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PAKISTAN BIOMEDICAL JOURNAL

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The Challenge of Communicating Biomedical Research to the Public

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Biomedical Research is a key field with the potential to significantly enhance medical results and save lives. It can be difficult to convey the results of this kind of research to the general audience, though. The public frequently has a limited knowledge of the research process and the significance of new findings, despite the significant work that researchers are performing in laboratories and clinics all around the world. Scientists and healthcare professionals are faced with a dilemma since they need to develop strategies for effectively sharing their research and interacting with the public in order to foster trust and understanding [1]. The intricacy of the topic is one of the major obstacles to effectively explaining biomedical research to the general audience. Many biomedical research studies use complex scientific terminology and technical jargon that is challenging for laypeople to comprehend. Additionally, some studies may deal with contentious subjects like gene editing or stem cell research, which might be difficult to convey in an understandable way. Scientists and healthcare professionals must discover ways to simplify complex concepts in order to make their results accessible to a larger audience [2]. The influence of the media and false information is another difficulty in explaining biomedical research to the general population. The media may be very helpful in spreading the word about new research findings, but it also has the potential to confuse and mislead the public by sensationalizing or misrepresenting study findings. Furthermore, the emergence of social media has facilitated the transmission of false information, posing difficulties for researchers who must traverse a sea of contradictory data and shifting degrees of public confidence [3]. Researchers and healthcare professionals must interact with the public in novel and creative ways in order to overcome these obstacles. For instance, they can share their study results with a larger audience through social media and other digital channels. To guarantee accurate and ethical reporting of their activities, they can also cooperate with journalists and media organizations. They can also collaborate with neighborhood associations and patient advocacy groups to foster public confidence and understanding. Overall, researchers and healthcare professionals must continually engage in and innovate to meet the issue of conveying scientific research to the general population. They can create a more educated and involved public and, in turn, enhance healthcare results and everyone's quality of life by figuring up fresh and efficient methods to share their work with the world.

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Review Article

Hypertension: Causes, Symptoms, Treatment and Prevention

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ABSTRACT

Hypertension "the silent killer is a common and significant disorder that can lead to many health complications. World health organization (WHO) declared the hypertension as the major cause of early mortality as it is directly or indirectly associated with risks of cardiovascular disorders, stroke, angina, kidney failure, diabetes and many more. The major causes of hypertension include obesity, decrease in physical activities, smoking and alcohol consumption. High blood pressure, possibly related to the age associated with the hearing impairment because of the subsequent vasoconstriction. After arthritis and hypertension, hearing loss is one of the most continual health issues of the older persons. Demographic factors and lifestyles are usually the variable factors due to which prevalence of arterial hypertension differs worldwide. These factors include nutritional habits and physical activities. A large number of antihypertensive and lipid-lowering drugs are being used to treat hypertension but it has been proved that changes in lifestyle are an easy way to treat hypertension.

INTRODUCTION

Hypertension is often called "the silent killer" because generally it has no any symptoms until major complications develop. Hypertension is a very frequent and major condition that can lead to many health problems or complicate. The risk of the cardiovascular mortality and morbidity is directly associated with hypertension. Risks of kidney failure, stroke, angina, early death or heart failure from a cause of cardiovascular are directly associated with hypertension [1]. Hypertension is estimated as one of the main issues of early death, according to World health organization (WHO).The relative risks of stroke as well as heart disease strongly associated with blood pressure. The people that aged 80 to 90 had a lower risk of 33 %. The People that aged 50 to59 had the risk of stroke (62%). High blood pressure is a risk factor for many different diseases, including heart disease, stroke, and kidney disease [2]. Hearing impairment is a very highly multifactorial process which is involving a multitude of extrinsic and intrinsic factors. A relationship between poor hearings in old age cardiovascular disease was proposed almost 40 years ago. The issue has been also discussed for almost half a century. The nature of the blood vessel and the distance from the heart are two things depend upon the blood pressure of the blood vessels. Arterial system has more blood pressure as comparable to the venous system. This is because the artery walls are very thicker and very less elastic while the vein walls are very thin and elastic. Hypertension causes the arteries to become hard and thick due to which oxygen and blood flow to the heart slows, a heart attack occurs due to the blockage of blood flow to the heart [3]. The normal range of blood pressure is 120/80 mmHg. The maximum BP of systolic BP during the ventricular systole- 120 mmHg and

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the range of systolic BP is 110-130 mm Hg. Is the least pressure of diastolic BP (DBP) is 80mmHg during the ventricular diastole and the range of 70-90 mmHg[4].

