NAFLD, PCOS, and metabolic diseases of the bones. Irisin is not only responsible for the disorders, but it also has the potential to be used as a biomarker for specific diseases. Humans and mice have both shown that myokine irisin promotes the browning of white adipose tissues while simultaneously increasing thermogenesis and energy expenditures. Irisin therapy reduces body weight while also increasing brown fat-specific gene expression in the patient. Irisin increases the risk of type 2 diabetes and cancer. Irisin levels were found to be lower in obese people who had NAFLD.

INTRODUCTION

Irisin is also given an additional name as Fibronectin type – III domain containing 5 (FNDC5). It was discovered a few years ago in 2012 in the muscles of mouse. Some very beneficial effects have been reported in the humans like thermoregulation and weight loss and is also secreted in the muscles of humans during exercise. Irisin is yield on cleavage of its originator Fibronectin type III domain containing 5 (FNDC5). The precursor FNDC5 weights about 23, 231 KDa. There is a difference in the KDa of transmembrane and cellular FNDC5, the cellule FNDC5 have molecular weight of 23KDa that is smaller than transmembrane having Molecular weight of 32 KDa. It has been well conserved through evolutionary process in the animals. Most of information about irisin has also been seen as similar in FNDC5 like gene and homology etc. [1]. It was reported that there is a protein that is formed by the cleavage of FNDC5 that is found in the cells of skeletal muscles. Browning of white fat is affected at a huge extent by this protein. After some days of continuous exercise, a huge increase in the volume of circulating irisin was also observed in both mice and humans [2]. The mineral density of bones is related to serum concentrations of irisin [3]. Some pathological conditions like osteoarthritis is also related to the lower serum concentrations of irisin [4]. Osteogenesis can also be induced by irisin; bone loss that is caused by the estrogen deficiency can be resisted by the deletion of FNDC gene.

Structure

Irisin mainly has three parts i.e., 29- amino acid signal peptide, 94- amino acid single FN III Fibronectin domain

DOI: https://doi.org/10.54393/pbmj.v6i07.905

and C- terminal The insertion of irisin in human beings has been reported as 50- amino acid N-terminal [5]. Basic structure and activity of irisin is shown in Figure 1.

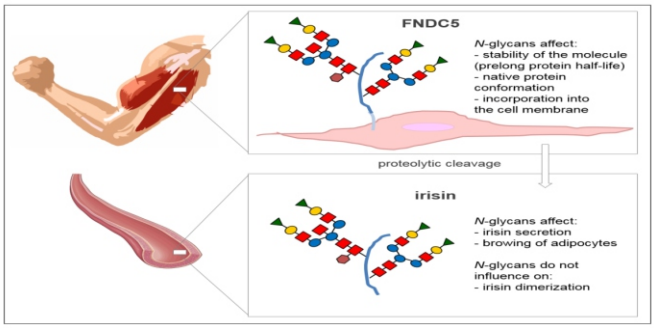


Figure 1: Showing the activity and the basic structure of irisin [6]

Origin of Irisin in the Human Body

The human FNDC5 gene's start codon was altered, and cells transfected with the mutant version of the gene translated only 1 % of the full-length protein [7]. Many scientists stated that translation of irisin is not started by a normal start codon (AUG) rather it is started by a manipulated start codon (non-canonical start codon). Animals having dense genetic arrangements like bacteria, fungi and mammalian cells that are affected by the viruses have the non-canonical start codons [8]. Non-canonical start codons have a much lower translational rate than the AUG start codon. The expression of proteins can be initiated by any of six non-canonical start codons [9]. Kim discovered three distinct FNDC5 transcripts with varying degrees of expression in various human tissues [10]. This is the first research to find irisin transcripts, and it will be the only one for the foreseeable future. Recent research, however, found that when employing all of the exons in these three transcripts, the annotated transcripts were not amplified [11]. Women suffering from osteoporosis have low serum irisin level [12]. Older women with minimal trauma hip fracture show lower serum irisin level and vice versa. For normal bone functioning irisin level is important and those having high hip density show high level of irisin [13]. In mice, bone formation rate is increased using recombinant irisin and it also alleviates the bone loss caused by ovariectomy and inflammatory disease in gastrointestinal tract. Irisin is sensitive to bone cells i.e. if a lower amount of irisin is introduced then it only effects the bone cells without browning of WAT [14]. Damage caused to bones because of disuse can also be restored with irisin [15]. Colaianni observed that differentiation of osteoblast promoted through irisin by MAPK and WNT pathways. Recombinant irisin act on osteoblast and activates 2 pathways i.e., P38 and ERK, the expression of ATF-4 increases and RUNX2 pathways activated [16]. The Wnt target genes activated when β-catenine moves in nucleus from cytoplasm [17].

Synthesis, Release and Regulation of Irisin

The precursor FNDC5 of irisin is the gene symbol for irisin. At protein level both FNDC5 and irisin show similarity in their characteristics but in some cases is not suitable. The gene responsible for the production of FNDC5 is located on the chromosome 1 in humans. The mRNA of FNDC5 in humans has 2099bp. The FNDC5 found in humans have 6 exons and 5 introns [18]. The transcriptional co-activator peroxisome proliferator-activated receptor-γ (PPAR γ) and the coactivator-1 α (PGC1α), activate the gene expression of FNDC5. The cleavage and release of irisin is similar to the cleavage and release of transmembrane polypeptides in epidermal growth factor (EGF) and transforming growth factor-α (TGF- α). The C-terminal moiety is glycosylated and proteolytically cleaved to liberate the 112-aa hormone that contains most of the FNIII repeat region and release the hormone (Figure 2) [19].

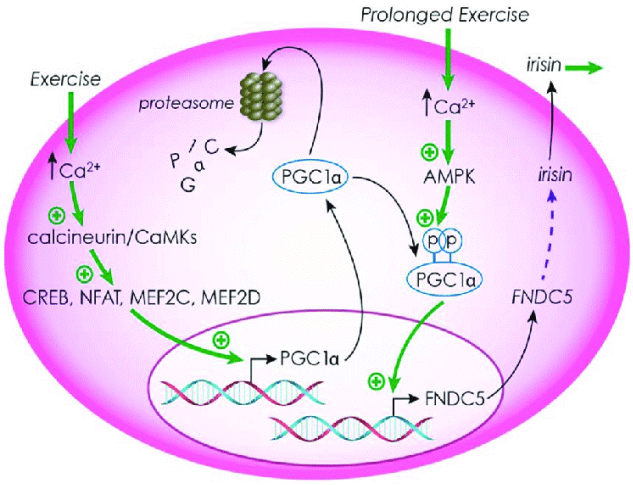


Figure 2: Showing the synthesis and release of irisin from muscle [20]

Irisin Detection Methods

Irisin is not only causing the disorders but can also be used as a biomarker for definite diseases [21]. ELISA is an antibody-antigen based detection method in human research, it is frequently utilized because of its quick detection speed, simple installation, and capacity to examine a large number of samples [22]. The irisin level in young athletes is tested higher than other individuals [23]. Ruan *et al.*, 2019 used both mass spectrometry and ELISA kits to examine the irisin level in cerebrospinal fluid but these kits failed to measure the irisin level in about 1/3rd of the sample while spectrometry measured it quantitatively [24]. The molecular weight of irisin was first calculated as 22kDa. The weight of irisin has ranged from 22 to 34 kDa, whereas the molecular weight was expected to be 12 kDa, Schumacher reported that irisin act like a dimer and its molecular weight by 24 kDa, which is not affected by glycosylation [25].

DOI: https://doi.org/10.54393/pbmj.v6i07.905

Irisin in Metabolic Diseases

In both humans and mice, the myokine irisin promotes browning of white adipose tissues while also increasing thermogenesis and energy expenses. Irisin causes many metabolic diseases like obesity, T2DM, lipid metabolism, diseases related to heart, NAFLD, PCOS and metabolic diseases of bone [26]. Irisin is the new health promoting hormone because in human muscles, FNDC5 gene is expressed during exercise and produces irisin. Irisin causes browning of white fat that enhances the metabolic uncoupling and increases the energy expenses. Two pathways i.e. p38 mitogen-activated protein kinase (MPAK) and extra cellular-signal regulated kinas (ERK) enhance this metabolic uncoupling. The maturation of preadipocytes into mature adipocytes decreases after the treatment of irisin and the gene expression remain unaffected. The MPAK and ERK-p38 pathways are triggered due to the increase expression of UCP1 protein. Agetr cold exposure the insulin-mediated glucose uptake of BAT rises ten times more than WAT [23]. The earlier studies show that expression and activity of PGC-1α and irisin level is lower in patients having T2DM. It was concluded that patients suffering from T2DM have lower level of circulating irisin than the normal persons [27].

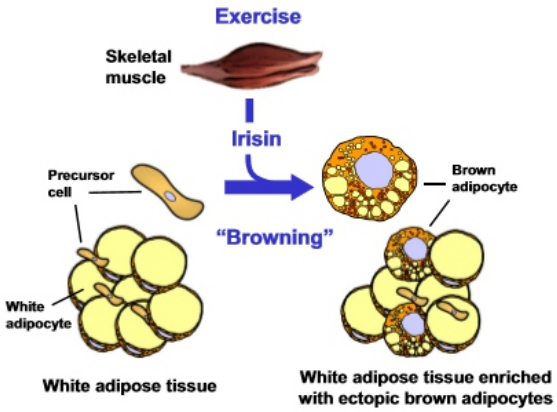


Figure 3: Showing the effect of irisin on adipose tissue [28]

Association between Serum Irisin Level and Exercise

Boström and colleagues reported that after exercise of about 10 weeks, the irisin level increases by 2 folds [29]. If the mouse runs downhill the level of irisin was reported to increases significantly but no change in the level of irisin was reported in uphill running after exercise of two weeks [30]. Changes that are induced by exercise are more complicated in patients suffering from metabolic disorders. In mice suffering from hyperthyroidism or hypothyroidism serum irisin level can be increased even after an acute exercise [31]. Huh reported that after 8 weeks of running there was no change in the serum irisin level in humans [5]. In later studies, it was observed that there is decrease in the level of serum irisin after a few weeks of exercise [32].

Stimulation of Browning of White Adipocytes

Ability of animals to resist body fat gain is connected to the quantity and activity of brown adipocytes. Some white adipose tissue (WAT) depots are easily converted into a "brown-like" state in particular circumstances, that is linked to mass decrease [33]. Irisin stimulates browning of WAT through unidentified mechanism. In mice, Recombinant irisin reduced body mass and improved glucose homeostasis [34]. Irisin increased the expression of uncoupling protein-1. Irisin-induced activation of the p38 MAPK and ERK signaling pathways may be responsible for this impact [35]. SB203580 and U0126 inhibition of the p38 MAPK and ERK inhibited upregulation of UCP-1 by irisin. Irisin also increased the expression of betatrophin, a recently discovered hormone that increases pancreatic β-cell proliferation and improves glucose tolerance [35]. Finally, our results show that irisin may help to reduce obesity and type 2 diabetes by increased expression of WAT browning-specific genes through the p38 MAPK and ERK pathways [36]. Obesity is by far the most common metabolic disorder that occurs in the world [37]. Obesity arises when a patient's calorie intake beyond their calorie expenditure, and it is defined by a rise in fatty tissue [38]. Overweight persons are more likely to get type 2 diabetes (T2DM) [39]. In terms of avoiding overweight, greater energy consumption has appeared as a possible and acceptable alternative [40]. While the exact mechanisms that control calorie expenditure are unknown, adipocytes play a very important role in mammalian energy balance and nutrient fluxes. White fat tissue and brown fat tissue are two kinds of adipose tissue that have received a lot of attention [41]. Brown adipocytes burn energy, whilst white adipocytes store it. Changes in BAT activity have been shown in numerous studies to have a significant impact on adaptive thermogenesis and glucose homeostasis [42]. Brown fat's thermogenic activity is primarily enabled by the presence of UCP-1, a mitochondrion uncoupling protein that decouples the electron transport chain from energy production, allowing potential energy received from food to be released as heat [43]. The transcriptional factor PPAR coactivator-1 (PGC-1), that can be stimulated by cold contact and/or -adrenergic signaling, regulates the expression of UCP-1 [44]. In various lab animals, browning of WAT compartments plays a protective role versus nutrition metabolic diseases like as obesity and diabetes [45]. It has 111 amino acids and a molecular weight of 22 kDa. PGC-1α and exercise have been shown to increase the expression of FNDC5, a type I transmembrane protein found in skeletal muscle. Irisin that is produced after Fndc5 is proteolyzed at amino acid positions 30 and 140. An increased level of irisin by an adenoviral vector enhances

DOI: https://doi.org/10.54393/pbmj.v6i07.905

complete body energy expenditure, causes minor weight loss, and improves glucose intolerance in high fat–fed mice [2]. The circulating level is mostly determined by age and skeletal muscle mass, with irisin levels in young male athletes are many times higher than in middle-aged obese women [5]. When compared to non-diabetic control participants, circulating irisin is noticeably lower in people with T2DM [27]. Furthermore, in chronic renal disease patients [46], plasma irisin levels are inversely connected with blood urea nitrogen levels and favorably connected with aerobic act in heart failure patients. Irisin, like hepatic fibroblast growth factor 21 [47] and cardiac natriuretic peptides, can now be considered a secreted protein that stimulates brown adipocyte thermogenesis [48]. However, very little known about the molecular processes and signaling pathways that irisin employs to produce the active brown-like adipocyte phenotype. Li *et al.*, in their study, developed a productive technique for the synthesis and purification of human recombinant irisin (r-irisin), and administered the r-irisin to mice through intraperitoneal injection. To better comprehend the fundamental "browning" mechanisms, they used r-irisin to treat primary adipocytes and 3T3-L1-derived adipocytes and looked at how it impacted the browning of fat cells [49]. In line with earlier research [50], their results showed that r-irisin treatment reduces body mass, elevates brown fat-specific gene expression in subcutaneous white adipose tissue, and enhances glucose tolerance in vivo. In cell culture assays, irisin increased the expression of genes related to brown fat by activating the p38 MAPK and ERK pathways [51].

T2DM

Adipocyte browning has been shown to be controlled by irisin, although its effects on lipid and glucose metabolism in T2DM remain mainly unclear. In diabetic mice Irisin's involvement in glucose consumption and lipid metabolism was examined. There was an increase in 18F-FDG accumulation and GLUT4 translocation in diabetic skeletal muscle when irisin was improved. Similarly, in myocytes, irisin enhanced glucose absorption grown in a high glucose medium. PEPCK and G6Pase inhibited by insulin in diabetic liver [52]. Diabetes mice treated with irisin showed a reduction in fat mass, total cholesterol serum, and triglyceride levels, but a rise in acetyl coenzyme fat tissue UCP1 expression and a carboxylase β-phosphorylation. Myocytes' lipid acid oxidation was also enhanced by Irisin. Irisin's effects on consumption of glucose and fatty acid β-oxidation in myocytes were reduced when AMPK was repressed. In hepatocytes irisin effect reduced on PEPCK and G6Pase by the inhibition of AMPK by a specific inhibitor [53]. By managing endoplasmic reticulum (ER) strain, irisin can enhance hepatic glucose and lipid regulation. It also boosts islet-cell activity and survival, therefore alleviating hyperglycemia, hyperlipidemia, and insulin resistance. If verified, irisin may decrease these defects in hepatic and islet functioning, which efficiently reduce T2DM risk [54]. In hepatocytes, irisin inhibits lipogenesis induced by palmitic acid and gluconeogenesis induced by glucosamine, through the PRMT3 and PI3K/Akt pathways, respectively [55]. In obese people with NAFLD, blood irisin levels were lower, which was connected to content of hepatic triglyceride. The islets of liver and the pancreas play important roles in T2DM pathogenesis, and are vulnerable to glucoli-potoxicity. The exact mechanism from which irisin controls hepatic glucose and lipid homeostasis as well as protects beside islet malfunction and death is remain unknown [56].

Irisin and Cancer

Exercise has been shown to lower the risk of many forms of cancer, prevent cancer progression, and have beneficial and healing effects on cancer. Changes in physical composition, hormone secretion, severe inflammatory state, and the immunity have all been postulated as potential moderators of exercise's anti-cancer benefits [57]. Obesity enhances inflammatory markers (IL-6 and TNF-), glucose intolerance, and adipokine productions, which together favor cancer cell proliferation and survival, whereas exercise has shown that anti-inflammatory benefits by decreasing TNF- expression. Considering that irisin has been linked to obesity, it makes sense that it could also be linked to cancer [58]. Increased irisin levels reduced lung cancer cell production, capability, and invasiveness via influencing the EMT, dramatically lowering the EMT markers (N-cadherin and vimentin) while raising E-cadherin expression. The Snail pathway, which is mediated by the PI3K/Akt pathway, was associated to this reduction in EMT. Irisin's effect on the PI3K pathway may also imply an inhibiting effect, which may also explain why cancer cell growth has reduced [59]. Irisin could be a new biomarker for breast cancer detection. 90% risk of breast cancer is reduced by increasing blood irisin level; whereas breast cancer patient had much lower irisin serum levels than healthy individuals. When the breast tumor cells are exposed to irisin, the caspase-3/7 increases and NF-κB movement suppressed thus cause the decrease in number of tumor cells [60]. The impact of irisin was studied using three different types of cells: non-malignant breast epithelial cells, malignant breast epithelial cells, and malignant aggressive breast epithelial cells. When aggressive breast tumour cells were exposed to irisin, there was an increase in caspase 3/7 and a reduction of NF-kB. Irisin increases caspase 3 activation and apoptosis, as well as the ability to fight cell death [61]. When the cells were exposed to irisin, there was an increase in doxorubicin

DOI: https://doi.org/10.54393/pbmj.v6i07.905

(a chemical reagent that is highly effective in cancer therapy) [62]. Irisin performs a protective role on bone against the breast cancer. It protects from the metastasis of breast cancer. Irisin and spinal irisin levels are inversely linked, indicating [60]. In osteocarcinoma cells irisin was able to inhibit the STAT3 pathway by reversing the IL-6-induced by [63]. Irisin's capacity to suppress EMT, capability, and growth of pancreatic cancer cell lines was further demonstrated when AMPK pathway is activated. Irisin suppresses the development of pancreatic cancer cells via the AMPK-mTOR pathway. Irisin's capacity to aim the AMPK pathway suggests that it may play a task in decreasing growth and modifying cancer metabolism [64]. Furthermore, irisin can be employed to detect for renal diagnosis of renal cancer, as irisin levels were significantly greater in individuals having kidney tumors; irisin also showed elevated particularly and sensitivity than other studied biomarkers [45]. The vitality of prostate cancer cells was lowered when different doses of irisin were applied to them [65]. In glioblastoma cells, irisin triggered G2/M cell cycle arrest and elevated p21 levels, inhibiting cell proliferation. Furthermore, through upregulating TFPI-2, irisin prevented glioblastoma cell invasion. Furthermore, irisin inhibited cancer in xenograft glioblastoma cells, and radiolabeled irisin revealed precise cancer cells-targeting capabilities in vivo.

CONCLUSIONS

As a result, our study identified irisin as a potential biochemical mechanism by which exercise reduces cancer progression. Irisin has the chance of growing as a molecular scanning and anticancer treatment drug.

Authors Contribution

Conceptualization: MFB, MKAK
Writing-review and editing: MFB, MKAK, M, MP, MA, UY, SS

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Aydin S. Three new players in energy regulation: preptin, adropin and irisin. Peptides. 2014 Jun; 56: 94-110. doi: 10.1016/j.peptides.2014.03.021.

[2] Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, *et al.* A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012 Jan; 481(7382): 463-8. doi: 10.1038/nature10777.

[3] Soininen S, Sidoroff V, Lindi V, Mahonen A, Kröger L, Kröger H, *et al.* Body fat mass, lean body mass and associated biomarkers as determinants of bone mineral density in children 6–8 years of age–The Physical Activity and Nutrition in Children (PANIC) study. Bone. 2018 Mar; 108: 106-14. doi: 10.1016/j.bone.2018.01.003.

[4] Yan J, Liu HJ, Guo WC, Yang J. Low serum concentrations of Irisin are associated with increased risk of hip fracture in Chinese older women. Joint Bone Spine. 2018 May; 85(3): 353-8. doi: 10.1016/j.jbspin.2017.03.011.

[5] Huh JY, Panagiotou G, Mougios V, Brinkoetter M, Vamvini MT, Schneider BE, *et al.* FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism. 2012 Dec; 61(12): 1725-38. doi: 10.1016/j.metabol.2012.09.002.

[6] Korta P, Pocheć E, Mazur-Biały A. Irisin as a multifunctional protein: implications for health and certain diseases. Medicina. 2019 Aug; 55(8): 485. doi: 10.3390/medicina55080485.

[7] Peng H, Wang Q, Lou T, Qin J, Jung S, Shetty V, *et al.* Myokine mediated muscle-kidney crosstalk suppresses metabolic reprogramming and fibrosis in damaged kidneys. Nature Communications. 2017 Nov; 8(1): 1493. doi: 10.1038/s41467-017-01646-6.

[8] Eisenberg AR, Higdon AL, Hollerer I, Fields AP, Jungreis I, Diamond PD, *et al.* Translation initiation site profiling reveals widespread synthesis of non-AUG-initiated protein isoforms in yeast. Cell Systems. 2020 Aug; 11(2): 145-60. doi: 10.1016/j.cels.2020.06.011.

[9] Hecht A, Glasgow J, Jaschke PR, Bawazer LA, Munson MS, Cochran JR, *et al.* Measurements of translation initiation from all 64 codons in E. coli. Nucleic Acids Research. 2017 Apr; 45(7): 3615-26. doi: 10.1093/nar/gkx070.

[10] Kim HK, Jeong YJ, Song IS, Noh YH, Seo KW, Kim M, *et al.* Glucocorticoid receptor positively regulates transcription of FNDC5 in the liver. Scientific Reports. 2017 Feb; 7(1): 43296. doi: 10.1038/srep43296.

[11] Albrecht E, Schering L, Buck F, Vlach K, Schober HC, Drevon CA, *et al.* Irisin: Still chasing shadows. Molecular Metabolism. 2020 Apr; 34: 124-35. doi: 10.1016/j.molmet.2020.01.016.

[12] Anastasilakis AD, Polyzos SA, Makras P, Gkiomisi A, Bisbinas I, Katsarou A, *et al.* Circulating irisin is associated with osteoporotic fractures in postmeno-

DOI: https://doi.org/10.54393/pbmj.v6i07.905

pausal women with low bone mass but is not affected by either teriparatide or denosumab treatment for 3 months. Osteoporosis International. 2014 May; 25: 1633-42. doi: 10.1007/s00198-014-2673-x.

[13] 13.Wu LF, Zhu DC, Tang CH, Ge B, Shi J, Wang BH, *et al.* Association of plasma irisin with bone mineral density in a large Chinese population using an extreme sampling design. Calcified Tissue International. 2018 Sep; 103: 246-51. doi: 10.1007/s00223-018-0415-3.

[14] Colaianni G, Cuscito C, Mongelli T, Pignataro P, Buccoliero C, Liu P, *et al.* The myokine irisin increases cortical bone mass. Proceedings of the National Academy of Sciences. 2015 Sep; 112(39): 12157-62. doi: 10.1073/pnas.1516622112.

[15] Tucci M, Stucci S, Felici C, Cafforio P, Resta L, Rossi R, *et al.* Cilengitide restrains the osteoclast-like bone resorbing activity of myeloma plasma cells. British Journal of Haematology. 2016 Apr; 173(1): 59-69. doi: 10.1111/bjh.13922.

[16] Qiao X, Nie Y, Ma Y, Chen Y, Cheng R, Yin W, *et al.* Irisin promotes osteoblast proliferation and differentiation via activating the MAP kinase signaling pathways. Scientific Reports. 2016 Jan; 6(1): 18732. doi: 10.1038/srep18732.

[17] Zhang J, Valverde P, Zhu X, Murray D, Wu Y, Yu L, *et al.* Exercise-induced irisin in bone and systemic irisin administration reveal new regulatory mechanisms of bone metabolism. Bone Research. 2017 Feb; 5(1): 1-4. doi: 10.1038/boneres.2016.56.

[18] Komolka K, Albrecht E, Schering L, Brenmoehl J, Hoeflich A, Maak S. Locus characterization and gene expression of bovine FNDC5: is the myokine irisin relevant in cattle? PloS One. 2014 Jan; 9(1): e88060. doi: 10.1371/journal.pone.0088060.

[19] Irving BA, Still CD, Argyropoulos G. Does IRISIN have a BRITE future as a therapeutic agent in humans? Current Obesity Reports. 2014 Jun; 3: 235-41. doi: 10.1007/s13679-014-0091-1.

[20] Gizaw M, Anandakumar P, Debela T. A review on the role of irisin in insulin resistance and type 2 diabetes mellitus. Journal of Pharmacopuncture. 2017 Dec; 20(4): 235.

[21] Park HS, Kim HC, Zhang D, Yeom H, Lim SK. The novel myokine irisin: clinical implications and potential role as a biomarker for sarcopenia in postmenopausal women. Endocrine. 2019 May; 64: 341-8. doi: 10.1007/s12020-018-1814-y.

[22] Elsen M, Raschke S, Sommerfeld M, Gassenhuber H, Eckel J. Comment on Wu and Spiegelman. Irisin ERKs the Fat. Diabetes. 2014 Sep; 63(9): e16. doi: 10.2337/db14-0776.

[23] Perakakis N, Triantafyllou GA, Fernández-Real JM, Huh JY, Park KH, Seufert J, *et al.* Physiology and role of irisin in glucose homeostasis. Nature Reviews Endocrinology. 2017 Jun; 13(6): 324-37. doi: 10.1038/nrendo.2016.221.

[24] Ruan Q, Huang Y, Yang L, Ruan J, Gu W, Zhang X, Zhang Y, *et al.* The effects of both age and sex on irisin levels in paired plasma and cerebrospinal fluid in healthy humans. Peptides. 2019 Mar; 113: 41-51. doi: 10.1016/j.peptides.2019.01.004.

[25] Ruan Q, Zhang L, Ruan J, Zhang X, Chen J, Ma C, *et al.* Detection and quantitation of irisin in human cerebrospinal fluid by tandem mass spectrometry. Peptides. 2018 May; 103: 60-4. doi: 10.1016/j.peptides.2018.03.013.

[26] Polyzos SA, Anastasilakis AD, Efstathiadou ZA, Makras P, Perakakis N, Kountouras J, *et al.* Irisin in metabolic diseases. Endocrine. 2018 Feb; 59: 260-74. doi: 10.1007/s12020-017-1476-1.

[27] Liu JJ, Wong MD, Toy WC, Tan CS, Liu S, Ng XW, *et al.* Lower circulating irisin is associated with type 2 diabetes mellitus. Journal of Diabetes and its Complications. 2013 Jul; 27(4): 365-9. doi: 10.1016/j.jdiacomp.2013.03.002.

[28] Villarroya F. Irisin, turning up the heat. Cell Metabolism. 2012 Mar; 15(3): 277-8. doi: 10.1016/j.cmet.2012.02.010.

[29] Timmons JA, Baar K, Davidsen PK, Atherton PJ. Is irisin a human exercise gene? Nature. 2012 Aug; 488(7413): E9-10. doi: 10.1038/nature11364.

[30] Murao M, Imano T, Akiyama J, Kawakami T, Nakajima M. Effect of single bout downhill running on the serum irisin concentrations in rats. Growth Factors. 2019 Nov; 37(5-6): 257-62. doi: 10.1080/08977194.2020.1742118.

[31] Samy DM, Ismail CA, Nassra RA. Circulating irisin concentrations in rat models of thyroid dysfunction—effect of exercise. Metabolism. 2015 Jul; 64(7): 804-13. doi: 10.1016/j.metabol.2015.01.001.

[32] Ozbay S, Ulupınar S, Şebin E, Altınkaynak K. Acute and chronic effects of aerobic exercise on serum irisin, adropin, and cholesterol levels in the winter season: Indoor training versus outdoor training. Chinese Journal of Physiology. 2020 Jan; 63(1): 21. doi: 10.4103/CJP.CJP_84_19.

[33] Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, *et al.* Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. The Journal of Clinical Investigation. 2011 Jan; 121(1): 96-105. doi: 10.1172/JCI44271.

[34] Collins S, Yehuda-Shnaidman E, Wang H. Positive and

DOI: https://doi.org/10.54393/pbmj.v6i07.905

negative control of Ucp1 gene transcription and the role of β-adrenergic signaling networks. International Journal of Obesity. 2010 Oct; 34(1): S28-33. doi: 10.1038/ijo.2010.180.

[35] Fernandez-Marcos PJ and Auwerx J. Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis. The American Journal of Clinical Nutrition. 2011 Apr; 93(4): 884S-90S. doi: 10.3945/ajcn.110.001917.

[36] Narkar VA, Downes M, Ruth TY, Embler E, Wang YX, Banayo E, *et al*. AMPK and PPARδ agonists are exercise mimetics. Cell. 2008 Aug; 134(3): 405-15. doi: 10.1016/j.cell.2008.06.051.

[37] Speakman JR. FTO effect on energy demand versus food intake. Nature. 2010 Apr; 464(7289): E1. doi: 10.1038/nature08807.

[38] Gesta S, Tseng YH, Kahn CR. Developmental origin of fat: tracking obesity to its source. Cell. 2007 Oct; 131(2): 242-56. doi: 10.1016/j.cell.2007.10.004.

[39] Park KW, Halperin DS, Tontonoz P. Before they were fat: adipocyte progenitors. Cell Metabolism. 2008 Dec; 8(6): 454-7. doi: 10.1016/j.cmet.2008.11.001.

[40] Frühbeck G, Becerril S, Sáinz N, Garrastachu P, García-Velloso MJ. BAT: a new target for human obesity? Trends in Pharmacological Sciences. 2009 Aug; 30(8): 387-96. doi: 10.1016/j.tips.2009.05.003.

[41] Cinti S. Adipocyte differentiation and transdifferentiation: plasticity of the adipose organ. Journal of endocrinological investigation. 2002 Nov; 25: 823-35. doi: 10.1007/BF03344046.

[42] Cederberg A, Grønning LM, Ahrén B, Taskén K, Carlsson P, Enerbäck S. FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. Cell. 2001 Sep; 106(5): 563-73. doi: 10.1016/S0092-8674(01)00474-3.

[43] Feldmann HM, Golozoubova V, Cannon B, Nedergaard J. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. Cell Metabolism. 2009 Feb; 9(2): 203-9. doi: 10.1016/j.cmet.2008.12.014.

[44] Barberá MJ, Schluter A, Pedraza N, Iglesias R, Villarroya F, Giralt M. Peroxisome proliferator-activated receptor α activates transcription of the brown fat uncoupling protein-1 gene: a link between regulation of the thermogenic and lipid oxidation pathways in the brown fat cell. Journal of Biological Chemistry. 2001 Jan; 276(2): 1486-93. doi: 10.1074/jbc.M006246200.

[45] Cao L, Choi EY, Liu X, Martin A, Wang C, Xu X, *et al*. White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. Cell metabolism. 2011 Sep; 14(3): 324-38. doi: 10.1016/j.cmet.2011.06.020.

[46] Choi YK, Kim MK, Bae KH, Seo HA, Jeong JY, Lee WK, *et al*. Serum irisin levels in new-onset type 2 diabetes. Diabetes Research and Clinical Practice. 2013 Apr; 100(1): 96-101. doi: 10.1016/j.diabres.2013.01.007.

[47] Wen MS, Wang CY, Lin SL, Hung KC. Decrease in irisin in patients with chronic kidney disease. PloS One. 2013 May; 8(5): e64025. doi: 10.1371/journal.pone.0064025.

[48] Hondares E, Rosell M, Gonzalez FJ, Giralt M, Iglesias R, Villarroya F. Hepatic FGF21 expression is induced at birth via PPARα in response to milk intake and contributes to thermogenic activation of neonatal brown fat. Cell Metabolism. 2010 Mar; 11(3): 206-12. doi: 10.1016/j.cmet.2010.02.001.

[49] Li SW, Sun Y, Donelan W, Yu H, Scian J, Tang D, *et al*. Expression, purification, and characterization of recombinant human pancreatic duodenal homeobox-1 protein in Pichia pastoris. Protein Expression and Purification. 2010 Aug; 72(2): 157-61. doi: 10.1016/j.pep.2010.04.004.

[50] Fernyhough ME, Vierck JL, Hausman GJ, Mir PS, Okine EK, Dodson MV. Primary adipocyte culture: adipocyte purification methods may lead to a new understanding of adipose tissue growth and development. Cytotechnology. 2004 Oct; 46: 163-72. doi: 10.1007/s10616-005-2602-0.

[51] Miegueu P, Cianflone K, Richard D, St-Pierre DH. Motilin stimulates preadipocyte proliferation and differentiation and adipocyte lipid storage. American Journal of Physiology-Endocrinology and Metabolism. 2011 Nov; 301(5): E758-66. doi: 10.1152/ajpendo.00089.2011.

[52] Kahn BB and Jeffrey S. Flier obesity and insulin resistance. Journal of Clinical Investigations. 2000 Aug; 106(4): 473-81. doi: 10.1172/JCI10842.

[53] Xin C, Liu J, Zhang J, Zhu D, Wang H, Xiong L, *et al*. Irisin improves fatty acid oxidation and glucose utilization in type 2 diabetes by regulating the AMPK signaling pathway. International Journal of Obesity. 2016 Mar; 40(3): 443-51. doi: 10.1038/ijo.2015.199.

[54] Chen N, Li Q, Liu J, Jia S. Irisin, an exercise-induced myokine as a metabolic regulator: an updated narrative review. Diabetes/metabolism Research and Reviews. 2016 Jan; 32(1): 51-9. doi: 10.1002/dmrr.2660.

[55] Park MJ, Kim DI, Choi JH, Heo YR, Park SH. New role of irisin in hepatocytes: The protective effect of hepatic steatosis in vitro. Cellular Signalling. 2015 Sep; 27(9): 1831-9. doi: 10.1016/j.cellsig.2015.04.010.

[56] Zhang HJ, Zhang XF, Ma ZM, Pan LL, Chen Z, Han HW,

DOI: https://doi.org/10.54393/pbmj.v6i07.905

et al. Irisin is inversely associated with intrahepatic triglyceride contents in obese adults. Journal of Hepatology. 2013 Sep; 59(3): 557-62. doi: 10.1016/j.jhep.2013.04.030.

[57] Calle EE and Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nature Reviews Cancer. 2004 Aug; 4(8): 579-91. doi: 10.1038/nrc1408.

[58] Lee CH, Woo YC, Wang Y, Yeung CY, Xu A, Lam KS. Obesity, adipokines and cancer: an update. Clinical Endocrinology. 2015 Aug; 83(2): 147-56. doi: 10.1111/cen.12667.

[59] Shao L, Li H, Chen J, Song H, Zhang Y, Wu F, *et al*. Irisin suppresses the migration, proliferation, and invasion of lung cancer cells via inhibition of epithelial-to-mesenchymal transition. Biochemical and Biophysical Research Communications. 2017 Apr; 485(3): 598-605. doi: 10.1016/j.bbrc.2016.12.084.

[60] Zhang ZP, Zhang XF, Li H, Liu TJ, Zhao QP, Huang LH, *et al*. Serum irisin associates with breast cancer to spinal metastasis. Medicine. 2018 Apr; 97(17): e0524. doi: 10.1097/MD.0000000000010524.

[61] Hanahan D and Weinberg RA. The hallmarks of cancer. Cell. 2000 Jan; 100(1): 57-70. doi: 10.1016/S0092-8674(00)81683-9.

[62] Gannon NP, Vaughan RA, Garcia-Smith R, Bisoffi M, Trujillo KA. Effects of the exercise-inducible myokine irisin on malignant and non-malignant breast epithelial cell behavior in vitro. International Journal of Cancer. 2015 Feb; 136(4): E197-202. doi: 10.1002/ijc.29142.

[63] Kong G, Jiang Y, Sun X, Cao Z, Zhang G, Zhao Z, *et al*. Irisin reverses the IL-6 induced epithelial-mesenchymal transition in osteosarcoma cell migration and invasion through the STAT3/Snail signaling pathway. Oncology Reports. 2017 Nov; 38(5): 2647-56. doi: 10.3892/or.2017.5973.

[64] Liu J, Song N, Huang Y, Chen Y. Irisin inhibits pancreatic cancer cell growth via the AMPK-mTOR pathway. Scientific Reports. 2018 Oct; 8(1): 15247. doi: 10.1038/s41598-018-33229-w.

[65] Tekin S, Erden Y, Sandal S, Yilmaz B. Is irisin an anticarcinogenic peptide? Medicine Science. 2015 Dec; 4(2): 2172-80. doi: 10.5455/medscience.2014.03.8210.

DOI: https://doi.org/10.54393/pbmj.v6i07.900

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 7 (July 2023)

Original Article

Hepatotoxicity Induced by Carbon Tetrachloride in Experimental Model

Faiza Munir[1] and Muhammad Khalil Ahmad Khan[1*]

[1]Department of Zoology, University of Okara, Okara, Pakistan

ARTICLE INFO

Key Words:

Carbon tetrachloride (CCl4), Hepatotoxicity, Liver, ALT, ALP, AST, Bilirubin

How to Cite:

Munir, F. ., & Khan, M. K. A. (2023). Hepatotoxicity Induced by Carbon Tetrachloride in Experimental Model: Hepatotoxicity Induced by Carbon Tetrachloride. Pakistan BioMedical Journal, 6(07). https://doi.org/10.54393/pbmj.v6i07.900

***Corresponding Author:**

Muhammad Khalil Ahmad Khan

Department of Zoology, University of Okara, Okara, Pakistan

dr.khalil@uo.edu.pk

Received Date: 9th July, 2023

Acceptance Date: 29th July, 2023

Published Date: 31st July, 2023

ABSTRACT

The present study is the first attempt to evaluate the hepatotoxicity induced by carbon tetrachloride (CCl_4) in experimental model. It poses a significant hazard to one's health. It is also one of the leading sources of toxicity in critical organs such as the lungs, kidneys, liver, and brain. **Objective:** To assess the hepatotoxicity of carbon tetra chloride in albino rats. **Methods:** The research was conducted at the Department of Zoology, University of Okara. The experiment was conducted at the animal home of the Department of Zoology, University of Okara. There were two groups created: a control group and an experimental group. The experimental group was treated with CCl_4. The rats were fed 30% diluted carbon tetrachloride with normal saline as a control group to test the harmful effect on the liver profile. This was accomplished through a 12-day trial. Sampling or dissection was done after 12 days. Rats were dissected, and their liver was punctured to obtain a blood sample and organ collection. After sampling was taken by puncturing the Rats' liver, the samples were examined by a machine called Micro-Lab 300. **Results:** Histopathological studies also proved that the liver of rats was damaged. The hepatotoxic dose of CCl_4 also raised the serum AST, ALP, ALT, and bilirubin levels. Total levels of AST, ALP, ALT, and Bilirubin were higher than usual, indicating that CCl_4 has a toxic effect on the liver profile of rats. **Conclusions:** This study suggested that CCl_4 induced toxicity in rat liver.