Family history is also associated with high risk of hypertension and hearing loss disease and it is reported in many studies that the risk is increased by two to seven folds due to family history of hypertension.

Causes

Obesity increases the risk of hypertension and heart diseases. Due to increase in BMI, the risk of hypertension also increases. Approximately 2.8 billion people have been reported to die due to obesity. In a study it was seen that a 10% increase in the body weight causes an increase in systolic blood pressure of about 7 mmHg [5]. Decrease in physical activities tend to have more risk of developing hypertension as compared to those who are physically fit [6].The use of tobacco is one of the major causes of hypertension. The lining of artery walls get damaged due to the chemicals that are present in tobacco. The arteries get narrow and increase the blood pressure [7]. Black have more risk of having hypertension as compared to the white. The prevalence of hypertension is about 13%in whites and about 23%in blacks[8]. The use of alcohol is also one of the risk factors for hypertension. Recent studies showed association of consumption of alcohol with hypertension and it may be a cause of essential hypertension[9].

Signs and Symptoms

High blood pressure, possibly related to the age associated with the hearing impairment because of the subsequent Vasoconstriction. Vasoconstriction of the inner ear blood vessels unfavorable effects on blood and supply oxygen in the inner part of the ear because the inner part of ear depend on oxidative metabolism, in the inner part of the ear the removal of oxygen is via to produce insufficiency in auditory sensitivity[10]. At least 28 million U.S. populations were hearing impairment. After arthritis and hypertension, hearing loss is one of the most continual health issues of the older persons. The effect of hearing loss on society will be increasing as baby boomers age because the age-specific prevalence of hearing loss and the number are increasing in older persons [11]. It was reported that there had a twofold accelerates in the speed at which men loss their hearing as compared to the women. It showed that gender and age are indeed related to the hearing impairment even in the groups without sign of hearing loss. It reported that males had a very significant age related drop in their hearing loss, while women did not show such patterns [12]. With aging, there are a higher number of chronic diseases. High blood pressure and hearing loss have very important prevalence in elderly populations. Since the study has shown that the arterial hypertension is an independent risk factor for the hearing loss[13].

Characteristics

Angiotensin-converting enzyme (ACE) gene is one of the entrant genes involved in rennin angiotensin-aldosterone system (RAAS). This system also involved to maintain the balance of fluid and electrolysis. The ACE enzyme involved the conversion of inactive angiotensin I into active angiotensin II. They also reduced bradykinin to sustain homeostasis of blood pressure [14]. Angiotensin I-converting enzyme (ACE) plays a vital role in the regulation of blood pressure and they consist of zinc metallopeptidase. Two types of homologous catalytic domains are present in the ACE. C-domains and N-domains, both are consisting of active catalytic sites. Which are suitable to cut bradykinin, and angiotensin I. The C-domain as compared to the N - domain of ACE is most efficient in cutting angiotensin I into vasopressor angiotensin II [15]. *ACE* is present as a membrane-bound enzyme in the different types of epithelial and endothelial cells, neuroepithelial cells and biological fluids in the form of circulating, such as amniotic, plasma and seminal fluids [16]. There are two isoforms of angiotensin converting enzyme. One of them is called somatic ACE because of its presence in the somatic tissue sit consist of very large proteins that is composed of 1300 amino acids. In the plasma membrane they are present in the soluble form, or it can be anchored through Tran's membrane domains in the plasma membrane. The other isoform is called as testicular form or germinal form. It is smaller proteins composed of 730 amino acids. Its molecular weight is 100-110KDa *ACE* gene of human is present on 17q23 chromosomes and it is about 21kb in size. Many different types of polymorphisms have been identified. There are about 160 polymorphisms whereas others are a result of a missense mutation. The most extensively studied of insertion/deletion polymorphism is present on the intron 25 and 26 exons. ACE II is a potent vasoconstrictor. It releases aldosterone by acting on the adrenal cortex and aldosterone in turn allows the kidney tubules to reabsorb more water and salts from urine [17]. The growth and proliferation of the cell are also stimulated by angiotensin11 by the help of different growth factors and cytokines [18]. The regulation of an angiotensin I-converting enzyme into angiotensin II then the cause the activation of the renin-angiotensin system which regulates the blood pressure. ACE has been associated with the cell proliferation, inflammation and angiogenesis. The most important system involved in the regulation of systemic blood pressure, glomerular filtration rate and renal blood flow is called the renin-angiotensin-aldosterone system. The renin–angiotensin system (RAS) or the renin–angiotensin–aldosterone system (RAAS) is hormone system which helps in the regulation of fluid balance and blood pressure. The angiotensin-converting