INTRODUCTION

Carbon tetrachloride (CCl_4) is a chlorinated hydrocarbon that is clear, colourless, volatile, and highly stable. Its inhalation can cause kidney and liver degeneration and central nervous system depression [1]. CCl_4 was first synthesised in 1839 by the French chemist Henri Victor Regnault by reacting chlorine and chloroform. Currently, it is mainly produced from methane. Recently, it has been widely used in fire extinguishers, as a precursor to refrigerants, and as a cleaning agent [2]. Although it is a common environmental pollutant, workers are at high risk of exposure to high levels of CCl_4 through inhalation and skin contact. On the other hand, the general population may be exposed to low levels of CCl_4 through inhalation in the atmospheric environment [3]. It is a well-known toxin frequently used in pre-clinical experiments for xenobiotic-induced hepatotoxicity. It induced hepatic damage in mice [4]. CCl_4-induced liver toxicity leads to necrosis and fatty liver conditions, ultimately causing tissue injury. The process is mediated by several underlying mechanisms, including metabolic activation, generation of reactive free radical metabolites, lipid peroxidation, covalent binding, and disruption of calcium homeostasis [5]. Carbon tetrachloride (CCl_4) is known for its hepatotoxic effects, causing severe liver damage such as necrosis and steatosis. This is due to the release of free radicals, including trichloromethyl (CCl3) and per oxy trichloromethyl ($OOCCl_3$) radicals, which produce lipid peroxides that damage cell membranes. It also alters enzyme activity and plays a significant role in liver damage. CCl_4 is an essential substance involved in tissue injury, and its

DOI: https://doi.org/10.54393/pbmj.v6i07.900

mechanism of hepatotoxicity, especially in necrosis and fatty liver, has been a challenging topic for researchers in various fields for the past 50 years [6, 7]. According to the World Health Organization (WHO), carbon tetrachloride causes hepatomas and hepatocellular carcinomas in mice and rats. These harmful effects are caused by toxic metabolites of CCl_4, which are produced by reactions catalysed by specific cytochrome enzymes such as CYP2E1 and CPY3A4. It should be noted that the doses required to induce liver tumours are higher than those required to induce cell toxicity [8]. Animal studies have indicated a positive correlation between CYP2E1 activity and the extent of liver injury due to CCl_4 exposure. However, no conclusive data exist on the relationship between CYP2E1 or CYP3A4 activity and human toxicity. Administration of CCl_4 changes liver tissue and causes an increased serum hepatic marker enzyme activity, which is associated with higher lipid peroxidation levels [9]. Carbon tetrachloride (CCl_4) is a potent toxic agent that affects multiple organs, including the kidneys, testicles, brain, heart, lungs, and especially the liver. It is a hepatotoxic solid with nephrotoxic and prooxidant properties, making it a commonly used agent to induce liver injury, hepatocellular carcinoma, hepatic fibrosis/cirrhosis, chemical hepatitis, renal failure, and nephrotoxicity in experimental animals [10]. In rats, administration of CCl_4-induced liver injury is characterised by significant changes in serum hepatic enzymes such as AST, ALT, and ALP, as well as changes in liver function biomarkers, oxidant parameters, and inflammation [11]. The study examined the effects of CCl_4 on male albino rats, looking specifically at hepatotoxicity, lipid peroxidation, and haematological changes. Results revealed increased alpha-fetoprotein levels and changes in hepatic function biomarkers, such as increased levels of transaminases (AST, ALT) [12]. CCl_4 is essential for investigating the underlying mechanisms of hepatotoxic effects, including fatty degeneration, fibrosis, hepatocellular death, and carcinogenesis. Its ability to damage hepatocytes is attributed to its similarity to the oxygen gradient in the liver lobule in CCl_4-induced injury. Thus, making it a valuable tool for understanding liver function and pathogenesis [13]. CCl_4-induced hepatotoxicity resulted in elevated plasma transaminase levels and liver damage. Women showed greater susceptibility to this form of liver injury than men [14]. After four weeks of CCl_4 administration, significant changes were observed in the liver. In the centrilobular area, hepatocytes underwent necrotic changes with infiltration of ceroid pigment-laden macrophages, causing inflammation. This region also shows increased hepatic stellate cells (HSCs) surrounding the central vein and displaying enlarged nuclei. Fibrotic changes in the centrilobular area were detected by reticulin and Sirius red staining [15]. CCl_4 is a highly effective compound for inducing hepatotoxicity in experimental rats and is commonly used for this purpose because of its hepatotoxic properties [16]. Because of its effectiveness, chemical toxin-induced liver damage has been extensively studied using animal models [17, 18]. When animals are injected with CCl_4 in their peritoneal cavity, CCL_3 interacts with key cellular molecules such as proteins, lipids, and nucleic acids that are structurally and biologically important. This interaction results in liver damage and dysfunctioning [19]. Twenty-four hours after CCl_4 injection, typical oxidative stress-induced centrilobular necrotic acute liver injuries were observed, including loss of body weight and development of small nodules with a corresponding increase in liver weight on gross examination with an increase in serum AST and ALT levels and notable deficiency of endogenous antioxidants and antioxidative enzymes, with centrilobular necrosis, and oxidative stress markers [20].

METHODS

CCl_4 was purchased freshly from a market. A 30% concentrated solution of CCl_4 was prepared using distilled water. The experimental model used in this study was the male albino Wistar adult mice weighing about 180-200g. They were purchased from a local market and were kept in the animal house of the Department of Zoology, University of Okara, approximately one week before the start of the experiment. The mice were housed in four to five cages under the standard conditions of 25-27°C, humidity minimum of 44% under 12 hours of light and dark. Rats were nourished with rat feed containing 20% of protein along with water available at the libitum. Rats were given CCL_4 doses in the daytime. Doses can only be given once the rats have attained a size of 30g for two weeks (Table 1). During the experiment, the mice were placed randomly, five per cage, divided into two groups, one of the control group and the other of the experimental group. The animals were randomly placed into 5 groups, with 3 rats in each group. The control group in which non affected animals not affected with CCl_4-intoxication. The experimental group in which the animals are operated with CCl_4 during solutions. The solutions were prepared just at the beginning of the experiment. The animals in the experimental group made exposed to the model substance carbon tetrachloride by oral consumption. Continuous for two weeks, given in day time.

Table1: List of Groups, Doses, Days and Amount of Dose

Groups	Doses	Days	Amount
Group 1	Normal Saline	12 Days	1 ml
Group 2	30 % CCl_4	12 Days	200mg/kg

DOI: https://doi.org/10.54393/pbmj.v6i07.900

The rats were euthanised with chloroform and weighed before dissection. Blood samples were collected in vacutainer tubes using 23 G1 syringes after a cardiac puncture. Dissected out heart, liver, and brain were washed with ice-cold saline. As for the requirements of tissue homogenisation, weighed organs were stored at -20°C after the dissection of 1x1 cm tissues was obtained. After this, they were placed in a petri dish containing 0.9% saline solution. For further processing, sections were placed in a glass containing 10% formalin solution. Blood samples were centrifuged for 15 minutes at 4°C at 10,000 rpm to isolate the serum. The diagnostic kits were used to estimate AST and ALT levels to analyse serum samples of rats. Took 800 ul of reagent (R1) and 100ul of the sample. Mixed and added 200µl reagent (R2) after 1 minute. Mixed and read absorbance at 340nm after 1 min. Read absorbance again after 1, 2, and 3 min. From the absorbance reading, calculated AA/min and multiplied by the corresponding factors (1745). ΔA/min x factor =ALAT (GPT)activity[U/1].

RESULTS

CCl_4 is a toxic compound that exerts toxic effects on the liver. The analysis showed that CCl_4 exposure disrupts regular physiological features by disturbing the standard values of ALP, AST, ALT, and bilirubin.

Table 2: Mean comparison between the healthy and experimental group

	Healthy N=15	Diseased N= 15
Total bilirubin	.52 00 ± 0.6188	1.2640 ± .04735
ALP	126.2000 ± 8.78212	153.40 ± 3.17850
AST	15.4000 ± 1.21420	53. 6 ±1.3876
ALT	16.4000 ± 1.36905	56.400 ± 1.526

The estimation level of bilirubin of the CCl_4 group (.5200 ± 0.6188) is usually increased than the standard control (1.2640 ± 0.04735). The estimation level of ALP in serum (126.200 ± 8.78212) is typically increased to the control level of (153.40 ± 3.17850). The estimation level of AST in serum (15.4000 ± 1.21420) is typically increased to the control level of (53.6 ± 1.3876). The estimation level of ALT in serum (16.4000 ± 1.36905) is generally increased to the control level of (56 ± 1.526)(Table 2).

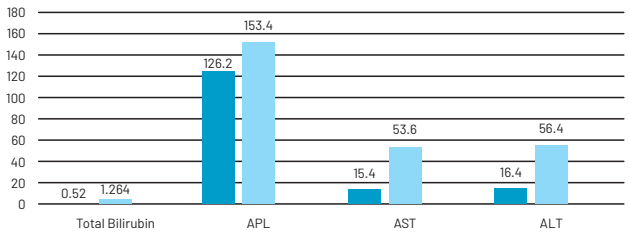


Figure 1: Mean Comparison of the Normal Group with the Diseased Group

The treatment group's descriptive statistics at the significant variation of (P>0.05) were done to determine the mean and standard deviation of Bilirubin, ALP, AST, and ALT. One sample t-test was applied to determine the confidence interval of the mean difference of bilirubin, ALP, AST, and ALT (Figure 1, Table 3-5).

Table3: Descriptive statistics values of Bilirubin, ALP, AST, ALT

	N	Minimum	Maximum	Mean	Std. Deviation
Bilirubin	15	.98	1.53	1.2640	.18337
ALP	15	133.00	168.00	153.4000	12.31027
AST	15	47.00	60.00	53.6000	5.38251
ALT	15	49.00	64.00	56.4000	5.91366

Table 4: One-Sample Test Show Mean, No of Bilirubin, ALP, AST, ALP

	N	Mean	Std. Deviation	Std. Error Mean
Bilirubin	15	1.2640	.18337	.04735
ALP	15	153.4000	12.31027	3.17850
AST	15	53.6000	5.38251	1.38976
ALT	15	56.4000	5.91366	1.52690

Table 5: Mean difference of Bilirubin, ALP, ALT, AST

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Bilirubin	26.697	14	.000	1.26400	1.1625	1.3655
ALP	48.262	14	.000	153.40000	146.5828	160.2172
AST	38.568	14	.000	53.60000	50.6193	56.5807
ALT	36.938	14	.000	56.40000	53.1251	59.6749

At 0.05 significant level (.536*), the value shows the association between the ALP and ALP. At 0.05 significant level (.613*), the value shows the correlation between the AST and ALT. At 0.01 significant level (-.715**) value shows the association between the AST and ALP (Table 6).

Table 6: Correlation between Bilirubin, ALP, AST, and ALT

		Bilirubin	ALP	AST	ALT
Bilirubin	Pearson Correlation	1	.536*	.304	.439
	Sig. (2-tailed)		.040	.271	.102
	N	15	15	15	15
ALP	Pearson Correlation	.536*	1	-.250	.613*
	Sig. (2-tailed)	.040		.370	.015
	N	15	15	15	15
AST	Pearson Correlation	.304	-.250	1	-.715**
	Sig. (2-tailed)	.271	.370		.003
	N	15	15	15	15
ALT	Pearson Correlation	.439	.613*	-.715**	1
	Sig. (2-tailed)	.102	.015	.003	
	N	15	15	15	15

DISCUSSION

CCl_4 is a toxic compound which exerts toxic effects on the liver. The analysis shows that CCl_4 exposure disrupts regular physiological features by disturbing the standard values of ALP, AST, ALT, and Bilirubin. The chemical industry commonly uses CCl_4 as an organic solvent, and it is also well-documented as an experimental inducer of

DOI: https://doi.org/10.54393/pbmj.v6i07.900

hepatotoxicity. In this study, mice treated with CCl_4 suffered damage to the hepatocyte membrane, releasing hepatocyte cytosolic enzymes. This was evidenced by significant increases in serum marker enzymes (AST, ALT, and ALP) associated with acute liver damage. Elevated serum levels of AST and ALT are specific markers and showed acute liver damage, while elevated ALP levels indicated hepatobiliary damage. ALT is the most specific marker of liver damage, while AST is abundant in cardiac muscle, kidney, testes, and skeletal muscle. ALP is abundant in growing bone. Therefore, elevated serum levels of these enzymes indicated disease affecting any extrahepatic tissues [21]. Studies have indicated that CCl_4 significantly affects ALT and AST serum levels. Elevation of these liver enzymes can be attributed to acute hepatocyte injuries caused by CCl_4 [22]. In assessing CCl_4-induced hepatotoxicity, serum AST, ALT, and ALP activity levels were used as indices. CCl_4-treated animals showed a significant increase in serum AST, ALT, and ALP activity levels compared to the standard group. This indicated that hepatotoxicity induced by CCl_4 was evident in the animals [23]. Elevated serum ALT and AST levels indicated severe liver damage and were significantly higher in the CCl_4-treated group than in the control group. This increase in ALT and AST activities resulted from CCl_4 exposure [24]. Control animals treated with CCl_4 exhibited a significant elevation in ALP levels. This finding was consistent with a study by Prakash and colleagues [25]. Serum levels of ALT and AST were measured and found to be significantly elevated after CCl_4 injection. Furthermore, the concentration of both ALT and AST peaked after injecting CCl_4 [26]. Elevated levels of ALT and AST serum activities and elevated bilirubin levels indicated early acute hepatic damage. In cases of CCl_4-induced hepatic cell injury, ALT and AST serum activities are markedly elevated. In contrast, bilirubin levels are elevated within 24 hours of treatment—these biochemical markers serve as essential indicators for early detection of hepatic damage [23]. Acute hepatotoxicity was observed due to increased serum AST and ALT activities by CCl_4. Our study found a significant reversal of AST and ALT level changes, consistent with previous findings [27]. In response to administering a toxic dose of CCl_4, the control group of rats showed elevated levels of serum transaminases, especially AST and ALT. These are essential indicators of liver damage and function [28].

CONCLUSIONS

The serum AST and ALT activity results from CCl_4-induced toxicity revealed significant increases in rats' livers. Serum enzyme assay (AST, ALT, and ALP) results of albino Wistar rats exposed to CCL_4-induced hepatotoxicity showed significant differences, and the serum AST, ALT, and ALP activity levels were significantly higher.

Authors Contribution

Conceptualization: FM, MKAK
Methodology: FM, MKAK
Formal analysis: FM, MKAK
Writing-review and editing: FM, MKAK

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Seifert WF, Bosma A, Brouwer A, Hendriks HF, Roholl PJ, van Leeuwen RE, *et al*. Vitamin A deficiency potentiates carbon tetrachloride-induced liver fibrosis in rats. Hepatology. 1994 Jan; 19(1): 193-201. doi: 10.1002/hep.1840190129

[2] Doherty RE. A history of the production and use of carbon tetrachloride, tetrachloroethylene, trichloroethylene and 1, 1, 1-trichloroethane in the United States: part 1—historical background; carbon tetrachloride and tetrachloroethylene. Environmental forensics. 2000 Jun; 1(2): 69-81. doi: 10.1006/enfo.2000.0010

[3] Liu F, Liu ZD, Wu N, Cong X, Fei R, Chen HS, *et al*. Transplanted endothelial progenitor cells ameliorate carbon tetrachloride–induced liver cirrhosis in rats. Liver Transplantation. 2009 Sep; 15(9): 1092-100. doi: 10.1002/lt.21845

[4] Moss O. Nutrition priorities: Diet recommendations in liver cirrhosis. Clinical liver disease. 2019 Oct; 14(4): 146. doi: 10.1002/cld.831

[5] Jeznach-Steinhagen A, Ostrowska J, Czerwonogrodzka-Senczyna A, Boniecka I, Shahnazaryan U, Kuryłowicz A. Dietary and pharmacological treatment of nonalcoholic fatty liver disease. Medicina. 2019 May; 55(5): 166. doi: 10.3390/medicina55050166

[6] Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Critical reviews in toxicology. 2003 Jan; 33(2): 105-36. doi: 10.1080/713611034

[7] Masuda Y. Learning toxicology from carbon tetrachloride-induced hepatotoxicity. Yakugaku zasshi: Journal of the Pharmaceutical Society of

DOI: https://doi.org/10.54393/pbmj.v6i07.900

Japan. 2006 Oct; 126(10): 885-99. doi: 10.1248/yakushi.126.885

[8] Fouw JD. Environmental Health Criteria 208, Carbon Tetrachloride. Geneva: World Health Organization. 1999:43-5.

[9] Oyinloye BE, Osunsanmi FO, Ajiboye BO, Ojo OA, Kappo AP. Modulatory effect of methanol extract of Piper guineense in CCl4-induced hepatotoxicity in male rats. International Journal of Environmental Research and Public Health. 2017 Sep; 14(9): 955. doi: 10.3390/ijerph14090955

[10] Unsal V, Cicek M, Sabancilar İ. Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Reviews on environmental health. 2021 Jun; 36(2): 279-95. doi: 10.1515/reveh-2020-0048

[11] Al-Harbi NO, Imam F, Nadeem A, Al-Harbi MM, Iqbal M, Ahmad SF. Carbon tetrachloride-induced hepatotoxicity in rat is reversed by treatment with riboflavin. International Immunopharmacology. 2014 Aug; 21(2): 383-8. doi: 10.1016/j.intimp.2014.05.014

[12] Meligi NM, Ismail SA, Tawfik NS. Protective effects of honey and bee venom against lipopolysaccharide and carbon tetrachloride-induced hepatoxicity and lipid peroxidation in rats. Toxicology Research. 2020 Sep; 9(5): 693-705. doi: 10.1093/toxres/tfaa077

[13] McCay PB, Lai EK, Poyer JL, DuBose CM, Janzen EG. Oxygen-and carbon-centered free radical formation during carbon tetrachloride metabolism. Observation of lipid radicals in vivo and in vitro. Journal of Biological Chemistry. 1984 Feb; 259(4): 2135-43. doi: 10.1016/S0021-9258(17)43327-8

[14] Lima CF, Fernandes-Ferreira M, Pereira-Wilson C. Drinking of Salvia officinalis tea increases CCl4-induced hepatotoxicity in mice. Food and chemical toxicology. 2007 Mar; 45(3): 456-64. doi: 10.1016/j.fct.2006.09.009

[15] Geerts AM, Vanheule E, Praet M, Van Vlierberghe H, De Vos M, Colle I. Comparison of three research models of portal hypertension in mice: macroscopic, histological and portal pressure evaluation. International journal of experimental pathology. 2008 Aug; 89(4): 251-63. doi: 10.1111/j.1365-2613.2008.00597.x

[16] Elachouri M. Ethnobotany/ethnopharmacology, and bioprospecting: Issues on knowledge and uses of medicinal plants by Moroccan people. Innatural products and drug discovery 2018 Jan 1 (pp. 105-118). Elsevier. doi: 10.1016/B978-0-08-102081-4.00005-8

[17] Rudnicki M, Silveira MM, Pereira TV, Oliveira MR, Reginatto FH, Dal-Pizzol F, *et al*. Protective effects of Passiflora alata extract pretreatment on carbon tetrachloride induced oxidative damage in rats. Food and Chemical Toxicology. 2007 Apr; 45(4): 656-61. doi: 10.1016/j.fct.2006.10.022

[18] Lee KJ, Choi JH, Jeong HG. Hepatoprotective and antioxidant effects of the coffee diterpenes kahweol and cafestol on carbon tetrachloride-induced liver damage in mice. Food and Chemical Toxicology. 2007 Nov; 45(11): 2118-25. doi: 10.1016/j.fct.2007.05.010

[19] Maghfur RM, Shahat MM, Soliman MM. Assessment of The Therapeutic Role of Mangifera indica Leaves Extract in Diabetic Albino Rats. Egyptian Academic Journal of Biological Sciences, B. Zoology. 2022 Oct; 14(2): 247-62. doi: 10.21608/eajbsz.2022.265796

[20] Lee YS, Cho IJ, Kim JW, Lee MK, Ku SK, Choi JS, *et al*. Hepatoprotective effects of blue honeysuckle on CCl4-induced acute liver damaged mice. Food science & nutrition. 2019 Jan; 7(1): 322-38. doi: 10.1002/fsn3.893

[21] Dike CC, Ezeonu FC, Maduk HC, Ezeokafor EN, Okpogba AN, Oguazu EC. Hepatotherapeutic Potentials of Sida corymbosa (Wire Weed) Ethanolic Leaf Extract on Carbon Tetrachloride (CCl4)-Induced Acute Hepatotoxicity in Male Albino Rats. European Journal of Pharmaceutical and Medical Research. 2018; 5: 146-55.

[22] Hu YY, Liu CH, Wang RP, Liu C, Zhu DY, Liu P. Protective actions of salvianolic acid A on hepatocyte injured by peroxidation in vitro. World Journal of Gastroenterology. 2000 Jun; 6(3): 402. doi: 10.3748/wjg.v6.i3.402

[23] Jiang W, Gao M, Sun S, Bi A, Xin Y, Han X, *et al*. Protective effect of L-theanine on carbon tetrachloride-induced acute liver injury in mice. Biochemical and biophysical research communications. 2012 Jun; 422(2): 344-50. doi: 10.1016/j.bbrc.2012.05.022

[24] Lu Y, Hu D, Ma S, Zhao X, Wang S, Wei G, *et al*. Protective effect of wedelolactone against CCl4-induced acute liver injury in mice. International immunopharmacology. 2016 May; 34: 44-52. doi: 10.1016/j.intimp.2016.02.003

[25] Prakash T, Fadadu SD, Sharma UR, Surendra V, Goli D, Stamina P, *et al*. Hepatoprotective activity of leaves of Rhododendron arboreum in CCl4 induced hepatotoxicity in rats. Journal of Medicinal Plants Research. 2008 Nov; 2(11): 315-20.

[26] Opoku AR, Ndlovu IM, Terblanche SE, Hutchings AH. In vivo hepatoprotective effects of Rhoicissus tridentata subsp. cuneifolia, a traditional Zulu medicinal plant, against CCl4-induced acute liver injury in rats. South African Journal of Botany. 2007 Jul; 73(3): 372-7. doi: 10.1016/j.sajb.2007.02.193

[27] Zhang S, Lu B, Han X, Xu L, Qi Y, Yin L, *et al*. Protection

DOI: https://doi.org/10.54393/pbmj.v6i07.900

of the flavonoid fraction from Rosa laevigata Michx fruit against carbon tetrachloride-induced acute liver injury in mice. Food and Chemical Toxicology. 2013 May; 55: 60-9. doi: 10.1016/j.fct.2012.12.041

[28] Janbaz KH, Saeed SA, Gilani AH. Protective effect of rutin on paracetamol-and CCl4-induced hepatotoxicity in rodents. Fitoterapia. 2002 Dec; 73(7-8): 557-63. doi: 10.1016/S0367-326X(02)00217-4

DOI: https://doi.org/10.54393/pbmj.v6i07.901

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 7 (July 2023)

Original Article

Identification and Characterization of Sesquiterpene Lactones as Potential Falcipain-2 Inhibitors

Sobia Rizwana[1], Muhammad Faisal Maqbool[1], Amara Maryam[1], Ejaz Bashir[2], Muhammad Ali[3], Muhammad Khan[1*], Bushra Nisar Khan[1], Hafiz Abdullah Shakir[1] and Muhammad Irfan[4]

[1]Institute of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan
[2]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Govt. College University, Faisalabad, Pakistan
[3]National University of Medical Sciences (NUMS), Rawalpindi, Pakistan
[4]Department of Biotechnology, University of Sargodha, Sargodha, Pakistan

ARTICLE INFO

Key Words:

Sesquiterpene Lactones, Falcipain-2, Anti-Malarial Activity, Molecular Docking, Binding Energy, Inhibition Constant

How to Cite:

Rizwana, S., Maqbool, M. F., Maryam, A. ., Bashir, E. ., Khan, M. ., Nisar Khan, B. ., Shakir, H. A. ., & Irfan, M. . (2023). Identification and Characterization of Sesquiterpene Lactones as Potential Falcipain-2 Inhibitors: Sesquiterpene Lactones as Potential Falcipain-2 Inhibitors. Pakistan BioMedical Journal, 6(07). https://doi.org/10.54393/pbmj.v6i07.901

***Corresponding Author:**

Muhammad Khan
Institute of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan
mkhan.zool@pu.edu.pk

Received Date: 6th July, 2023
Acceptance Date: 27th July, 2023
Published Date: 31st July, 2023

ABSTRACT

Drug resistance affects the most effective anti-malarial medications, hence finding new, unique bioactive compounds with strong anti-malarial activity is extremely desirable. Falcipain-2 (2GHU) is a protease of *plasmodium falciparum* and considered as an important target to design antimalarial drugs. **Objective:** To identify potential novel falcipan-2 inhibitor for effect treatment of malaria. **Methods:** Molecular docking analysis was performed by using different bioinformatic tools to check the interaction between the Alantolactone and Brevilin A and falcipain-2 (2GHU). **Results:** Alantolactone and Brevilin A show a strong affinity to bind with 2GHU with binding energy values -7.2kcal/mol and -8.1kcal/mol respectively. Moreover, results of ADMET and cytotoxicity analysis showed that both investigated compounds strongly followed the Lipinski rule of five for drug-likeness and are quite safe to be used as an antimalarial drug. **Conclusions:** Both of the studied sesquiterpene lactones may inhibit falcipain-2, according to the results of our molecular docking study, but Brevilin A is predicted to be the most effective inhibitor because it forms strong hydrogen bonds with the protein's amino acid residues and has lower values for binding energy and inhibition constant. Therefore, new anti-malarial medications can be created from these two bioactive sesquiterpene lactone molecules to overcome the resistance of *plasmodium falciparum* against already clinically approved drugs.

INTRODUCTION

Malaria is a well-known parasitic infection throughout the world. As it has been almost eradicated from temperate regions, many travelers from temperate zone each year visited tropical areas, where still malaria exists as a major cause of morbidity. Eukaryotic single-celled microorganisms of the genus Plasmodium are malarial parasites. Only four parasitic species of the genus Plasmodium including, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* may infect people. *Falciparum P.* is the main cause of malaria fatalities in young children in Africa and is the agent of severe, potentially fatal malaria. By being bitten by an infected female *Anopheles* mosquito, malaria is spread [1]. *Plasmodium falciparum* is a protozoan parasite that kills at least one million children each year. Simple malaria is associated with periodic fevers and chill that mirror the

DOI: https://doi.org/10.54393/pbmj.v6i07.901

intraerythrocytic cycle. Multiple additional diseases, such as lactic acidosis, cerebral malaria (caused by infected erythrocytes adhering to the brain's endothelium), and severe anemia, are associated with severe malaria [2]. In 2010, malaria is thought to have caused 216 million illnesses and 655,000 mortalities in 108 countries with a combined population of about 3 billion people [3]. More than two billion people, or more than 40% of the world's population, is at risk of contracting malaria, and according to data collected by the World Health Organization (WHO) between 1999 and 2004, 1.1 to 1.3 million people die from malaria each year globally. Sixty percent of Pakistan's 161 million residents, or 95 million people, reside in areas where malaria is endemic [4]. Malaria re-emerged as an epidemic in the 1970s after being eradicated in the 1960s. In recent years, floods that affected almost 20 million people in over 60 districts have contributed to an increase in malaria cases [5]. The number of malaria cases reported nationwide in 2008 was 2.6 million, with a yearly fatality rate of 50,000 [4]. In 2010, the Eastern Mediterranean region reported over a million microscopy-confirmed malaria cases, 22% of which originated in Pakistan [4]. 50,000 people die from malaria-related causes each year in Pakistan, despite the existence of a well-established malaria control program, with 37% of cases reportedly occurring in areas near the Iranian and Afghan borders [6]. All the symptoms and pathologies connected with malaria are brought on by the asexual blood stage in which parasites infect the mature red blood cell [2]. Possible medications including Chloroquine and other related Quinolones (such as Hydroxychloroquine), Quinine, Primaquine, Mefloquine, Sulfonamides, and Artesunate & Artemether (Artemisinin analogs) is used to treat malaria to eliminate the parasite. But *Plasmodium falciparum* has become immune to all these medications. By dissolving erythrocyte proteins, most notably hemoglobin, Falcipain-2 (FP-2), a papain-family (C1A) cysteine protease of *Plasmodium falciparum*, greatly contributes to the development of the illness in the host. As FP-2 and its paralogs prevent parasite maturation, these proteins may make interesting targets for the development of brand-new anti-malarial drugs. The search for strong, focused, and efficient FP-2 inhibitors has been slowed down by a dearth of structural knowledge [7]. The plant has great medicinal importance due to the presence of secondary metabolites such as flavonoids, polyphenols, sesquiterpene lactones, alkaloids, etc. [8-10]. Sesquiterpene lactones are a group of secondary metabolites that are typical of the Compositae but sporadic in other angiosperm groups and even in some liverworts. Recent studies have proven that a prominent structural component of sesquiterpene lactones is an unsaturated lactone group, which has anti-tumor, cytotoxic, anti-microbial, and phytotoxic activities [11-15]. Drug designing, a branch of bioinformatics, uses molecular docking, which is the interaction of two or more molecules to create a stable adduct. Based on the ligand and target's binding properties, it can predict the three-dimensional structure of any complex. Virtual screening, bioremediation, drug discovery, protein de-orphaning, prediction of binding sites (blind docking), protein-protein interactions, mechanisms of enzymatic reactions, studies of structure-function, and protein engineering are all applications of molecular docking [16-18]. In our research, we used molecular docking to work on two sesquiterpene lactones; Alantolactone and Brevilin A, as falcipain-2 inhibitors to check whether they are effective anti-malarial drugs or not.

METHODS

Here we applied Molecular Docking analysis to find the interaction between falcipain-2 and two sesquiterpene lactones; Alantolactone and Brevilin A. AutoDock vina 4.2.1 was used for analyzing protein-ligand interaction. AutoDock mgl tool 1.5.6 and pyMOL2.4 were used for obtaining PDBQT format of protein and ligand. Finally, docking results were visualized by using Discovery Studio visualizer 2.5 and Ligplot+ 4.5.3. Molecular Docking (MD) involves the following steps. 1) Preparation of Protein Molecule. 2) Preparation of Ligand. 3) Molecular Docking protocol. 4) Visualization of Protein-Ligand complex. The following steps involve the preparation of protein for MD. The crystal structure of falcipain-2, with the PDB code of 2GHU at a resolution of 2.25 Å, was obtained from the RCSB PDB (https://www.rcsb.org/), the Research Collaboratory for Structural Bioinformatics Protein Data Bank. The structure was downloaded and saved in the protein data bank file format (PDB). Afterward, the protein was prepared by using "Autodock MGL tool (ADMGLT). Polar hydrogens were added, Kollman charges were injected, and water molecules were removed. Finally, the protein's PDB format was changed to the PDBQT format. The following steps involve the preparation of ligands for MD: The 3D crystal structure of two sesquiterpene lactones; Structure-data file (SDF) formats of alantolactone and brevilin A were downloaded from the Pubchem database on an online server (https://pubchem.ncbi.nlm.nih.gov/). For educational purposes, ligands were first translated from SDF format into PDB format using Pymol. Using ADVMGLT, the PDB format of the acquired ligands was converted into the PDBQT format required for MD. Molecular docking analysis of 2GHU with Alantolactone and Brevilin A was done by using AutoDock 4.2.1. The grid box was prepared by using 40 x 40 x 40 points in X, Y, and Z dimensions with center 51.151, 7.351, and -28.801 via ADMGLT. The ligand-

DOI: https://doi.org/10.54393/pbmj.v6i07.901

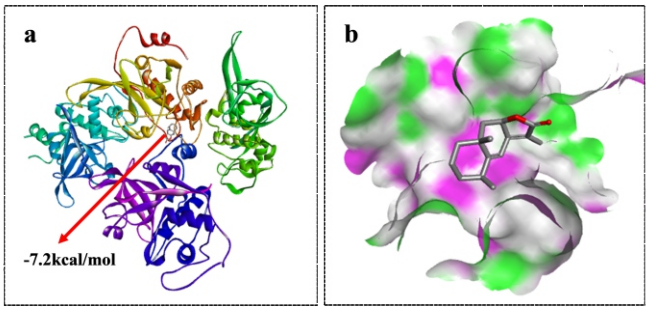
2GHU complex obtained because of MD was visualized by using Discovery Studio Visualizer. The conformation that had the lowest binding energy was considered the most stable conformation of the ligand with respect to the protein. In this work, medication similarity and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profile were predicted using a pkCSM web server. An open database server called pkCSM provides information on the pharmacokinetics of medicines. Ligand structures were retrieved from PubChem and was analyzed with pkCSMs. The toxicity ADMET module's server database was chosen to operate on [19].

RESULTS

Biova Discovery Studio Visualizer (Free verison) was used to examine the intricate interactions of the optimal conformation. The interaction of falcipain-2 and alantolactone showed that alantolactone showed hydrophobic interactions with three amino acids of falcipain-2; Pi-Sigma bond with TRP206 at a bond distance of 3.69181Å, Alkyl bond with CYS42 at a bond distance of 4.70387Å and Pi-Alkyl bond with HIS174 at a bond distance of 5.28753Å. For falcipain-2 and brevilin A interaction, we found that brevilin A showed hydrogen bonding with two amino acids of falcipain-2; ASN173 at a bond distance of 2.40334 Å and TRP206 at a bond distance of 2.50892Å as mentioned in Table 1 and shown in Figure 1 and Figure 2.

Table 1: Binding affinities, Inhibition constant, and interacting amino acid residues of 2GHU interacting with studied Sesquiterpene lactones

Ligands (Sesquiterpene Lactones)	Binding Energy	Inhibition Constant	Interacting Amino Acid Residues	
	(kcal/mol)	(μM)	H-bonds	Hydrophobic interactions
Alantolactone	-7.2	5.205	-	TRP206 (3.69181Å) CYS42 (4.70387Å) HIS174 (5.28753Å)
Brevilin A	-8.1	1.136	ASN173 (2.40334 Å) TRP206 (2.50892 Å)	-



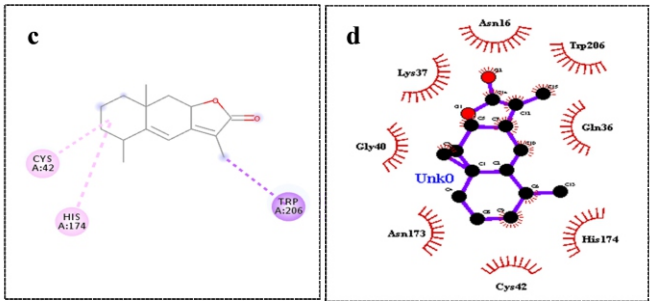


Figure 1: Alantolactone and 2GHUinteraction. (a) Output of AutoDock Vina visualized by Discovery Studio Visualizer shows the interaction of binding-site residues of chain A of 2GHU with Alantolactone. (b) Stick model showing Donor and acceptor regions of 2GHU interacting with Ligand (Pink color indicating H-bond donor region and green color indicating H-bond acceptor region of 2GHU). (c) Two-dimensional diagram showing the type of interaction formed between 2GHU and Alantolactone (the purple dotted line is representing the Pi-Sigma bond formed between TRP206 residue of 2GHU and Alantolactone, one light pink dotted line indicating Alkyl bond formed between CYS42 residue of 2GHU and Alantolactone, other light pink dotted line indicating Pi-Alkyl bond formed between HIS174 residue of 2GHU and Alantolactone. (d) Ligplot analysis of 2GHU –Alantolactone interaction mediated by the hydrophobic interactions (Hydrophobic interactions are shown by an arc with spokes pointing towards the ligand they are interacting with and interacting atoms of ligand with spokes radiating back)

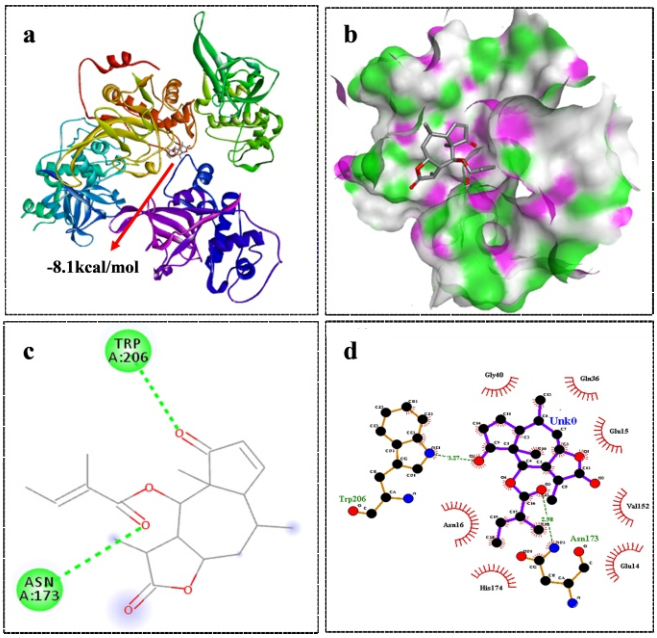


Figure 2: Brevilin A and 2GHU interaction. (a) Output of AutoDock Vina visualized by Discovery Studio Visualizer shows the interaction of binding-site residues of chain A of 2GHU with Brevilin A. (b) Stick model showing Donor and acceptor regions of 2GHU interacting with Ligand (Pink color indicating H-bond donor region and green color indicating H-bond acceptor region of 2GHU). (c) Two-dimensional diagram showing the type of interaction formed between 2GHU and Brevilin A (the green dotted lines are representing the hydrogen bonds formed

DOI: https://doi.org/10.54393/pbmj.v6i07.901

between TRP206 and ASN173 residues of 2GHU and Brevilin A. (d) Ligplot analysis of 2GHU –Brevilin A interaction mediated by the hydrogen bonds (The hydrogen bond is represented by Green amino acid residue)

No matter how well a candidate molecule binds to the receptor, if it is poorly absorbed or is eliminated from the body too slowly, it is useless. Therefore, Lipinski's rule of five was applied to forecast drug-likeness, which is always necessary. This rule states that any ligand that satisfies the criteria of molecular weight 500 Dalton, number of H-bond acceptors 10, number of H-bond donors 5, and lipophilicity expressed as logP 5 is regarded to be drug-like [19]. Our both selected ligands obeyed this rule. Predicting human pharmacokinetic qualities is crucial for the drug-designing process since it aids in the identification and advancement of potential candidate molecules for the clinic [19]. One of the crucial steps for figuring out a drug's bioavailability after oral administration is absorption in the small intestine. It is important to note that sesquiterpene lactones have intestinal absorbance values of more than 30%, indicating their ease of absorption. Additionally, both compounds had water solubility (log S) greater than 5, reflecting their solubility in water at 25 °C (Table 2) (Muhammad *et al.*, 2021). Our findings also demonstrated that none of the compounds we chose are P-glycoprotein II inhibitors, despite this being a crucial component of pharmacokinetics research. Instead, they are P-glycoprotein I inhibitors. Therefore, it may be inferred that these medications may act as secure and efficient adjuvants to any pharmaceutical medication against falcipain-2. The dosage of drug needed for even distribution in blood and plasma is shown by the steady-state volume of distribution (VDss). If Log VDss is less than 0.5, it is considered low; if it is larger than 0.45, it is considered excessive. A higher VDss value indicates that the medication is distributed more widely in the plasma than in the tissue, while a lower VDss value indicates that the drug has a weak ability to diffuse or cross the cell membrane [19]. Our results show that alantolatone has a value near 0.45 and brevilin a has value of more than -0.5. alantolactone can be good candidate in respect to distribute uniformly in blood plasma (see Table 2). Drug metabolizing enzymes can also have an impact on pharmacokinetic interactions. The human body's key detoxification enzyme, cytochrome P450, is found mostly in the liver. The pharmacokinetics of medications that are processed by these enzymes can be disturbed by any change in their activity. Cytochrome P2D6 and cytochrome P3A4 are the two most significant isoforms of cytochrome P450 [19]. Brevilin A does not inhibit these enzymes, according to Table 2, and solely interferes with the CYP3A4 substrate. Therefore, it can be inferred that brevilin A is safe when used as an adjuvant with other medications. Total clearance measures the amount of medication removed from plasma or blood. Clearance (the process of removing drugs) is produced by both the kidney and the liver. Total clearance Log (CLtot) forecasts the sum of hepatic and renal clearance. It is crucial to establish steady-state concentrations by figuring out dosing rates and is related to bioavailability. When the medicine is taken at the right concentration and is bioavailable, a steady state level is reached. The greater the CLtot value, the faster the excretion process of the compound [19]. Table 2 shows the total clearance values from which their rate of excretion can be predicted. Moreover, it can be seen that brevilin A is not the substrate of renal OCT2. Protein transporter OCT2 (organic cation transporter 2) is essential for renal medication clearance. OCT2 substrates and OCT2 inhibitors can interact and cause negative effects [19]. Protein transporter OCT2 (organic cation transporter 2) is essential for renal medication clearance. OCT2 substrates and OCT2 inhibitors can interact and cause negative effects [19]. Table 2 reveals that both compounds do not have mutagenic effects. Determining a medication's capacity to cause liver damage is a crucial component of drug development research. From Table 2, both compounds are not hepatotoxic.

Table 2: Selected ligands' ADMET analyses utilizing the pkCSM online database server

ADMET	parameters	alantolactone	Brevilin a
Absorbance	Water solubility (log S) mol/L	-3.993	-4.007
	Intestinal absorption %	97.057	99.538
	P-glycoprotein I inhibitor	Yes	Yes
	P-glycoprotein II inhibitor	No	No
Distribution	Log VDss (L/kg)	0.361	0.066
Metabolism	CYP2D6 substrate	No	No
	CYP3A4 substrate	Yes	Yes
	CYP2D6 inhibitor	No	No
	CYP3A4 inhibitor	Yes	No
Excretion	Total clearance (log ml/min/kg)	1.076	1.174
	Renal OCT2 substrate	Yes	No
Toxicity	AMES	No	No
	Max. tolerable dose (human) (log mg/kg/day)	0.042	-0.081
	Hepatotoxicity	No	No

LIPINSKY'S rule of five is a rule designed to check the drug-likeness of any compound before purposing it as a drug molecule. Results of ADME analysis show that both selected molecules strongly obeyed the LIPINSKY'S rule of five without any violation as shown in Table 3. This suggested that both molecules can be used as a drug molecule against any disease.