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enzyme can cut other proteins, including bradykinin. Bradykinin causes blood vessels to dilate which cause blood pressure decreases. Inactivates bradykinin, cutting by the angiotensin-converting enzyme, which help with blood pressure increase [2]. The Figure 1 showed the renin angiotensin aldosterone system.

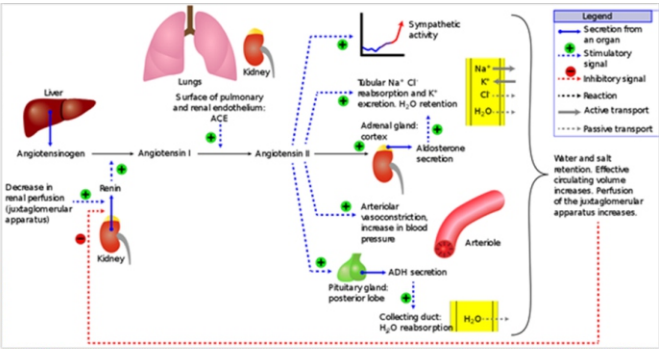

Figure 1: Renin angiotensin aldosterone system [19]

Wright and his colleagues were the first to report this polymorphism by using PCR reaction. A set of primers that flank the insertion sequence was used. Studies based on family showed that by using this PCR, there may be I/D heterozygote mistyping possibility. About 4% to 5% of I/D genotype can be mistyping, an extra PCR amplification reaction was made for confirmation of DD genotype that was obtained in first PCR, including insertion specific primer. Many studies use the combination of standard and confirmatory PCR reactions [20]. Different studies were made that association of ACE I/D polymorphism with diabetes mellitus. The Caucasians women failed to show any relationship between diabetes and ACEI/D polymorphism. As a result, *ACE* cannot be considered as an independent factor for diabetes [21]. *ACE* homologues have been found in rabbit, chimpanzee, mice and drosophila melanogaster. *ACE* gene is located in the Noncoding region due to which it becomes a functional variable. DD variant is through to be related to hypertension due to increase in level of *ACE* activity. Due to aging and abnormality in blood pressure, different variants showed different chances of developing hypertension. ACE I/D polymorphism is also associated with many other diseases such as diabetes mellitus, Alzheimer's disease, Parkinson's disease, diabetes retinopathy and cancer [21]. Two third of the United States population have controlled blood pressure at a threshold of 160/95 mmHg and the range in Canada in 49%. Spain showed 23% control rate while England showed 38%control in blood pressure [22]. The aim of this study is to determine the relationship between hearing loss and hypertension in Bahawalpur, Pakistan. In the interaction of angiotensin-converting enzyme (*ACE*) gene polymorphisms as modifiers, and the possible relationship between hearing loss and hypertension.

Classification

Basically, hypertension has two types, primary hypertension or secondary hypertension. Both types of hypertension account for about 90% of all hypertension cases. Primary hypertension is hypertension, which the cause is unknown this is also called essential hypertension. This is the most common type of hypertension [2]. Secondary hypertension is caused by another disease. It is due to hyperaldosteronism unrestricted levels of aldosterone hormone, which are causes kidneys to maintain higher amounts than normal amount of water and salts, which increases your blood pressure and increases your blood volume [23]. It is also known as renal hypertension. In this type of hypertension, once the root cause is treated, blood pressure usually returns to normal or is significantly lowered. Diseases that may be a cause for high blood pressure, alcohol addiction, thyroid dysfunction, sleep apnea, may be chronic kidney disease, and others [2]. Prehypertension is not considered as a disease, but it indicates that individuals suffering. Since it may have a risk of developing stage 3 and stage 4 hypertension [24]. There are four different types of stages of high blood pressure or hypertension [25] if your systolic blood pressure is between 140 and 159 or your diastolic pressure is between 90 and 99, you are considered to be in hypertension stage 1. If systolic blood pressure between140/90 or diastolic blood pressure is between 159/99 are considered as stage 2 or mild hypertension. If systolic blood pressure between160/100 or diastolic blood pressure is between 179/109 are considered as stage 3 or moderate hypertension. Stage 4 or severe hypertension is 180/110 or higher (Table 1).