DOI: https://doi.org/10.54393/pbmj.v6i07.901

Table 3: Drug linkess of Brevilin A and Alantolactone

Serial Number	Standard Values of LIPINSKY'S rule	ADMET values for Brevilin A	ADMET values for Alantolactone
H-bond donors	not over 5	0	0
H-bond acceptors	not over 10	5	2
Molecular weight	less than 500 g /mol	346.42 g/mol	232.32 g/mol
High lipophilicity	LogP not over 5	2.62	3.20
Molar refractivity range	40-130	93.47	67.95

DISCUSSION

The fundamentals of rational drug design involve using structural information and understanding lignad-protein attaching operations to investigate the possibility of discovering novel therapeutic targets. Therefore, having a thorough knowledge of the nature of recognitions and interactions at the molecular level are also very important because it will provide you insights into creating, developing, and discovering new medications. For investigating interactions and patterns of binding of proteins and ligands, MD is a widely used computer method. In the current investigation, MD was carried out using AutoDock Vina, a grid-based method. According to the docking analysis, the two sesquiterpene lactones under study alantolactone and brevilin A have a strong potential to attach to chain A of the 2GHU and thereby decrease its function. To achieve better outcomes, the docking was carried out three times. For each ligand and macromolecule, the molecular docking yielded nine postures, from which the optimal position was chosen based on the affinities of the binding partners. That protein-ligand complex is considered more stable whose binding energy value is less. 2GHU strongly binds with Brevilin A by making hydrogen bonds and with Alantolactone by making hydrophobic interactions with a binding affinity value of -8.1 and -7.2 kcal/mol respectively and inhibition constant (Ki) 1.136 and 5.205µM respectively. The Ki value reveals the amount of drug required to reach the 50% inhibition. Wang *et al.*, in 2014 screened 50 natural compounds as an inhibitor of 2GHU, out of these 50 compounds, FP-2 is shown to be moderately inhibited by 10 natural products with various scaffolds, with IC50 values ranging from 3.18 to 68.19 µM [20]. These inhibitors can be divided into three classes: caffeats (compounds 1, 2 and 8 having inhibition constant values 3.18, 3.77, and 53.12µM, respectively), flavonoid glycosides (compounds 5, 6 and 10 having inhibition constant values 15.74, 17.13 and 68.19µM, respectively) and flavonoids (compounds 3, 4, 7 and 9 having inhibition constant values 5.23, 9.12, 44.81 and 56.92µM, respectively) [8]. Out of these 10 compounds, not a single compound has an inhibition constant value less than that of our best-docked compound Brevilin A having an inhibition constant value of 1.136µM. Only two compounds from caffeats (compounds 1 & 2) have inhibition constant values more than that of our second docked compound Alantolactone having an inhibition constant value of 5.205µM. One of the sesquiterpene lactones we have investigated, alantolactone, interacts with 2GHU through hydrophobic interactions with the Pi-Sigma residues TRP206, CYS42, and HIS174 at bond distances of 3.69181Å, 4.70387Å, and 5.28753Å, respectively. Brevilin A interacts with 2GHU by making hydrogen bonds with ASN173 and TRP206 residues at a bond distance of 2.40334Å and 2.50892Å, respectively, while other investigated sesquiterpene lactones do not [14]. Findings of *in silico* study clearly show that both studied sesquiterpene lactone compounds are proved to be good inhibitors of falcipain-2. Among these two compounds, Brevilin A is the best inhibitor of falcipain-2.

CONCLUSIONS

Finding new anti-malarial medications is urgently needed in light of *Plasmodium falciparum's* evolving resistance to various anti-malarial medications. Falcipain-2 is crucial to the parasite life cycle. In the current study, we used molecular docking to study two sesquiterpene lactones, alantolactone and brevilin A, as falcipain-2 inhibitors. As a result of their interactions with several residues of 2GHU, including TRP206, CYS42, HIS174, and ASN173, our findings indicated that both of the compounds under consideration might interact with the binding pocket of that molecule. Alantolactone and Brevilin A's interactions with 2GHU resulted in binding energies of -7.2 and -8.1 kcal/mol, respectively. The binding energies of alantolactone and brevilin A in association with 2GHU yielded inhibition constant values of 5.205 and 1.136 M, respectively. Our findings demonstrated the effectiveness of both substances as potent falcipain-2 inhibitors, particularly Brevilin A, which is anticipated to be the most effective inhibitor of falcipain-2 among all the natural substances investigated to date. These results should be validated by additional in vivo and in vitro research before these two bioactive sesquiterpene lactone compounds are turned into brand-new anti-malarial medications.

Authors Contribution

Conceptualization: MK, EB
Methodology: MFM, SR
Formal analysis: SR, MFM
Writing-review and editing: MK, AM, BNK, HAS, MI

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

DOI: https://doi.org/10.54393/pbmj.v6i07.901

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Snow RW, Korenromp EL, Gouws E. Pediatric mortality in Africa: *plasmodium falciparum* malaria as a cause or risk? The Intolerable Burden of Malaria II: What's New, What's Needed. American Journal of Tropical Medicine and Hygiene. 2004 Aug; 71(2): 1-10. doi: 10.4269/ajtmh.2004.71.16.

[2] Weatherall DJ, Miller LH, Baruch DI, Marsh K, Doumbo OK, Casals-Pascual C, *et al.* Malaria and the red cell. Hematology, ASH Education Program. 2002 Jan; 2002(1): 35-57. doi: 10.1182/asheducation-2002.1.35.

[3] White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp, AM. Malaria. The Lancet; 383(9918): 723-35. doi: 10.1016/S0140-6736(13)60024-0.

[4] Khattak AA, Venkatesan M, Nadeem MF, Satti HS, Yaqoob A, Strauss K, *et al*. Prevalence and distribution of human *Plasmodium* infection in Pakistan. Malaria Journal. 2013 Dec; 12(1): 1-8. doi: 10.1186/1475-2875-12-297.

[5] Williams O and Meek S. Malaria: country profiles. Department of International Development; 2011. [Last cited: 13th Jul 2023]. Available at: https://assets.publishing.service.gov.uk/media/57a08abbed915d622c00088f/malaria-country-profiles.pdf.

[6] Kakar Q, Khan MA, Bile KM. Malaria control in Pakistan: new tools at hand but challenging epidemiological realities. EMHJ-Eastern Mediterranean Health Journal. 2010; 16 (Supp. 2010), 54-60. doi: 10.26719/2010.16.Supp.54.

[7] Hogg T, Nagarajan K, Herzberg S, Chen L, Shen X, Jiang H, *et al*. Structural and functional characterization of Falcipain-2, a hemoglobinase from the malarial parasite *Plasmodium falciparum*. Journal of Biological Chemistry. 2006 Sep; 281(35): 25425-37. doi: 10.1074/jbc.M603776200.

[8] Gul S, Maqbool MF, Zheng D, Li Y, Khan M, Ma T. Alpinetin: a Dietary Flavonoid with Diverse Anticancer Effects. Applied Biochemistry and Biotechnology. 2022 Sep; 194(9): 4220-43. doi: 10.1007/s12010-022-03960-2.

[9] Maqbool MF, Ashaq A, Maryam A, Khan M, Ali MA, Shakir HA, *et al*. Unraveling the Anticancer Components of Centipeda minima and their Cellular Targets in Human Cancers. Journal of Biologically Active Products from Nature. 2022 Jan; 12(1): 42-64. doi: 10.1080/22311866.2021.2016485.

[10] Zafar E, Ahsan Z, Maqbool MF, Zaman A, Gul S, Maryam A, *et al*. Alantolactone: A Potential Multitarget Drug candidate for Prevention of SARS-CoV-2 Cell Entry. Journal of Molecular Docking. 2022 Dec; 2(2): 1-5.

[11] Rodriguez E, Towers GH, Mitchell JC. Biological activities of sesquiterpene lactones. Phytochemistry. 1976 Jan; 15(11): 1573-80. doi: 10.1016/S0031-9422(00)97430-2.

[12] Khan M, Maryam A, Saleem MZ, Shakir HA, Qazi JI, Li Y, *et al.* Brevilin A induces ROS-dependent apoptosis and suppresses STAT3 activation by direct binding in human lung cancer cells. Journal of Cancer. 2020 Apr; 11(13): 3725. doi: 10.7150/jca.40983.

[13] Saleem MZ, Nisar MA, Alshwmi M, Din SR, Gamallat Y, Khan M, *et al*. Brevilin A inhibits STAT3 signaling and induces ROS-dependent apoptosis, mitochondrial stress and endoplasmic reticulum stress in MCF-7 breast cancer cells. OncoTargets and Therapy. 2020 Jan; 13: 435-50. doi: 10.2147/OTT.S228702.

[14] Wang J, Li M, Cui X, Lv D, Jin L, Khan M, *et al*. Brevilin A promotes oxidative stress and induces mitochondrial apoptosis in U87 glioblastoma cells. OncoTargets and Therapy. 2018 Oct; 11: 7031-40. doi: 10.2147/OTT.S179730.

[15] Rasul A, Khan M, Ali M, Li J, Li X. Targeting apoptosis pathways in cancer with alantolactone and isoalantolactone. The Scientific World Journal. 2013 Jan; 2013: 248532. doi: 10.1155/2013/248532.

[16] Muhammad S, Maqbool MF, Al-Sehemi AG, Iqbal A, Khan M, Ullah S, *et al.* A threefold approach including quantum chemical, molecular docking and molecular dynamic studies to explore the natural compounds from Centaurea jacea as the potential inhibitors for COVID-19. Brazilian Journal of Biology. 2023 Sep; 83: e247604. doi: 10.1590/1519-6984.247604.

[17] Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. Current Computer-Aided Drug Design. 2011 Jun; 7(2): 146-57. doi: 10.2174/157340911795677602.

[18] Chaudhary KK and Mishra N. A review on molecular docking: novel tool for drug discovery. JSM Chemistry. 2016 Aug; 4(3): 1029. doi: 10.47739/2334-1831/1029.

[19] Muhammad S, Hassan SH, Al-Sehemi AG, Shakir HA, Khan M, Irfan M, *et al*. Exploring the new potential antiviral constituents of Moringa oliefera for SARS-COV-2 pathogenesis: An in silico molecular docking and dynamic studies. Chemical Physics Letters. 2021 Mar; 767: 138379. doi: 10.1016/j.cplett.2021.138379.

[20] Wang L, Zhang S, Zhu J, Zhu L, Liu X, Shan L, *et al.* Identification of diverse natural products as falcipain-2 inhibitors through structure-based virtual screening. Bioorganic & Medicinal Chemistry Letters. 2014 Mar; 24(5): 1261-4. doi: 10.1016/j.bmcl.2014.01.074.

DOI: https://doi.org/10.54393/pbmj.v6i07.910

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 7 (July 2023)

Original Article

Evaluating the Hematological Profile of Pregnant Women and the Role of Folic Acid Supplementation in the Third Trimester

Kainaat Zafar[1], Amina Shahid[1] and Imran Qadeer[2]

[1]Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan
[2]Sundas Foundation, Lahore, Pakistan

ARTICLE INFO

Key Words:
Hematological profile, folic acid, Third trimester

How to Cite:
Zafar, K. ., Shahid, A. ., & Qadeer, I. . (2023). Evaluating the Hematological Profile of Pregnant Women and the Role of Folic Acid Supplementation in the Third Trimester: Role of Folic Acid Supplementation in the Third Trimester. Pakistan BioMedical Journal, 6(07). https://doi.org/10.54393/pbmj.v6i07.910

***Corresponding Author:**
Kainaat Zafar
Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan
kainatzafar18@gmail.com

Received Date: 4th July, 2023
Acceptance Date: 24th July2023
Published Date: 31st July, 2023

ABSTRACT

Folic acid, the significant vitamin used as supplementation during the third trimester of pregnancy, if not provided in adequate amounts, can lead to chronic diseases. Neural tube development requires folic acid during gastrulation, and its deficiency may lead to the transformation of normal mucosa into a neoplastic condition. **Objectives:** To evaluate the pregnant woman's complete blood count (CBC) during the third trimester of pregnancy. **Methods:** Twenty-four (n=24) females were selected for the study during their third trimester of pregnancy to assess their haematological profiles by taking folic acid as a supplement. A 3-cc blood sample from the median cubital vein was taken from these females, immediately transferred to yellow-capped vacutainers and stored in ice bags. The serum was separated by centrifugation at 1000-2000 rpm for 2 minutes. The supernatant was separated as serum and transferred into vials for diagnostic tests. **Results:** The study suggested that folic acid significantly affects the woman's Complete Blood Count (CBC) profile. In short, folic acid raises the values of CBC during the third trimester. **Conclusions:** Folic acid improves haematological parameters during pregnancy.

INTRODUCTION

Folic acid supplementation has been an essential aspect of prenatal and early pregnancy care worldwide for over two centuries. This is because folic acid supplements have been found to significantly reduce the risk of fetal neural tube defects, which has been a tremendous public health success story in many countries. The incidence of these disorders has decreased significantly after the introduction of food fortification with folic acid [1]. Folic acid supplementation during pregnancy is a widely recognised dietary intervention that protects against neural tube defects and fetal developmental abnormalities. Folate deficiency during conception and pregnancy can lead to abnormal growth, making maternal supplementation a critical step to ensure healthy fetal development [2]. Health authorities recommend folic acid supplementation for conception purposes in all trimesters at the onset of pregnancy [3]. In recent years, there has been significant coverage of the relationship between folic acid supplementation and the risk of neural tube defects (NTDs) and other congenital disabilities sensitive to folic acid. During gastrulation, which occurs in the third week of pregnancy, the dorsal side of the fetus undergoes a remarkable transformation into the neural tube. The neural tube is completely closed by day 28 after conception. Women are given high doses of folic acid to develop the neural tubes to achieve a high serum volume during pregnancy. Researchers have examined the risk of neural tube defects about polymorphisms in the gene methylene

DOI: https://doi.org/10.54393/pbmj.v6i07.910

tetrahydrofolate reductase (MTHFR) [4]. MTHFR genotype, particularly MTHFR TT, influences the risk of neural tube defects by reducing folate concentrations [4]. The role of elevated homocysteine (Hcy) levels and vascular malformations as predictors of pre-eclampsia has been extensively investigated. The primary purpose of the prenatal brain is to control hypertension and prevent pre-eclampsia [5]. The lack of essential food fortification containing folate has raised concerns about cancer risks. Studies have shown that high blood folate levels can lower the risk of colon polyps or cancer. On the other hand, folate deficiency may make normal mucosa more vulnerable to neoplastic transformation [6]. Serum lactic dehydrogenase is involved in adverse drug reactions associated with folate deficiency. According to the findings of Chanarin, Mullen and Anderson in 1958, increased turnover of marrow cells can lead to folate deficiency [7]. Patients with megaloblastic anaemia showed elevated lactate dehydrogenase levels in their plasma [8]. Folate deficiency and excessive supplementation can lead to sudden changes in both mother and offspring's methylome and phenotypic characteristics. Maternal folic acid supplementation has been found to affect the development of asthma, cancer, insulin resistance, autism, and behaviour/attention problems in children. Maternal nutrition plays an important role both before and after birth. Low intake of dietary folate or plasma folate levels during the second trimester of pregnancy usually does not affect birth size. However, low folic acid intake during early pregnancy can hinder fetal brain development and lead to hyperactivity and attention problems in childhood. High levels of maternal plasma folate and serum vitamin B-12 have been associated with the development of atopic dermatitis. Finally, serum lactate dehydrogenase activity can indicate the severity of folate deficiency in haematological diseases, which experienced increased bone marrow activity [9] in pregnant women later in life. Of those who choose to have it, about 5% experience degenerative conditions, such as Crohn's disease. This condition affects folic acid absorption in the ileum, leading to potential complications during pregnancy [4]. Imbalanced folate levels, too low or too high, can cause epigenetic changes that raise concerns about potential effects on the phenotype of both mother and offspring in the short or long term [10]. High folate levels in the blood are associated with a lower risk of colon polyps and cancer. However, when administered in the presence of neoplastic foci, folic acid supplementation may induce colorectal carcinogenesis. In contrast, folate deficiency has an inhibitory effect overall [11].

METHODS

This observational study involved 24 women divided into two groups based on folic acid intake before and during pregnancy. Group A included 10 women who took folic acid regularly during their pregnancy, while Group B included 14 women unaware of the importance of folic acid intake. All participants were evaluated during their third trimester, and their blood samples were analysed using CBC markers to determine desired outcomes.

Table 1: Study design

Groups	No. of Females	Characteristics
Group A	10	Taking folic acid
Group B	14	Not taking folic acid
Total	24	

All participants were requested to sign the consent forms before blood sampling. A 3cc blood sample was taken from the median cubital vein using a 5cc syringe, then transferred immediately to the yellow-capped vacutainers and stored in ice bags. The sample was centrifuged at 1000-2000 rpm for 2 minutes to isolate serum. The supernatant was isolated as serum and then transferred to vials for diagnostic tests. Serum was used to analyse hemolytic profiles, including White Blood Cells (WBCs), Red Blood Cells (RBCs), Platelets (PLT), Hemoglobin (Hb), Hematocrit (HCT), Mean Platelet Volume (MPV), Lymphocytes, and Granulocytes. Sysmex is an automated and computerised haematology analyser designed for in vitro diagnosis of clinical conditions. It can accurately measure 17 haematological parameters like WBCs, RBCs, Hb, HCT, and PLT, making it a valuable tool for clinicians and laboratory professionals. Whole blood component testing has revealed beneficial insights into various disease states, including anaemia, leukaemia, allergic reactions, and viral, bacterial, and parasitic infections. Platelets were electronically assayed and sized using antibody detection. Hematocrit (HCT) was measured as the ratio of total RBC volume to whole blood using joint height detection, while haemoglobin is converted to methemoglobin and read photometrically at 555 nm. Statistical analysis was performed on the data of 24 participants during their 3rd trimester of pregnancy using the independent sample t-test.

RESULTS

A descriptive analysis of WBC levels was observed among the participants. The p-value (0.05) showed significant results for WBC, and the Mean and SEM for WBC of Group B participants came out to be 10.3993 ± 1.09802 while comparing the Group A participants with Group B participants, as given in Table 2.

Table 1: Study design

Parameter	Groups	N	Mean ± SE	p-value
WBC	Group A	10	5.9900 ± 0.21367	0.05
	Group B	14	10.3993 ± 1.09802	

DOI: https://doi.org/10.54393/pbmj.v6i07.910

The value of the Mean and SEM of white blood cells among Group A people came out to be ~6.0000; among Group B people, the value was between 10.0000-12.0000. The p-value (0.05) showed significant results for Group B lymphocytes, as shown in Table 3. The Mean and SEM for lymphocytes were 2.2471±0.49386 while comparing Group A with Group B participants.

Table 3: Independent sample test results for lymphocytes.

Parameter	Groups	N	Mean ± SE	p-value
Lymphocytes	Group A	10	2.2000± 0.00000	0.05
	Group B	14	2.2471±0.49386	

The p-value (0.05) showed significant results for granulocytes in patients. The Mean and SEM for granulocytes for Group B people were 6.7829±0.98279 while comparing Group B with Group A participants as given in Table 4.

Table 4: Independent sample test results for granulocytes

Parameter	Groups	N	Mean ± SE	p-value
Granulocytes	Group A	10	4.6600± 0.00000	0.05
	Group B	14	6.7829±0.98279	

The value of the Mean and SEM of granulocytes for Group A participants ranged between 4.0000-5.0000, and for Group B, the value was between 6.0000-7.0000. The p-value (0.05) showed significant results for RBCs among the participants, as shown in Table 5. The Mean and SEM for GRA was 4.1957±0.10316 for Group B while comparing Group A with Group B participants.

Table 5: Independent sample test results for RBC

Parameter	Groups	N	Mean ± SE	p-value
RBC	Group A	10	4.6000± 0.00000	0.05
	Group B	14	4.1957±0.10316	

The value of the Mean and SEM of red blood cells among Group A people was 4.6000, and in Group B, the value came out to be 4.2000. The p-value (0.05) showed significant results for RBC among Group B participants. The Mean and SEM for haemoglobin were 10.7000±0.33725 while comparing Group A with Group B participants, as given in Table 6.

Table 6: Independent sample test results for Haemoglobin

Parameter	Groups	N	Mean ± SE	p-value
Haemoglobin	Group A	10	14.9000± 0.18014	0.05
	Group B	14	1 0.7000±0.33725	

The value of the Mean and SEM of haemoglobin among Group A people ranged between 14.0000- 16.0000, and in Group B, the value was between 10.0000-12.0000.The p-value (0.05) showed significant results for HCT in Group B, as given in Table 7. The Mean and SEM for HCT were 30.4993±1.54082 while comparing Group A with Group B participants.