Table 1: Classification of blood pressure [26]

Classification	Systolic BP (mmHg)	Diastolic BP (mmHg)
Stage 1 Normal	<120	<80
Stage 2 Prehypertension	120-139	80-89
Stage 3	140-159	90-99
Stage 4	>180	>110

Statistics

Demographic factors and lifestyles are usually the variable factors due to which prevalence of arterial hypertension differs worldwide. These factors include nutritional habits and physical activities. According to estimation this fluctuation is also due to geographic regions locations. In developing countries like Australia and US where literacy rate is quite high, hypertension increases from 62% to 72% [27]. In South Asian region, studies have found that most (53%, 71%, and 57%) of individuals taking medications for hypertension have uncontrolled BP in Bangladesh 53%, Pakistan 71%, and Sri Lanka 57%. The rate of hypertension is expected to rise in these developing countries due to

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sociodemographic changes and low literacy rate and availability of healthcare facilities. This indicates the necessity for instant health care actions for targeting known hurdles and for enhancing approach to required hypertension care service, particularly in low socioeconomic status communities living in South Asia [28]. In South Asia, situation is quite alarming as developed country like China is estimated to have only 8% and India having 6% control rates in administrating hypertension. Currently, 1 billion people worldwide are estimated to have hypertension (>140/90 mmHg), it is predicted that this number will increase to 1.56 billion by 2025 [29]. In Pakistan same situation can be seen as National Health Survey calculated that hypertension affects 18% of adults and 33% of adults above 45 years old. Similar report shows that in Pakistan approximately 18% of people have hypertension and one out of three people (over the age of 40) are at high risk of wide range of diseases [29].

Treatment

Different studies have provided the magnitude of treatment of hypertension. Current studies have indicated that prescription in a population has changed due to changes in the pattern of anti-hypertensive drugs. For example, in USA, immediate aggravation in the ratio of anti-hypertensive prescription for angio-tensin converting enzyme-inhibitors and blockers of calcium channel and in the same way decrease ratio of prescription for diuretics [30]. At this moment studies are going on for antihypertensive and lipid-lowering treatment for preventing heart attack. But the surveillance system has given finite data for treating hypertension by changing lifestyle to some extent. Changes in lifestyle seem an easy way to treat hypertension and it attracts health care providers and patients both. However it is very hard to maintain the aims of this therapy [31]. For accomplishing these goals food should be consumed by hypersensitive patients that have low calories and salt content. Public education campaigns should be started for promoting healthy lifestyle, for example, good nutrition, modification in alcohol intake and physical activity should be increased. Health care providers should motivate patients and at the same time ensure that alternating lifestyle modification interventions instead of pharmacological therapy should not decrease level of hypertension control in population [31].

Prevention

To eradicate all hypertension related diseases in the population, preventive measures must be taken along with the treatment. Measures for primary prevention are similar and in use for non-pharmacological treatment of hypertension. Similarly, measures like reduction of risk accompany and boost each other. Many people in community are attracted to and pursue primary prevention measures because at least some change in blood pressure will yield fundamental health benefits.

Future perspectives

Genetic diversity plays a crucial role in response generation against antihypertensive medications. Genome-wide associations studies (GWAS) have increased our awareness in identify genes associated with the development of hypertension and expecting responses against antihypertensive agents. Genomic studies can provide more knowledge in the risk assessment and progression of diseases associated with hypertension.

CONCLUSIONS

Therefore lowering of blood pressure by 2 mm Hg generally in community can have vast outcome of annual decrease in stroke, coronary heart disease and all-causes of mortality of about 6%, 4% and 3% respectively [32]. In the same way, if a hypertension patient has a 2-3 mmHg average reduction in their high normal blood pressure, then it will result in a 25-50% reduction in the occurrence of hypertension. [33] This accessible prospective for the well-being gives primary prevention of hypertension its significance and make it an important target for the community.

Conflicts of Interest

The authors declare no conflict of interest.

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Review Article

A Comprehensive Review on Therapeutic Properties of *Bombax ceiba*

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ABSTRACT

Plants have played a significant role in traditional medicine for treating a wide range of human ailments. Among the many medicinal herbs used in Unani medicine, *Bombax ceiba* Linn. has been employed for centuries. This herbaceous plant is renowned for its impressive height, reaching approximately 150 feet. It can be found in temperate and tropical regions of Australia, Africa, and, Asia with occurrences in India at altitudes of up to 1500 meters. The indigenous communities and forest dwellers extensively utilize various components of this plant, including the root, flower, gum, leaf, prickles, stem bark, fruit, seed, and heartwood, to address diverse diseases. Ethnobotanical research reveals that *Bombax ceiba* Linn. is effectively employed in the treatment of ailments such as diarrhea, boils, wounds, leprosy, acne, and various other skin conditions. Furthermore, it has been used as an anthelmintic since ancient times. Through scientific investigations, the presence of numerous beneficial properties has been confirmed in different parts of this plant, thus validating its traditional medicinal use. These properties include hypotensive, antioxidant, pain-relieving, anti-inflammatory, antipyretic, antiangiogenic, antioxidant, antibacterial, antidiabetic, hepatoprotective, anticancer, and anti-helicobacter pylori properties.

INTRODUCTION

Throughout history, nature has been an invaluable source of therapeutic agents, leading to the discovery of groundbreaking drugs derived from natural origins [1]. India, often referred to as the "Botanical Garden of the world," is renowned for its abundant variety of therapeutic plants. These plants have played a crucial role in advancing material medica, contributing significantly to human well-being and promoting a healthy, disease-free life [2, 3]. The preservation of our health is of utmost importance, and India holds a significant position in this regard. With its rich traditions in medicine and culture, India has a profound legacy of utilizing medicinal plants that continues to be respected even in modern times. The old systems of medicine in India, like Ayurveda, Unani, and Siddha, are vital in maintaining the well-being of individuals [4]. Plants have long been recognized for their remarkable biochemical capabilities and have been integral to the field of phytomedicine for centuries [4]. Different parts of plants, including bark, leaves, flowers, roots, fruits, and seeds, serve as potential sources of natural compounds with medicinal properties [1]. In the tropical regions of India Silk Cotton Tree, scientifically known as *Bombax ceiba*, holds significant importance as a medicinal plant. This tall deciduous tree exhibits a straight buttressed trunk and expansive branches. According to Ayurveda, *Bombax ceiba* is renowned for its proven medicinal properties and finds

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applications in numerous formulations. Virtually every part of this plant possesses medicinal value, with the roots and flowers being particularly noteworthy for their efficacy in treating various ailments [2]. Belonging to the Bombacaceae family, *Bombax ceiba* Linnaeus is a notable species among approximately 26 species and nearly 140 pantropical classes. It is usually referred to as Semal, Simbal, Simul, Indian kapok, Katsavar, Indian *bombax*, or Red Silk cotton tree. In India, it can be found at altitudes of up to 1500 meters. This tree thrives in both dry and moist deciduous forests, as well as in the vicinity of rivers, particularly in peninsular India. *Bombax ceiba* prefers well-drained soils, especially deep sandy loams, and is characterized as a fast-growing species that requires ample sunlight. It flourishes in regions with annual rainfall ranging from 50 to 460 centimeters, which is evenly distributed throughout the year [3]. *Bombax ceiba* is a deciduous tree that can reach heights of up to 45 meters. Its trunk is straight and is supported by buttresses measuring 1-2 meters in height. The bark has a gray color with white mottling and is approximately 20-30 mm thick. The branches are arranged horizontally in a whorled pattern. The leaves are alternately arranged and have a digitately-compound structure with small lateral stipules. The central axis of the leaf, known as the rachis, is smooth and measures 12-25 cm in length. The leaflets, usually 5-7 in number, are elliptic or elliptic-obovate in shape, with dimensions of 10-20 x 2-6 cm. The leaf margins are smooth, and the entire leaf surface is free from hairs [5]. The flowers of *Bombax ceiba* are bisexual and have a dark crimson color. They are large, with a diameter of 6-7 cm, and can appear individually or in clusters of 2-5 flowers. The calyx, which forms the outer part of the flower, has a bell-shaped structure, and its lobes measure about 3-4 x 3 cm. The outer surface of the calyx is smooth, while the inner surface is covered in silky hairs. The petals, numbering 5, are fleshy and elliptic-obovate, with measurements of 8.5-18 x 3.5-5 cm. The tree produces a substantial number of stamens, ranging from 65 to 80, which can reach lengths of 3-7.5 cm. The ovary is conical and covered in fine hairs, and it contains 5 compartments with numerous ovules. The style, the elongated part of the female reproductive structure, is longer than the stamens [6-8]. The fruit of *Bombax ceiba* is a capsule that measures 8-10 x 3 cm. It has a cylindrical shape and is covered in soft, tomentose hairs. As it matures, the capsule turns blackish and becomes glabrous. Inside the fruit, numerous seeds are present. These seeds are pyriform (pear-shaped), smooth, and have a dark brown color. They are surrounded by a distinctive white cotton-like material [5, 7].