Table 7: Independent sample test results for HCT

Parameter	Groups	N	Mean ± SE	p-value
HCT	Group A	10	46.8400+ 0.16746	0.05
	Group B	14	30.4993±1.54082	

The Mean and SEM of HCT of Group A people ranged between 40.0000-50.0000; in Group B, the value was 30.0000. The p-value (0.05) showed significant results for PLT in Group B, as shown in Table 8. The Mean and SEM for PLT were 227.0714±18.50615 while comparing the Group A people with Group B participants.

Table 8: Independent sample test results for platelets.

Parameter	Groups	N	Mean ± SE	p-value
Platelets	Group A	10	252.8000 + 7.36025	0.05
	Group B	14	227.0714±18.50615	

The mean and seam of platelets among Group A people was 250.0000; in Group B, the value was between 200.0000-250.0000. The p-value (0.05) showed significant Mean Platelet Volume (MPV) results in Group B people. The Mean and SEM for PLT were 8.5620 + 0.10653 while comparing Group A with Group B.

Table 9: Independent sample test results for mean platelet volume(MPV)

Parameter	Groups	N	Mean ± SE	p-value
Platelets	Group A	10	8.5620 + 0.10653	0.05
	Group B	14	227.0714±18.50615	

The value of Mean and SEM of mean platelet volume among Group A people was between 8.4000-8.6000, and among Group B participants, the value was between 8.4000-8.6000, as given in Table 9.

DISCUSSION

The above investigation was intended to ponder the impact of folic acid on the haematological profile and suggested that folic acid significantly affects the CBC profile [12]. Determination of the blood folate and folic acid status aimed at posting fortification. The results showed a significant effect of folic acid on CBC, reducing the effect of anaemia [13]. Taking folic acid supplements is a common and helpful way to protect against neural tube defects and support fetal development during pregnancy. Ensuring adequate folate levels before and during pregnancy prevents abnormal growth of the child [2]. The WBCs value showed a significant effect of folic acid on CBC. The primary function of folic acid is the formation of red and white blood cells in the body. With typical pregnancy, blood volume grows, causing an affiliated hemodilution. With pregnancy, plasma volume extends furthermore, causing paleness. This outcome physiologically chopped down haemoglobin (Hb), hematocrit (Hct), and RBC values. [14]. Past investigations proposed that genetic and environmental factors added to the aetiology of extreme introvertedness [15]. A previous study discovered 95 of the

DOI: https://doi.org/10.54393/pbmj.v6i07.903

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 7 (July 2023)

Original Article

Evaluation of Lipid Profile in *H. Pylori* Infected Coronary Artery Disease Patients

Mehk Memon[1], Nosheen Aghani[1], Waseem Akram[2], Ghulam Qadir[3], Mehwish Memon[4] and Mahrish Memon[5]

[1]Department of Biochemistry, Peoples University of Medical and Health Sciences for Women, Nawabshah, Pakistan
[2]Department of Pathology, Peoples University of Medical and Health Sciences for Women, Nawabshah, Pakistan
[3]Department of Surgery, Peoples University of Medical and Health Sciences for Women, Nawabshah, Pakistan
[4]Bakhtawar Ameen Medical College, Multan, Pakistan
[5]Institute of Physiotherapy and Rehabilitation Sciences, Peoples University of Medical and Health Sciences for Women, Nawabshah, Pakistan

ARTICLE INFO

Key Words:

Triglycerides (TG), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Total Cholesterol (TG)

How to Cite:

Memon, M. ., Aghani, N. ., Akram, W. ., Qadir, G. ., Memon, M. ., & Memon, M. (2023). Evaluation of Lipid Profile in H. Pylori Infected Coronary Artery Disease Patients: Evaluation of Lipid Profile in Coronary Artery Disease Patients. Pakistan BioMedical Journal, 6(07). https://doi.org/10.54393/pbmj.v6i07.903

***Corresponding Author:**

Nosheen Aghani
Department of Biochemistry, Peoples University of Medical and Health Sciences for Women, Nawabshah, Pakistan
nosheenaghani@gmail.com

Received Date: 1st July, 2023
Acceptance Date: 24th July, 2023
Published Date: 31st July, 2023

ABSTRACT

Increase in low density lipoprotein level and decrease in high density lipoprotein level result to coronary artery disease. Metabolism of lipids regulated during host response to *H. pylori* infection. **Objective:** To analyze the serum levels of lipid profile in *H. pylori* infected coronary artery disease patients. **Methods:** It was a comparative Cross-Sectional study. This study was done at the Department of Biochemistry, Peoples University of Medical Health Sciences for Women (PUMHSW) from 1st July 2022 to 15th December 2022. A sample of 60 subjects was divided into 2 groups. Group A (Control) comprised of 30 subjects and group B (cases) of 30 subjects. 5 mL of blood from each participant was collected under aseptic conditions. For the Lipid profile, 2 mL of the blood was collected in the Gel test tubes. A Spectrophotometer was used to perform the lipid profile. *For the data analyzes SPSS Version-22.0 was used.* ***Results:*** In this study we found that *Helicobacter pylori* positive subjects have higher levels of serum LDL.C, Triglycerides and total cholesterol. The outcomes of present research showed that *H. pylori* is associated with low level of HDL-C. The present study results shown an association among *H. pylori* infection and coronary artery possibility influence. **Conclusions:** We concluded in this study that serum levels of lipid profile become worse in positive *H. pylori* infected patients as compared to the control group participants which were negative *H. pylori* with coronary artery disease.

INTRODUCTION

H. pylori is a gram-negative bacterium, initially sequestered in gastric mucosa by Marshall and Warren in 1983 [1]. *H. pylori* had known to be the cause liable of maximum of the cases of gastric mucosal damage. Mostly *H. pylori* infection does not harm but most of times they are responsible for stomach ulcer and also ulcer of small intestine [2]. Coronary artery disease progresses once the main blood vessels come to be damaged or diseased. Cholesterol comprising deposits in arteries and inflammation leads to coronary artery disease [3]. The relation of coronary artery disease and *H. pylori* infection is based on 3 main evidences. Microbial, pathological and epidemiological mechanism of postulation [4]. There are many ways in which infection organism introduce and enhances atherosclerosis. This goes through protruding invasion into vessels wall causing response of inflammation which enhances macrophages, lymphocytes, cytokines production and factors of tissue growth [5, 6].

DOI: https://doi.org/10.54393/pbmj.v6i07.903

Lipopolysaccharides releases endotoxins due to systemic effect of infection cause release of lipopolysaccharides which ultimately damage to epithelium increase in cytokines with enhancing inflammatory parameters and stimulate procoagulants which leads to ischemia and thrombosis which all predisposes towards coronary artery disease [7]. Increase in low density lipoprotein level and decrease in high density lipoprotein level result to coronary artery disease. Metabolism of lipids regulated during host response to *H. pylori* infection [8]. Lipids show host defense with lipoproteins for infectious particles like endotoxins. This is mediated through cytokines like TNF a, interleukin 6, interleukin1 and interferon. Cytokines also decreases lipoprotein lipase activity and clearance of triglycerides and also increase very low-density lipoprotein levels [9].

METHODS

This study was done at the Department of Biochemistry Peoples University of Medical Health Sciences for Women Nawabshah Shaheed Benazirabad (PUMHSW) from 1st July 2022 to 15th December 2022 along the cooperation with Medicine OPD/Ward PMCH. The analysis of sample had been done at diagnostic and research laboratory PUMHSW, Shaheed Benazirabad (SBA). Study design was comparative cross sectional. Sample technique was non probability purposive sampling. Both male and female subjects were included in the study which were diagnosed cases of Myocardial infarction from age 35 to 65 years with *H. pylori* infection and coronary artery disease. The patients with hepatic carcinoma, renal or hepatic failure and using drugs that affect the *H. pylori* were excluded. The sample size of study was based upon 60 subjects divided into two groups. Group A (Control) comprised of 30 participants with *H. pylori* negative coronary artery disease and group B (Cases) comprised of 30 participants with *H. pylori* positive coronary artery disease. We collected complete medical data and pertinent information from every subject through filling out a proforma. All participants gave verbal and written agreement after being informed about the study's purpose. Blood sample collection was done by venipuncture of the participants. 5 mL of blood from each participant was taken under aseptic conditions. For the Lipid profile, 2 mL of the blood was collected in the Gel test tubes. The blood was centrifuged for 10 minutes at 3500 rpm, fractionated, and conveyed to eppendorf cups before being stored at -20^{0}c until analysis. The material was allowed to reach room temperature before being utilized. A Spectrophotometer was used to perform the lipid profile. *For the data analyzes SPSS Version 22.0 was used. Results were shown as mean and standard deviation.* Total cholesterol, HDL, LDL, TAG was performed using spectrophotometer. Lipoproteins are fractioned, after centrifugation, the supernatant contains chylomicron, which may be detected using the CHOD-PAP assay and lipid clearing factor (LCF) while LDL and VLDL were fractionated and precipitated by addition of polyethylene glycol (PEG). *This study had been approved by the Review Committee of PUMHSW Nawabshah.*

RESULTS

Total 60 cases of coronary artery disease were analyzed and they were equally divided in to two groups. Group A, *H. pylori* negative (n=30) and group B, *H. pylori* positive (n=30) subjects. Table 1 shows the age distribution of the study participants. In group A the mean age of study subjects was 46.7±5.7 years. In group B the mean age of study subjects was 55.7±9.6 years. The other main finding of the study was that the *H. pylori* positive subjects were older than the negative subjects. There was statistically significant difference of age between group A and group B subjects shown in Table 1.

Table 1: Distribution of Subjects According to Age in Years n=60

Group A H. Pylori -Ve	Group B H. Pylori +Ve	t-value	p-value
46.7±5.7	55.7±9.6	16.51	0.03

The mean and standard deviation of lipid profile of study groups is shown in Table 2. The mean triglycerides of group A subjects was 177.5±77.25 while in group B the mean triglycerides were 232.01±65.53. Subjects with *H. pylori* positive have significant higher triglyceride concentrations. In group A subjects the mean low-density lipoproteins were 110.3±21.6 while in group B was 126.94±49.8. Low density lipoprotein concentration was significantly lower in subjects with *H. pylori* negative groups. The mean high-density lipoprotein level in group A subjects was 43.4±10.11 while in group B the mean high-density lipoprotein level was 40.3±11.76. Subjects with *H. pylori* positive have insignificantly decreased high density lipoproteins concentrations compared with control group. The mean total cholesterol level in group A subjects was 165.35±31.40 while in group B total cholesterol level was 179.47±46.31. Subjects with *H. pylori* positive have significantly higher total cholesterol concentrations compared with control group subjects.

Table 2: Comparison of Lipid Profile Variation in *H. Pylori* –Ve And *H. Pylori* +Ve

Variables	Group A H. Pylori –Ve	Group B H. Pylori +Ve	p-value
Triacylglycerol (mg/dl)	177.52±77.25	232.01±65.53	0.01
HDL-C (mg/dl)	43.4±10.11	40.3±11.76	0.69
LDL-C (mg/dl)	110.3±21.6	126.94±49.8	0.001
Total Cholesterol (mg/dl)	165.35±31.40	179.47±46.31	0.02

DISCUSSION

In this study we found that *Helicobacter pylori* positive subjects have higher levels of serum LDL.C, Triglycerides

DOI: https://doi.org/10.54393/pbmj.v6i07.903

and total cholesterol. These results are supported by Kucukazman *et al.*, who found total cholesterol and LDL concentrations increased in *H. pylori* positive patients than in negative *H. pylori* patients [10]. Similarly Sung *et al.*, also reported the increased levels of TG, TC and LDL but decreased levels of HDL-C in *H. pylori* infected patients [11]. The results of present study showed that *H. pylori* is associated with low level of HDL-C. Hoffmesister *et al.*, and Takashima *et al.*, demonstrated that *H. pylori* causes low HDL-C level [12, 13]. The results of present study consistent to the study done by Maleki*et al.*, who found in his study that there is a relationship in *H. pylori* infection and cardiovascular diseases [14]. Higher occurrence of *H. pylori* was found among CAD patients. When related to *H. pylori* triglyceride levels were increased in positive than that in negative cases, on the other hand HDL-C levels were in positive cases. Shimamoto *et al.*, estimated the association between *H. pylori* infection and the serum lipid profile revealed that *H. pylori* infection is positively associated with LDL-C, TC, and TG and negatively associated with HDL-C [15]. Findings of the current study showed similar results. Guzman *et al.*, reported that gastric *H. pylori* infection does not have significant relation with the presence of dyslipidemia [16]. The alteration of the serum lipid profile was discreetly higher in the patients infected by *H. pylori* but they were not statistically significant. Hissun *et al.*, reported that the serum of level of total cholesterol were significantly increased in group which had *H. pylori* positive and coronary artery disease, while in other group which had *H. pylori* positive without coronary heart disease the serum levels of LDL was significantly increased. These results are inconsistent with the present study [17]. Nam *et al.*, estimated increased low-density lipoprotein (LDL) and decreased high-density lipoprotein (HDL) than *H. pylori*-negative group which was comparative to the present study [18]. Abdu *et al.*, reported that serum LDL levels were high in *H. pylori* positive coronary artery disease patients as compared to the *H. pylori* negative patients which was similar to the present study findings [19]. The results of present study were consistent to the study of Longo-Mbenza *et al.*, who found in his study that there is a relationship in *H. pylori* infection and cardiovascular diseases [20].

CONCLUSIONS

The present study results shown a relation between *H. pylori* infection and coronary artery risk factors. *H. pylori* infection affects the development of cardiovascular disease as it introduces the chronic long-term infection in epithelium, which leads to locally and systematically inflammation. *H. pylori* infection enhances the risk of cardiovascular disease by decreasing the level of HDL concentration and it may be understood as a risk factor of developing atherosclerosis.

Authors Contribution

Conceptualization: MM[1], NA, WA
Methodology: GQ, MM[2], MM[3]
Formal analysis: GQ, MM[2], MM[3]
Writing-review and editing: MM[1], NA, WA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFRENCES

[1] Vafaeimanesh J, Hejazi SF, Damanpak V, Vahedian M, Sattari M, Seyyedmajidi M. Association of *Helicobacter pylori* infection with coronary artery disease: is Helicobacter pylori a risk factor? The Scientific World Journal. 2014 Jan; 2014: 516354. doi: 10.1155/2014/516354.

[2] Haeri M, Parham M, Habibi N, Vafaeimanesh J. Effect of *Helicobacter pylori* infection on serum lipid profile. Journal of Lipids. 2018 Jun; 2018: 6734809. doi: 10.1155/2018/6734809.

[3] Al-Ghamdi A, Jiman-Fatani AA, El-Banna H. Role of Chlamydia pneumoniae, *Helicobacter pylori* and cytomegalovirus in coronary artery disease. Pakistan Journal of Pharmaceutical Sciences. 2011 Apr; 24(2): 95-101.

[4] Temesgen GB, Menon M, Gizaw ST, Yimenu BW, Agidew MM. Evaluation of lipid profile and inflammatory marker in patients with gastric *Helicobacter pylori* infection, Ethiopia. International Journal of General Medicine. 2022 Jan; 15: 271-8. doi: 10.2147/IJGM.S345649.

[5] Abdu A, Cheneke W, Adem M, Belete R, Getachew A. Dyslipidemia and associated factors among patients suspected to have *Helicobacter pylori* infection at Jimma University Medical Center, Jimma, Ethiopia. International Journal of General Medicine. 2020 Jun; 13: 311-21. doi: 10.2147/IJGM.S243848.

[6] Nam SY, Ryu KH, Park BJ, Park S. Effects of *Helicobacter pylori* infection and its eradication on lipid profiles and cardiovascular diseases. Helicobacter. 2015 Apr; 20(2): 125-32. doi: 10.1111/hel.12182.

[7] Jukic A, Bozic D, Kardum D, Becic T, Luksic B, Vrsalovic M, *et al*. *Helicobacter pylori* infection and severity of coronary atherosclerosis in patients with

DOI: https://doi.org/10.54393/pbmj.v6i07.903

chronic coronary artery disease. Therapeutics and Clinical Risk Management. 2017 Jul; 13: 933-8. doi: 10.2147/TCRM.S142193.

[8] Ndebi ME, Guimtsop YA, Tamokou JD. The assessment of risk factors, lipid profile, uric acid and alanine aminotransferase in *Helicobacter pylori*-positive subjects. International Journal of Research in Medical Sciences. 2018 Sep; 6(9): 2889. doi: 10.18203/2320-6012.ijrms20183623.

[9] Sharma V and Aggarwal A. *Helicobacter pylori*: Does it add to risk of coronary artery disease. World Journal of Cardiology. 2015 Jan; 7(1): 19. doi: 10.4330/wjc.v7.i1.19.

[10] Kucukazman M, Yavuz B, Sacikara M, Asilturk Z, Ata N, Ertugrul DT, *et al.* The relationship between updated Sydney System score and LDL cholesterol levels in patients infected with *Helicobacter pylori*. Digestive Diseases and Sciences. 2009 Mar; 54(3): 604-7. doi: 10.1007/s10620-008-0391-y.

[11] Sung KC, Rhee EJ, Ryu SH, Beck SH. Prevalence of *Helicobacter pylori* infection and its association with cardiovascular risk factors in Korean adults. International Journal of Cardiology. 2005 Jul; 102(3): 411-7. doi: 10.1016/j.ijcard.2004.05.040.

[12] Hoffmeister A, Rothenbacher D, Bode G, Persson K, März W, Nauck MA, *et al.* Current infection with *Helicobacter pylori*, but not seropositivity to Chlamydia pneumoniae or cytomegalovirus, is associated with an atherogenic, modified lipid profile. Arteriosclerosis, Thrombosis, and Vascular Biology. 2001 Mar; 21(3): 427-32. doi: 10.1161/01.ATV.21.3.427.

[13] Takashima T, Adachi K, Kawamura A, Yuki M, Fujishiro H, Rumi MA, *et al.* Cardiovascular risk factors in subjects with *Helicobacter pylori* infection. Helicobacter. 2002 Apr; 7(2): 86-90. doi: 10.1046/j.1083-4389.2002.00064.x.

[14] Maleki VS, Zarkesh EH, Behjati M. *Helicobacter pylori's* evasion of the immune system could establish an inflammatory environment that potentially induces the development of coronary artery disease. Jundishapur Journal of Microbiology. 2013Jan; 6(3): 242-7. doi:10.5812/jjm.5038.

[15] Shimamoto T, Yamamichi N, Gondo K, Takahashi Y, Takeuchi C, Wada R, *et al.* The association of *Helicobacter pylori* infection with serum lipid profiles: An evaluation based on a combination of meta-analysis and a propensity score-based observational approach. PLoS One. 2020 Jun; 15(6): e0234433. doi: 10.1371/journal.pone.0234433.

[16] Guzman E, Montes P, Monge E. Relation between Patients with Gastric *Helicobacter Pylori* Infection and Dyslipidemia. Journal of Gastrointestinal Disorders and Liver Function. 2015 Oct, 1(1): 1-4. doi: 10.15436/2471-0601.15.004.

[17] Hissun Y, Modawe GA, Abdrabo AE. Association between *H. pylori* and coronary heart disease among Sudanese patients. International Journal of Informative Research and Review. 2016 Jan; 3: 1589-93.

[18] Nam SY, Ryu KH, Park BJ, Park S. Effects of *Helicobacter pylori* infection and its eradication on lipid profiles and cardiovascular diseases. Helicobacter. 2015 Apr; 20(2): 125-32. doi: 10.1111/hel.12182.

[19] Abdu A, Cheneke W, Adem M, Belete R, Getachew A. Dyslipidemia and associated factors among patients suspected to have *Helicobacter pylori* infection at Jimma University Medical Center, Jimma, Ethiopia. International Journal of General Medicine. 2020 Jun; 13: 311-21. doi: 10.2147/IJGM.S243848.

[20] Longo-Mbenza B, Nsenga JN, Ngoma DV. Prevention of the metabolic syndrome insulin resistance and the atherosclerotic diseases in Africans infected by *Helicobacter pylori* infection and treated by antibiotics. International Journal of Cardiology. 2007 Oct; 121(3): 229-38. doi: 10.1016/j.ijcard.2006.12.003.