Bombax ceiba Morphology

According to ethnobotanical surveys and traditional systems of medicine like Ayurveda, various parts of semal, including seeds fruits, roots, leaves, bark, flowers, and gum, have been identified for their medicinal properties. The Semal tree is a tall deciduous tree that can reach heights of up to 40 meters. It features horizontally spreading branches, and the young stems are adorned with hard prickles. The bark of the tree displays a grey-brown or silver-grey color and is adorned with sharp conical prickles. The leaves are large, spreading, and possess a smooth texture. They consist of lanceolate leaflets, typically numbering 3-7, with smooth margins. The flowers are abundant and characterized by their red color. They appear when the tree is leafless and are distinguished by multiple stamens arranged in 5 bundles, each containing 9 to12 stamens, along with an internal bundle of 15 stamens. The fruits resemble capsules, are brown in color, and can reach lengths of up to 15 millimeters. They contain numerous black seeds that are smooth and can vary in color, ranging from black to grey. These seeds are enveloped in extended white wool, which has an uneven obovoid shape, a smooth and oily texture, and is densely covered in silky hair. Additionally, the tree produces a gum known as semul gum, which ranges in color from light brown to opaque or dark brown [2, 4].

Figure 1: *Bombax ceiba* tree in bloom, showcasing its vibrant flowers during the months of April to May

The seeds of *Bombax ceiba* are rich in essential amino acids. The gum obtained from *Bombax ceiba* upon hydrolysis yields various sugars and derivatives. The gum can be used as a substitute for gum tragacanth. Methylated *Bombax ceiba* gum, upon hydrolysis, produces different methylated sugars. Extracts of *Bombax ceiba* flowers and their solvent fractions have undergone physicochemical and preliminary phytochemical screening using standard tests [8, 9].

Bombax as Antioxidant

Bombax ceiba, is a medicinal plant belonging to the Bombacaceae family [10]. The plant extracts of B. ceiba have been studied for their antioxidant, anti-inflammatory, and antibacterial properties. They have shown potential in managing diabetes and hyperglycemia. While certain parts

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of B. ceiba flowers are consumed in Thai culture, limited research has been conducted to explore their biological and pharmacological effects. Chemical analysis of B. ceiba leaf and flower extracts has revealed many flavonoids, saponins, tannins, terpenoids, and cardiac glycosides. Notably, the flower extracts exhibit significant concentrations of total phenolics and flavonoids. In vitro studies have demonstrated the antioxidant and anti-diabetic activities of the flower extracts, which contain phytochemicals such as anthocyanin, rutin, quercetin, and apigenin [11]. In recent years, various chemical-based tests have been developed and modified to detect antioxidant activities. Primary antioxidants play a vital role in inhibiting oxidation by interrupting chain reactions and neutralizing free radicals. Secondary antioxidants act as metal chelating agents, helping to restore primary antioxidants and stabilize singlet oxygen. These tests have diverse applications in evaluating antioxidant properties [12]. Flavonoid compounds, known as secondary natural antioxidants, have been found to exhibit stronger inhibitory effects on glucosidase and amylase compared to acarbose in vitro. The flavonoids and phenolics present in B. ceiba flower extracts have the potential to suppress the activity of glucosidase and amylase, which could aid in the management of diabetes. Additionally, a 50/50 ethanolic extract of B. ceiba stem bark and flowers has shown hypoglycemic effects, with mangiferin being attributed to the reduction of fasting blood glucose levels [12]. Nitrite, a highly toxic compound found in high levels in leafy and root vegetables, can have adverse effects such as methemoglobinemia through the oxidation of hemoglobin [13]. In a specific study, B. ceiba extract exhibited excellent scavenging properties against nitric oxide radicals. Phenolic compounds, particularly flavonoids, are known not only as scavengers of free radicals but also for their ability to stabilize nitric oxide, peroxynitrite, and reactive oxygen species during lipid peroxidation [14].