DOI: https://doi.org/10.54393/pbmj.v6i07.867

PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
Volume 6, Issue 7 (July 2023)

Original Article

Impact of Drinking Water on People's Health and Water Borne Diseases

Qamar Yasmeen[1*] and Summaira Yasmeen[2]

[1]Department of Biochemistry, Independent Medical College (IMC), Faisalabad, Pakistan
[2]Computational Medicine-Digital Health, Berlin Institute of Health, Charité- Universitätsmedizin, Berlin, Germany

ARTICLE INFO

Key Words:
Drinking Water, Health, Water Borne Diseases, Sanitation

How to Cite:
Yasmeen, Q. ., & Yasmeen, S. . (2023). Impact of Drinking Water on People's Health and Water Borne Diseases: Impact of Drinking Water on People's Health. Pakistan BioMedical Journal, 6(07). https://doi.org/10.54393/pbmj.v6i07.867

***Corresponding Author:**
Qamar Yasmeen
Department of Biochemistry, Independent Medical College (IMC), Faisalabad, Pakistan
qammar1@hotmail.com

Received Date: 16th May, 2023
Acceptance Date: 12th July, 2023
Published Date: 31st July, 2023

ABSTRACT

Poor water quality is a result of a variety of sources, including human, animal and industrial wastes, by consuming such unhygienic water, there is a risk of contracting waterborne diseases and infections. **Objective:** To explore impact of drinking water on health of people and related waterborne diseases due to poor quality of drinking water. **Methods:** From March 2022 to June 2022, a cross-sectional study was carried out to assess the drinking water quality and any concomitant health concerns. The areas with the highest illness ratios were selected using convenient sampling technique. Total 277 participants both male and female participated in this study. Multiple choice questions (MCQs) and rating questions on a Likert scale were the two formats used in questionnaire. **Results:** According to survey area demographic data, 84.1% of respondents were living in joint families, and 63.5% of respondents were male. However, the respondents' literacy rate was below average. Motor pumps made up the majority of the water supply (60.6%). The majority of participants (84.8%) firmly believed that the quality of the water they consume has an impact on their health. The majority of respondents (56.3%) stated that water-borne illnesses such cholera, typhus, and stomach ailments affected children in their community. **Conclusions:** It was determined that the majority of the population reported higher disease development and expressed dissatisfaction with the quality of their drinking water. Also, education and economic conditions of a person can play an important role in health management and more access to better quality of drinking water.

INTRODUCTION

Pure water is very essential vital. liquid for continuation of life on earth, around 97% water is occurring in sea (oceans) which is not benefited for drinking purpose, only 03% is (fresh) water ,2.97% which is covered by glacier and ice capes. The residual slight share of 0.3 is accessible as a surface and ground water [1]. Pure and harmless drinking water is fundamental need of human being's health. Pure & safe drinking water is too fundamental right of human's beings. (Fresh) water now-a-days a limiting reserve in maximum part of the world. In the next phase of century, it will become scarce commodity as zig zag population, urbanization and climate change is being worst in the world [2]. According to a research study, which holds the water's sample of Pakistan's 34 urbane cities. This research showed the result that the water samples are composed of bacteria, viruses, and also showed some extremely poisonous elements. 2, 000,000 people of Pakistan are affected by drinking water (contaminated) and hygienic conditions. In Pakistan nearly 4,000,000 infants, and children died every year, due to drinking of polluted water (water borne pathogens), and poor hygienic and sanitations conditions. Drinking water and poor hygienic conditions leads to diseases rate of 50% and 30%deaths in the country. The people who belong to slum urban areas have been severely stimulated by polluted water (water borne pathogens) as in Sargodha 1000 people affected by diarrhea and typhoid and other stomach diseases [3]. However (TAZA) water assets are unequally and unevenly circulated many areas of the (world) are facing enormously lack of water. Safe and pure (PANI) is basic component of

DOI: https://doi.org/10.54393/pbmj.v6i07.867

life and polluted and water borne pathogens are hazards to humanism's life. As mankind human health based on the supplies of pure, safe & satisfactory water approachable and trustworthy water's drinking supplies. Safe and pure water as well as hygienic and sanitation conditions sustainability is becoming more difficult to achieve in 21th century as boom of population is going on worst. The condition is too serious because truth is that in 1956, 67% of the worldwide populace lived in to country side parts, 33% in urban areas .1996 this transformed to 54% rural & 46 % urbane. This will lead in 2026 as 41% countryside and 59% in urban areas [4, 5]. According to latest study, diseases are the major concern and problem, upsetting the well-being worth and prestige illness by self is medicinal situation which can't be separated by communal, racial, & economics upbringings. Value of foods and drinking, foods patterns, human life style, financial conditions also approach to health centers these are entirely aspects have an impact on the patterns and the intensity of diseases in society [6]. Because of presence of various types of pollutants (organic pollutants, inorganic pollutants, pathogens, suspended solids, nutrients and agriculture pollutants, thermal, radioactive, and other pollutants) access to safe pure drinking water is lacking for most of peoples/population in Pakistan. According to a research report ratio of population who are unable to have access to safe drinkable water raised from 38.3 million to 52.8 million in ten years (2005 to 2015) [7, 8]. Deaths and disease ratio (40 and 30%) occur due to contaminated drinking water with industrial waste water and municipal sewage according to an analysis. consequently, the assessment of health risks brought on by environmental contamination by pollutants has recently received considerable attention on a global scale [9]. Presence of microorganisms (bacteria, protozoa, and virus) in drinking water in developing countries are cause of waterborne diseases (typhoid, giardiasis, intestinal worms, diarrhea, cryptosporidium infections, and gastroenteritis). It is also reason of death (90%) of small children below age 5 in developing countries like Pakistan each year. In Pakistan diarrhea a waterborne disease due to infection of pathogen in drinking water is basic reason of mortality in young children and infants [10]. Due to the country's expanding population, excessive water use is a result of household, agricultural, and industrial demands. Water quality has declined and has become dangerous and unfit for drinking as a result of widespread discharge of untreated industrial and municipal waste water in fresh water sources (streams, rivers, lakes, and ponds). Water contamination issue is rising in Pakistan at alarming rate due to excessive consumption of pesticides/insecticides and fertilizers [11].

Very few cities have been analyzed for quality check of drinking water in Pakistan. Limited data was available about drinking water quality in district Sargodha. People awareness should be assessed about quality control and contamination of water. There was ominous need of development of monitoring agencies and well-equipped labs for quality check of drinkable water. Therefore, this study was designed for determination of people's awareness about water contamination and water borne diseases.

METHODS

A cross-sectional study was carried out from March 2022 to June 2022, to assess the drinking water quality and associated health concerns. The study was conducted in three of the tehsils in the Sargodha District. This study included a total of 277 individuals, both male and female. Inclusion Criteria: Individuals of areas with low quality of drinking water and high frequency of water borne diseases for at least six months and were willing to participate in the study. Exclusion criteria: individuals who were not willing to be voluntary participants of the study. Data on water-borne infections/diseases were collected from DHQ Sargodha (the district headquarters) and other health facilities. The areas with the highest illness ratios were selected using convenient sampling technique. The questionnaire contained two types of questions: multiple choice (MCQ) questions and rating questions on a five-point Likert scale (1=strongly disagree, 2=disagree, 3=no opinion, 4=agree, 5=strongly agree). The five categories were divided into three new groups with the labels agree, disagree, and have no opinion in order to simplify statistical analysis. SPSS software (Statistical Package for the Social Sciences, version 23.0, IBM®) was used for the analysis after the data were entered into Microsoft Excel. Through Cronbach's alpha, the validity and reliability of the data were assessed. For all variables, descriptive statistics were applied using frequency and percentage.

RESULTS

Cronbach's alpha (0.73), the reliability of the data is high which indicates data is reliable. Demographic information of the survey area revealed that 63.5 % respondents were males (Table 1). Literacy rate cannot be considered up to the mark as majority of the respondents were primary and matric pass (70% and 65%) then FA, BA and MA 53%, 42 % and 45 % respectively. Most of the respondents (84.1%) respondents were living in joint family setup. Majority of the respondents (55.6%) were earning their income on daily wages basis and higher monthly income percentage was 59.6% within range of 31000-4500. Majority of the people (78%) were living in their own houses.

DOI: https://doi.org/10.54393/pbmj.v6i07.867

Table 1: Demographic characteristics of the participants

Parameters	Participants	Frequency (%)
Gender	Male	176(63.5)
	Female	101(36.5)
Age	28-32	20(7.2)
	33-37	41(14.8)
	38-42	30(10.8)
	43-47	59(21.3)
	48-52	41(14.8)
	53 or above	86(31.0)
Education	Illiterate	2(1)
	Primary	70(25.4)
	Matric	65(21.8)
	F. A	53(19.6)
	B. A	42(15.4)
	M.A and above	45(16.8)
Family Type	Joint	233(84.1)
	Nuclear	44(15.9)
Nature of job	Regular	51(18.4)
	Contract	50(18.1)
	Self-employed	22(7.9)
	Daily wages	154(55.6)
Monthly income (PKR)	16000-30000	18(6.5)
	31000-45000	165(59.6)
	46000-60000	47(17.0)
	Any other	47(17.0)
Residential status	Personal	216(78)
	On rent	15(5.4)
	Govt.	46(16.6)

According to table 2 for water consumption 60.6 % of population used electric pumps as main source of drinking water. While, 19.5 %, 19.1 % and less than 1 % of respondents used water supply, hand pump and buy from market respectively. When asked about which type of drinking water they use, majority (57.8%) replied ground water, while 35% used filtered water as drinking source and less than 1% consume bottle drinkable water

Table 2: Source and type of drinking water

Sources		Frequency (%)
Drinking water	Hand Pump	53 (19.1)
	Motor Pump	168(60.6)
	Water Supply	54(19.5)
	Buy from Market	2(0.7)
	Total	277(100)
Type of drinking water	Bottled	2(0.7)
	Filtered	97(35)
	Ground Water	160(57.8)
	Stored Tank	18(6.5)
	Total	277(100)

As shown in Figure 1. Percentage of stomach diseases (64%) due to impurity of drinking water was quite high followed by 16%, 15%, 4% and 1% typhus, cholera, skin problems and other infectious diseases respectively.

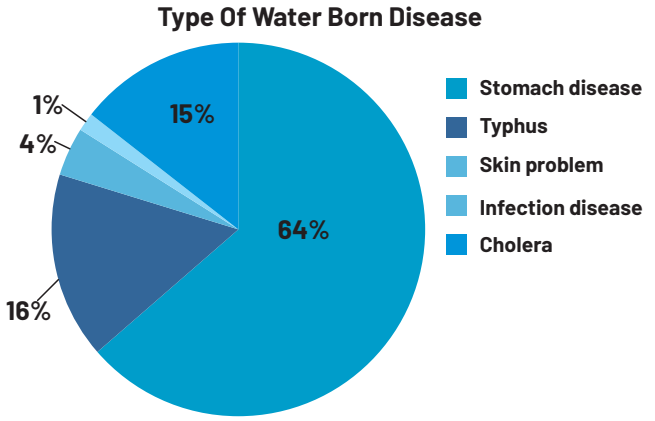


Figure 1: Percentage of water borne diseases

As per table 3, drinking water has major impact on health and health related issue in that area of district Sargodha, 95.7% of the respondents said that drinking water poor quality is the major cause of water borne diseases. 84.8% of the view that drinking water has direct impact on people's health. 37.2% were agreed that diarrhea is major disease due to poor quality of drinking water in their area. 56.3% of the respondents said that children in their area suffered from water borne diseases due to drinking water. 72.6% were of the opinion that drinking water caused stomach disease. Majority 40.1% have never testified drinking water quality from laboratories. 22% of the respondents said that there are not adequate sanitation facilities in their area. The majority of participants (66.6%) expressed dissatisfaction with the quality of the water they were drinking.

Table 3: People's perception about drinking water and its effects on health

Sources	Agree (%)	No opinion (%)	Disagree (%)
Drinking water is the major cause of waterborne diseases in your area	265(95.7)	8 (2.9)	4(1.4)
Drinking water has direct impact on one's health	235(84.8)	6(2.2)	36(13)
Diarrhea is a common disease in your area	103(37.2)	148(53.4)	26(9.3)
Typhus is a common disease in your area	79 (28.5)	137(49.5)	61(22)
children suffered from different diseases related to drinking water	156(56.3)	117(42.2)	4(1.4)
Skin related diseases are common due to drinking water	28 (10.1)	173(62.5)	76 (27.4)
Drinking water cause stomach diseases	201(72.6)	68 (24.6)	8(2.8)
You ever testified water you drink, from laboratories	40 (14.4)	126 (45.5)	111 (40.1)
Literate people are more conscious about type of their drinking water	213 (77)	32 (11.5)	32(11.2)
You are used to visit a dietician frequently	6 (2.2)	109(39.4)	162(58.5)

DOI: https://doi.org/10.54393/pbmj.v6i07.867

Government health services are easily available in your area	59 (21.3)	158(57)	60 (21.6)
Government hospitals provide satisfactory health services to patient	79 (28.5)	124(44.8)	74(26.7)
Healthy life style leads to better health	97 (35)	164(59.2)	16(5.8)
Adequate sanitations facilities are available in slum areas	53 (19.1)	163(58.8)	61(22)
Are you satisfied with quality of drinking water?	34 (12.3)	75 (27.1)	168(60.6)

DISCUSSION

The Aim of this study research was to investigate, impact of drinking water upon the health status of the people and type of water borne diseases due to low quality drinking water. The study finds out that the health of people is affected by the type of water they use and the source of water which is used for drinking water. Many epidemiological researches and outburst inquiries have also found link between poor water class and health illnesses [12, 13]. The people who use ground water or hand pump water are more vulnerable to diseases as compared to others. In the Pakistan, for example, 19 outbreaks of gastroenteritis with an infectious etiology connected with drinking water were described in the 2-year period 2001-2002 [14]. One of the objectives of the study was to describe the impact of hygienic and sanitation conditions on the health of the people. The study finds out that people have less access to hygienic and sanitation conditions. Only 25% of the population in Pakistan has access to clean drinking water, according to an analysis. It badly affects the health of people by spreading water-borne pathogens and resulting in water-borne diseases. In another study in Rawalpindi and Islamabad fecal contamination and pathogen were found in drinking water which indicate poor hygiene and sanitation conditions [15]. The study also finds out the people who had poor health had also less access to proper sanitation facilities. The study gives an insight into the impact of income level of the people onto their health. The study finds that people with high income levels have more and better approach to both, healthy Drinking water and cleanness facilities. Income which is a financial feature and have linked with diseases, illness and basic health like other factors that influence to them and have a direct link with fundamental health and illness. Poor educational techniques and skills, unhealthy residence and highly nutrition prices in the setting of low and uneven earnings creates bad health and illness [16]. Recent data from Pakistan show unequivocally that there is still a need to improve the availability of clean water and sanitary facilities for both urban and rural populations, even in the most remote places. It also emphasizes that drinking water is frequently contaminated with Salmonella, E. coli, Enterobacter, and Clostridium, but that lack of general public awareness is also a big factor in people's poor health [17 18]. In study area, Typhoid, cholera, gastro, abdominal discomfort, are major water borne diseases which respondents reported in their families. Our results were consistent with a study in district Abbottabad, where typhoid, stomach problems, skin infection, allergies and diarrheal diseases were found due poor quality of drinking water and sanitation conditions [19]. Similarly, in another analysis in Karachi, Pakistan outbreak of diseases like malaria, typhoid, diarrhea, helicobacter pylori were identified as water borne diseases due to contamination in drinking water. The situation is made worse by quickly expanding population and the general lack of knowledge among the populace regarding clean drinking water. Access to drugs and proper healthcare services is hampered by historical socioeconomic inequality and illiteracy[20].

CONCLUSIONS

According to the study, the type of drinking water, its low quality, and its unsafe sanitation conditions have an impact on people's health and are also the root of various water-borne diseases that are serious threats to both adults and children alike. A combination of economic, social, ecological, and environmental factors affect health standards, illness, and diseases. As a result, attention must be paid to both enhancing health and preventing sickness. The actions made in this regard should take into account all of the problem's aforementioned dimensions.

Authors Contribution

Conceptualization: QY, SY
Methodology: QY, SY
Formal Analysis: SY
Writing-review and editing: QY, SY

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFRENCES

[1] Zulfiqar H, Abbas Q, Raza A, Ali A. Determinants of safe drinking water in pakistan: a case study of faisalabad. Journal of Global Innovations in Agricultural and Social Sciences. 2016 Apr; 4(01): 40-5. doi: 10.17957/JGIASS/4.1.731.

[2] Zahid J. Impact of clean drinking water and sanitation on water borne diseases in Pakistan. 2018 May: 1-23.

[3] Kahlown MA and Tahir MA. Quality Analysis of Bottled

DOI: https://doi.org/10.54393/pbmj.v6i07.867

Mineral Water. PCRWR Report. Pakistan Council of Research in Water Resources, Islamabad, Pakistan. 2001.

[4] Khan FJ and Javed Y. Delivering access to safe drinking water and adequate sanitation in Pakistan. Pakistan Instiute of Development Economics. 2007 May: 1-92.

[5] Patoli AA, Patoli BB, Mehraj V. High prevalence of multi-drug resistant Escherichia coli in drinking water samples from Hyderabad. Gomal Journal of Medical Sciences. 2010 Jun; 8(1): 23-6.

[6] Khalid S, Shahid M, Dumat C, Niazi NK, Bibi I, Gul Bakhat HF, *et al*. Influence of groundwater and wastewater irrigation on lead accumulation in soil and vegetables: Implications for health risk assessment and phytoremediation. International Journal of Phytoremediation. 2017 Nov; 19(11): 1037-46. doi: 10.1080/15226514.2017.1319330.

[7] Shakoor MB, Bibi I, Niazi NK, Shahid M, Nawaz MF, Farooqi A, *et al*. The evaluation of arsenic contamination potential, speciation and hydrogeochemical behaviour in aquifers of Punjab, Pakistan. Chemosphere. 2018 May; 199: 737-46. doi: 10.1016/j.chemosphere.2018.02.002.

[8] Shakoor MB, Niazi NK, Bibi I, Murtaza G, Kunhikrishnan A, Seshadri B, *et al*. Remediation of arsenic-contaminated water using agricultural wastes as biosorbents. Critical Reviews in Environmental Science and Technology. 2016 Mar; 46(5): 467-99. doi: 10.1080/10643389.2015.1109910.

[9] Sanneh ES, Sanneh ES. Access to Safe Drinking Water. Systems Thinking for Sustainable Development: Climate Change and the Environment. Springer. 2018:25-31. doi: 10.1007/978-3-319-70585-9_3.

[10] Khalil S. Drinking Water Quality Challenges of Pakistan. Pakistan Journal of Applied Economics. 2013 Jul; 23(1): 55-66.

[11] Niazi NK, Bibi I, Shahid M, Ok YS, Burton ED, Wang H, *et al*. Arsenic removal by perilla leaf biochar in aqueous solutions and groundwater: an integrated spectroscopic and microscopic examination. Environmental Pollution. 2018 Jan; 232: 31-41. doi: 10.1016/j.envpol.2017.09.051.

[12] Ahmad M, Jamal A, Tang XW, Al-Sughaiyer MA, Al-Ahmadi HM, Ahmad F. Assessing potable water quality and identifying areas of waterborne diarrheal and fluorosis health risks using spatial interpolation in Peshawar, Pakistan. Water. 2020 Jul; 12(8): 2163. doi: 10.3390/w12082163.

[13] Soomro M, Khokhar M, Hussain W, Hussain M. Drinking water Quality challenges in Pakistan. Pakistan Council of Research in Water Resources, Lahore. 2011 Apr: 17-28.

[14] Nasir A, Nasir MS, Shauket I, Anwar S, Ayub I. Impact of samanduri drain on water resources of Faisalabad. Advances in Environmental Biology. 2016 Jan; 10(1): 155-60.

[15] Murtaza F, Muzaffar M, Mustafa T, Anwer J. Water and sanitation risk exposure in children under-five in Pakistan. Journal of Family & Community Medicine. 2021 May; 28(2): 103. doi: 10.4103/jfcm.jfcm_149_21.

[16] Tabassum RA, Shahid M, Dumat C, Niazi NK, Khalid S, Shah NS, *et al*. Health risk assessment of drinking arsenic-containing groundwater in Hasilpur, Pakistan: effect of sampling area, depth, and source. Environmental Science and Pollution Research. 2019 Jul; 26: 20018-29. doi: 10.1007/s11356-018-1276-z.

[17] Javed A and Kabeer A. Enhancing waterborne diseases in Pakistan & their possible control. American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS). 2018; 49(1): 248-56.

[18] Amin R, Zaidi MB, Bashir S, Khanani R, Nawaz R, Ali S, *et al*. Microbial contamination levels in the drinking water and associated health risks in Karachi, Pakistan. Journal of Water, Sanitation and Hygiene for Development. 2019 Jun; 9(2): 319-28. doi: 10.2166/washdev.2019.147.

[19] Jabeen S, Mahmood Q, Tariq S, Nawab B, Elahi N. Health impact caused by poor water and sanitation in district Abbottabad. Journal of Ayub Medical College Abbottabad. 2011 Mar; 23(1): 47-50.

[20] Kumar P, Arshad F, Shaheen SK, Nadeem A, Islam Z, Essar MY. Water sanitation in Karachi and its impact on health. Annals of Medicine and Surgery. 2022 May; 77: 103688. doi: 10.1016/j.amsu.2022.103688.

DOI: https://doi.org/10.54393/pbmj.v6i07.904

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 7 (July 2023)

Case Study

A Case of Lung Abscesses Secondary to Mucormycosis in a Diabetic Female Patient

Rabia Seher Alvi[1*], Kamran Khan Sumalani[1], Nausheen Saifullah[1], Sadhna Priya[1], Saifullah[1] and Rabia Javed[1]

[1]Jinnah Postgraduate Medical Center (JPMC), Karachi, Pakistan

ARTICLE INFO

Key Words:
Fungal Infections, Tuberculosis, Bronchoscopy

How to Cite:
Alvi, R. S., Sumalani, K. K. ., Saifullah, N., Priya, S. ., Saifullah, & Rabia Javed. (2023). A Case of Lung Abscesses Secondary to Mucormycosis in a Diabetic Female Patient: Lung Abscesses Secondary to Mucormycosis. Pakistan BioMedical Journal, 6(07). https://doi.org/10.54393/pbmj.v6i07.904

***Corresponding Author:**
Rabia Seher Alvi
Jinnah Postgraduate Medical Center (JPMC), Karachi, Pakistan
rabiaseheralvi@yahoo.com

Received Date: 14th May, 2023
Acceptance Date: 18th July, 2023
Published Date: 31st July, 2023

ABSTRACT

Mucormycosis is a rapidly advancing and hazardous form of opportunistic infection usually starts in nose and/or paranasal sinuses after inhaling fungal spores. This infection is caused by Mucorales fungi which belong to the Zygomycetes class. The incidence of Mucormycosis is approximately 1.7 cases in 1 million. They more frequently exist in rhinomaxillary area and is a rare pulmonary disease of an opportunistic fungi, which is difficult to diagnose with an unpredictable response to treatment. They usually appear in individuals with immunocompromised states such as diabetes, long term use of corticosteroids, immunosuppressive therapy for solid organ and hematopoietic cell transplantation, and disorders like neutropenia are notably common risk factors. As a potential for relatively life-threatening condition, the disorders warrant an anticipatory approach. We report here a case of pulmonary mucormycosis in uncontrolled diabetes presented with bilateral cavitary lesions in lungs that was first misdiagnosed as case of Pulmonary TB, later diagnosis was made on the basis of results of bronchoscopic biopsy, and was started on amphotericin and followed up with improvement of her symptoms.

INTRODUCTION

Mucormycosis is an uncommon invasive fungal infection of Mucorales class fungus, which is an order of filamentous fungi of family Mucoraceae. It is much more non frequent compared to other opportunistic infections like candida and aspergillus. There are six most common reported presentations of an infection of Mucormycosis including rhino cerebral infection, pulmonary, cutaneous or gastrointestinal infection as well as disseminated disease and some rather rare presentations [1]. Individuals who are living with Type 2 Diabetes, neutropenia, systemic administration of corticosteroids, chemotherapy, hematological malignancies (i.e. leukemia and lymphoma) are the frequent immunocompromised states in our setup [2]. Pulmonary invasion of mucormycosis mainly results from inhalation of sporangiospores of the fungi or in some cases through hematogenous and/or lymphatic spread of the microscopic organism. Presenting complains tend to be nonspecific like cough, chest pain, dyspnea, fever and hemoptysis [3]. It is somewhat difficult to make diagnosis when the lungs are involved particularly when no reliable serological test, PCR or skin tests exists for Mucormycosis. Culture specimen of some other organism also does not rule out the concurrent mucormycosis infection. When in doubt, the clinician has to rely on histopathological specimen from affected soft tissue to make the definite diagnosis [4].

CASE REPORT

A 60-year-old female, came to outpatient clinic with the

DOI: https://doi.org/10.54393/pbmj.v6i07.904

chief complaints of productive cough and hemoptysis, significant weight loss for 4 months, generalized body weakness and lethargy for 1 month. Her previous medical history includes treated case of pulmonary tuberculosis on clinical grounds and uncontrolled diabetes for five years, her average fasting sugar levels were 342mg/dl and 3-month sugars control report are 13.9% HBA1C and drug history include noncompliance to oral hypoglycemic drugs and insulin. Patient was vitally stable, admitted to pulmonology ward for further proceedings. On General Physical Examination she was pale and skinny, on Chest examination there were crepitations in the right 4th to 6th ICS and left 3rd and 4th ICS. Her CBC was requested which revealed her hemoglobin to be 9.6gm/dl, platelets in range of 590/L and total WBC count of 8700 cells/dl. Biochemical investigations showed a BUN of 14.1mg/dl and creatinine 0.7mg/dl. Chest radiograph and CT chest contrast demonstrated bilateral cavitary lesions with air fluid levels in left upper lobe and right middle lobe suggestive of lung abscesses (Figure 1 and Figure 2). Sputum workup was done for microscopy and culture that came out to be negative.

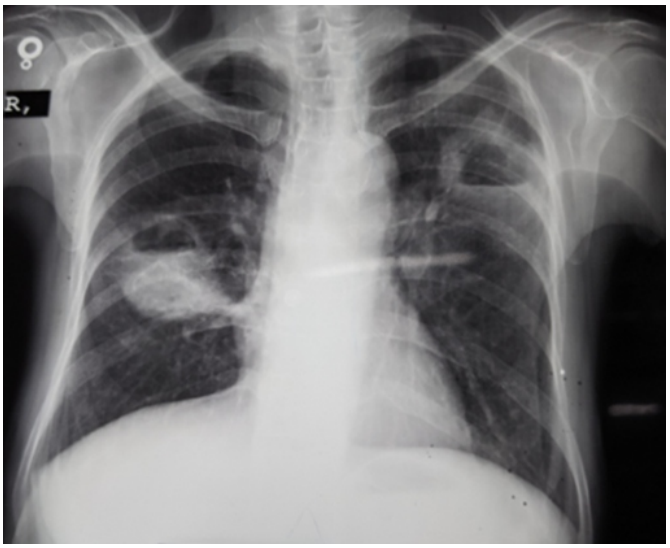


Figure 1: Shows bilateral lung abscesses

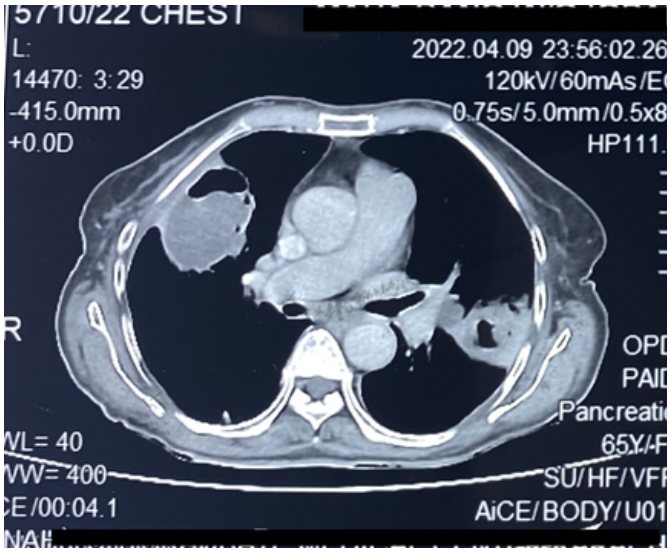


Figure 2: Demonstration of bilateral lung abscesses in the mediastinal window of CT chest

Bronchoscopy was performed that showed granulation tissue and grey white mucoid material seen in lateral segment of the right middle lobe blocking the airway lumen, left bronchial tree appeared to be normal with no sign of any endobronchial lesion or mucosal abnormalities, (Figure 3).

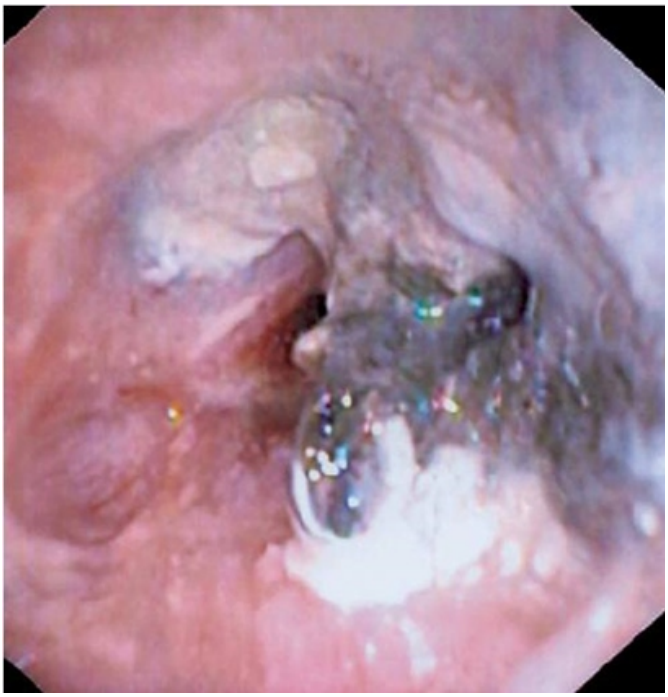


Figure 3: Granulation tissue with grey white mucoid material seen in lateral segment of right lower lobe

Bronchoalveolar lavage analysis showed inflammatory cells and degeneration of lining though cytology came out to be negative for any evidence of malignancy. Culture was also performed on cells from lavage with negative growth

DOI: https://doi.org/10.54393/pbmj.v6i07.904

after 5 days. Examination of AFB smear under light microscope, gene Xpert, AFB culture; all came out to be negative. Tissue sample were taken from lateral segment of middle lobe and sent for the pathological examination. Microscopic description showed bronchial mucosa in abundant necrotic debris containing numerous broad aseptate ribbon like fungal hyphae suggestive of Mucormycosis. Tissue culture showed no growth. After confirmation Amphotericin B 5mg/kg IV q24hr started and followed up at 6 weeks with recovery of clinical symptoms and radiologic improvement. (Figure 4 and Figure 5).

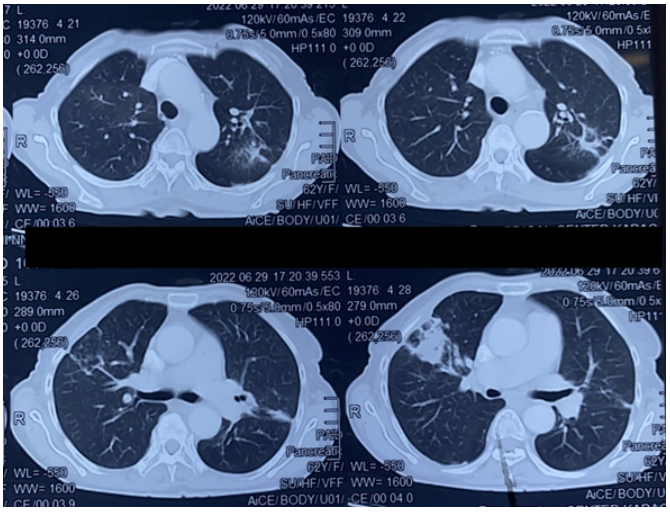


Figure 4: Radiologic improvement

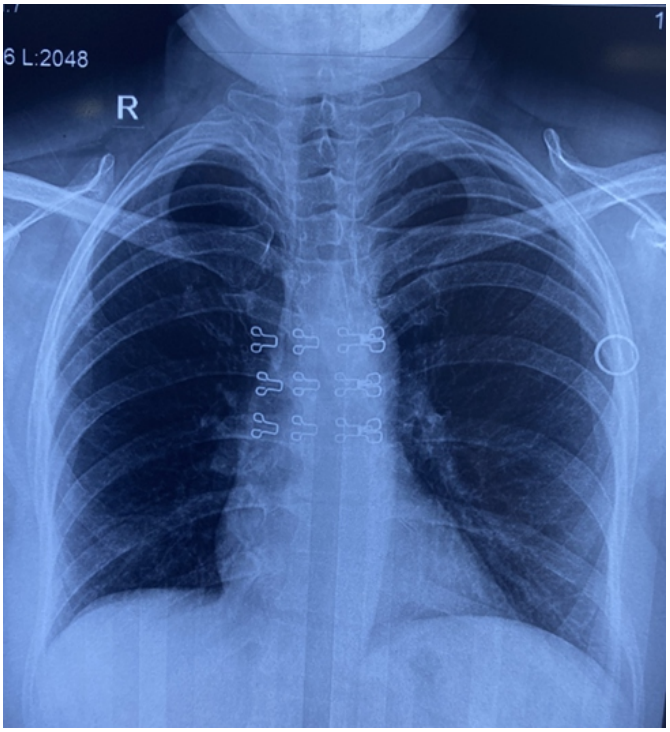


Figure 5: Follow-up chest X-ray shows dissolution of both abscesses

DISCUSSION

The present content reports the case of a middle aged diabetic female presented with chief respiratory complaints productive cough, dyspnea and weight loss with bilateral cavitary lesions on imaging and was misdiagnosed and treated as a case of pulmonary Tuberculosis but no response to the treatment although being compliant to it, admitted for bronchoscopic diagnostic workup ,that confirmed the growth of Mucorales thus was started on amphotericin and followed up with improvement of her symptoms and radiological improvements. Mucormycosis is the name given to group of infections caused by a fungus belonging to the order of Mucorales in taxonomic classification. It is highly invasive and is associated with high mortality, with the most common cause (65%) being Rhizopus [5]. Mucormycosis occurs almost exclusively in diabetic or immuno-compromised patients through inhalation of spores [6]. There is no evidence of the transmission of this organism from person to person. Pulmonary symptoms are usually nonspecific including but not always limited to cough, chest pain, dyspnea and fever. Symptoms usually occur due to endobronchial lesions, and complications related to airway obstruction. Hemoptysis usually results from vascular involvement and can sometimes be fatal [3]. Radiographically, a range of findings may be present and are mostly nonspecific or with abnormal chest radiographs result are present in >80% of patients [7]. The findings may include nonspecific pulmonary infiltrates or nodules, and cavitated lesions which appear as 'halo' sign and Reversed halo sign [9]. Cavitary lesions with or without air-crescent signs are infrequent. The air-crescent sign which appear in almost 40% of the cases, generally portrays a poor prognosis if surgical intervention is delayed [8]. Also, cavitation may be more common in COVID-19 associated mucormycosis [9]. The definitive diagnosis of mucormycosis is dependent on histopathology and direct microscopy as well as culturing the organism through various clinical pathological and biopsy specimens. However cellular identification through sputum or Broncho-alveolar Lavage is unpredictable and cytology samples may come out to be negative most of the times [10, 11]. Thus, the most common method used for diagnosis of pulmonary mucormycosis is identification of characteristic fungal hyphae through microscopic examination of specimens obtained via flexible fiber-optic bronchoscopy [12]. The hyphae appear broad, non-septate filaments with branches at right angle on histopathological identification. The differentiation can be made from Aspergillus hyphae which shows regular, septate, and acute angle branching [13]. Treatment options for pulmonary mucormycosis involves a combination of

DOI: https://doi.org/10.54393/pbmj.v6i07.904

various surgical expurgations of the involved tissues in combination with antifungal therapy. However, in addition to those, removal of predisposing factors for infection are also necessary which may include strictly controlled blood sugar levels, correction of metabolic acidosis, administration of a chelating agent like deferoxamine, and correction of immunosuppressive state due to therapy or neutropenia etc. The antifungal agent; Amphotericin B in lipid formulation given intravenously is the drug of choice for initial therapy [14]. After the desired response is obtained the step-down therapy is initiated in the form of Posaconazole or Isavuconazole. In our case, we started the patient on amphotericin B (IV) with a dose of 5mg/kg daily alongside good control of sugars after which the scans were repeated that showed resolution of cavitary lesions with satisfactory control of symptoms.

CONCLUSIONS

Mucormycosis, though uncommon, is a serious infection affecting predominantly immunocompromised population. Non resolving Pneumonia and demonstration of cavities/abscesses on chest imaging could give a clue on further evaluation of such invasive fungal infections rather than predominant diseases like pulmonary tuberculosis in our system. To ensure better prognostic outcomes early diagnosis leading to prompt treatment that may include surgery as well as antifungal agents could result in better outcome and survival. Final diagnosis should be made on pathologic evidence of the organism's septate hyphae in damaged tissue after negative growth and culture of sputum and BAL samples. The physician should always have pulmonary Mucormycosis as his differentials in all the patients presenting with non-specific chronic pulmonary symptoms with negative cultures and carry out above mentioned promising diagnostic methods to ensure administration of correct treatment in the first presentation in order to promote better management for rare fungal infections in our setup.

Authors Contribution

Conceptualization: RSA, KKS
Methodology: NS, SP S
Formal analysis: NS, SP, S
Writing-review and editing: RSA, KKS, NS, SP, S, RJ

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Pennell E, Pecson I, Nakamura C, Galvis AE. Pulmonary mucormycosis in an adolescent female with type 1 diabetes mellitus. IDCases. 2018 Jan; 14: e00474. doi: 10.1016/j.idcr.2018.e00474.

[2] Serris A, Danion F, Lanternier F. Disease entities in mucormycosis. Journal of Fungi. 2019 Mar; 5(1): 23. doi: 10.3390/jof5010023.

[3] Spellberg B, Edwards Jr J, Ibrahim A. Novel perspectives on mucormycosis: pathophysiology, presentation, and management. Clinical Microbiology Reviews. 2005 Jul; 18(3): 556-69. doi: 10.1128/CMR.18.3.556-569.2005.

[4] Mohammadi A, Mehdizadeh A, Ghasemi-Rad M, Habibpour H, Esmaeli A. Pulmonary mucormycosis in patients with diabetic ketoacidosis: a case report and review of literature. Tuberk Toraks. 2012 Jan; 60(1): 66-9. doi: 10.5578/tt.2464.

[5] Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, *et al.* Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clinical Infectious Diseases. 2005 Sep; 41(5): 634-53. doi: 10.1086/432579.

[6] Kim Y, Lee KS, Jung KJ, Han J, Kim JS, Suh JS. Halo sign on high resolution CT: findings in spectrum of pulmonary diseases with pathologic correlation. Journal of Computer Assisted Tomography. 1999 Jul; 23(4): 622-6. doi: 10.1097/00004728-199907000-00025.

[7] Donado-Uña JR, Díaz-Hellín V, López-Encuentra A, Echave-Sustaeta JM. Persistent cavitations in pulmonary mucormycosis after apparently successful amphotericin B. European Journal of Cardio-Thoracic Surgery. 2002 May; 21(5): 940-2. doi: 10.1016/S1010-7940(02)00081-7.

[8] Wang XM, Guo LC, Xue SL, Chen YB. Pulmonary mucormycosis: a case report and review of the literature. Oncology Letters. 2016 May; 11(5): 3049-53. doi: 10.3892/ol.2016.4370.

[9] Chung JH, Godwin JD, Chien JW, Pipavath SJ. Case 160: pulmonary mucormycosis. Radiology. 2010 Aug; 256(2): 667-70. doi: 10.1148/radiol.10081907.

[10] Guarner J and Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. Clinical Microbiology Reviews. 2011 Apr; 24(2): 247-80. doi: 10.1128/CMR.00053-10.

[11] Garg R, Marak RS, Verma SK, Singh J, Prasad R. Pulmonary mucormycosis mimicking as pulmonary tuberculosis: a case report. Lung India: Official Organ of Indian Chest Society. 2008 Jul; 25(3): 129. doi:

DOI: https://doi.org/10.54393/pbmj.v6i07.904

10.4103/0970-2113.59595.

[12] Lee FY, Mossad SB, Adal KA. Pulmonary mucormycosis: the last 30 years. Archives of Internal Medicine. 1999 Jun; 159(12): 1301-9. doi: 10.1001/archinte.159.12.1301.

[13] Song JG, Kang SH, Jung BW, Oh HS, Kim MJ, Lee SH. Invasive pulmonary aspergillosis histologically mimicking mucormycosis. The Ewha Medical Journal. 2016 Apr; 39(2): 65-8. doi: 10.12771/emj.2016.39.2.65.

[14] Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SC, Dannaoui E, Hochhegger B, *et al*. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. The Lancet infectious diseases. 2019 Dec; 19(12): e405-21.