Bombax as Anti-Inflammatory

Chronic inflammation is associated with conditions like gastritis, rheumatoid arthritis, atherosclerosis, inflammatory bowel disease (IBD), and cancer. Excessive production of free reactive nitric oxide (NO) by inducible NO synthase (iNOS) can contribute to inflammatory disorders and autoimmune diseases like rheumatoid arthritis. Compounds that inhibit the overproduction of NO are often referred to as anti-inflammatory drugs. However, synthetic anti-inflammatory drugs available in the market can have significant toxic side effects and may lead to a recurrence of inflammation upon discontinuation [15]. Plants provide a diverse range of secondary metabolites with potential anti-inflammatory activity, targeting various molecular pathways. Exploring the anti-inflammatory properties of natural products offers a promising approach to developing new drugs with reduced risks of side effects [16]. *Bombax malabaricum*, a plant species, has been found to exhibit significant antifungal activity against the fungus responsible for ringworm infections when extracted from its leaves. Catechutannic acid is present in the bark gum of this plant, while mangiferin, an antioxidant and analgesic compound, has been isolated from its leaves. The roots of the plant contain several compounds, and the root bark is a source of lupeol, β-sitosterol, isohemigossypol-1-methyl ether, and 7-hydroxycadalene [16, 17].

Bombax as Antibacterial

The antibacterial activity of methanol extracts from *Salmalia malabarica* was found to be potent against multi-drug resistant strains such as *Salmonella typhi*, *Staphylococcus aureus*, *Micrococcus luteus* (Gram-positive), *Escherichia coli*, and *Pseudomonas aureginosa* (Gram-negative) bacteria [18]. The N-hexane and methanol extracts of *Bombax malabarica* flowers also exhibited significant antimicrobial activity. Gram-positive bacteria including *S. aureus*, *Bacillus cereus*, *E. coli*, and *Vibrio cholerae* showed high susceptibility to the methanol extract of *B. malabarica* flowers. Furthermore, the extract demonstrated antifungal activity against *Cryptococcus neoformans* but did not exhibit effectiveness against *C. albicans* [19]. In the case of *Bombax ceiba* extracts, certain fractions showed greater susceptibility against bacterial strains such as *B. subtilis, S. aureus, E. coli, P. aeruginosa*, as well as fungal strains *A. niger and C. albicans*. The bark extract in different solvents exhibited susceptibility against *A. niger and C. albicans*, with ethanol showing the highest activity followed by acetone and aqueous extracts. However, petroleum ether and chloroform extracts did not show any effect. *E. coli* showed the highest sensitivity among the tested microbes, with a larger zone of inhibition observed in the carbon tetrachloride fraction compared to the n-hexane and chloroform fractions [17].

Hepatoprotective Activity

The aqueous extract of *B. malabarica* has shown protective effects against CCl4-induced hepatotoxicity. Specifically, the xylem of the stem and root play a role in protecting the liver from histological changes associated with CCl4-induced damage, such as fatty degeneration, cell necrosis, ballooning necrosis, lymphocytes, and Kupffer cell aggregation. Mangiferin, isolated from *B. malabarica*, has also been found to alleviate liver damage caused by CCl4. Furthermore, mangiferin has been shown to reverse alterations in liver biochemical markers induced by antitubercular medicines (Isoniazid and Rifampicin) and paracetamol, although it does not have an impact on liver histology [20]. Excessive and chronic alcohol consumption can lead to severe liver injury. The therapeutic effects of aqueous methanol extract from *Bombax ceiba*

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(Bombacaceae) on liver steatosis were investigated in a study that lasted eight weeks and involved seven groups. One group served as the control, while the other six groups were subjected to various conditions. One group was fed a fatty diet, another received ethanol and a high-fat diet, and each group was administered the same dose of fluvastatin (2 mg/kg/d). Another group received oral administration of BCE extract (200 mg/kg/d), and the final group did not receive any treatment. The BCE extract contributed to weight loss and enhanced hepatic function in alcohol-induced liver injury. The extract significantly reduced malondialdehyde (MDA) levels and increased hepatic antioxidants. Furthermore, it led to a significant decrease in triglycerides (TG), LDL (LDL), and total cholesterol (TC) levels. Histopathological analysis revealed that BCE treatment reversed alcohol-induced fatty alterations. Phenolic compounds and flavonoids present in BCE possess anti-alcoholic, anti-steatosis, anti-inflammatory, and antioxidant properties, which contribute to its therapeutic potential [21].

Anticancer Activity

The antiproliferative activity of diethyl ether (DE) and light petroleum (PE) extracts from *Bombax ceiba* flowers was assessed against seven human cancer cell lines, including ACHN, COR-L23, A549, Caco-2, Huh-7D12, and C32 [22-25]. Both DE and PE extracts displayed strong inhibition of tumor cell viability, particularly against ACHN cells, in a dose-dependent manner. The IC50 values for PE and DE were determined as 45.5 µg/mL and 53.2 µg/mL, respectively [26]. Flavonoid-rich extracts obtained from *B. ceiba* flowers were screened for their impact on fatty acid synthase (FAS) in various cancer cells [23]. FAS is known to be overexpressed and hyperactive in certain cancers. The *B. ceiba* extract exhibited significant inhibition of FAS activity across different cancer cells. Among the tested cells, N87 gastric cancer cells exhibited the lowest FAS activity, while A549 lung cancer cells showed the highest. The flavonoid-rich extract demonstrated inhibitory effects on FAS with a minimum inhibitory concentration of 247.98 µg/mL, using A549 cells [26]. The methanolic extract of *B. ceiba* demonstrated minimal cytotoxicity in the Vero cell line based on a mitochondrial activity assay [24]. The anticancer potential of the methanol extract from *B. ceiba* root was evaluated using a brine shrimp lethality bioassay, with vincristine sulfate as the standard cytotoxic agent. The LC50 (50% mortality) and LC90 values for the crude extract were determined as 3.90 µg/mL and 150.0 µg/mL, respectively [25]. Additionally, the methanolic extract of *B. ceiba* leaves exhibited antioxidant activity and showed significant enhancements in neutrophil adhesion, carbon clearance from blood, delayed-type hypersensitivity (DTH) response, and protection against cyclophosphamide-induced myelosuppression. The extract also demonstrated increased cell death in the HL-60 cell line, as observed through the MTT assay. Furthermore, elevated caspase-3 activity and an increase in the sub-G1 population were observed in the presence of the methanolic extract of *B. ceiba* leaves [26].

Anti-Diabetic Activity

Shamimin, obtained from the leaves of *Bombax ceiba* at a dosage of 500 mg/kg, has been identified as a hypoglycemic agent in rats [27]. The hydromethanolic (2:3) extract of *Salmalia malabarica* sepals demonstrated a significant reduction in Fasting Blood Sugar and Glycated Hb (HbA1C) levels in STZ-induced diabetic rats. This extract also restored the activity of specific carbohydrate metabolic enzymes and countered the hyperactivity of glucose-6-phosphatase in the liver and skeletal muscle, which were impaired due to STZ induction. Moreover, the extract alleviated elevated oxidative stress levels and restored SGOT and SGPT levels [28]. The n-hexane fraction of this hydromethanolic extract also exhibited significant hypoglycemic and hypolipidemic effects. Additionally, the n-hexane fraction increased serum insulin levels and hemoglobin concentration while decreasing glycated hemoglobin levels. This fraction was also found to be beneficial in preserving the islets of Langerhans in diabetic rats [29]. Certain compounds present in *Bombax ceiba*, such as quercetin and epicatechin, were identified as potent inhibitors of the α-glucosidase enzyme, with inhibitory rates of 50.5% and 48.3%, respectively [30]. Furthermore, glucosylxanthone derived from the plant has been investigated as a potential target for new anti-diabetic medication, particularly due to its inhibitory effect on DPPIV. In silico binding studies revealed that glucosylxanthone and its analogues exhibited comparable binding activity to FDA-approved medicines and other compounds under research. Inhibiting DPPIV could lead to increased serum Glucagon-like Peptide-1 (GLP-1), resulting in a net hypoglycemic effect. Additionally, the aqueous (at a dose of 100 mg/kg) and ethanolic (at a dose of 200 mg/kg) extracts of *Bombax malabarica* bark demonstrated beneficial effects in alloxan-induced diabetic rats [30]. The anti-diabetic activity observed of the extract could be attributed to the antioxidant properties induced by compounds such as isoorientin, vitexin, isomangiferin, quercetin, hexoside, mangiferinisovitexin, and nigricanside [31]. In a study conducted on the therapeutic potential of a standardized extract derived from *Bombax ceiba* leaves (BCE) was investigated in rats with type 2 diabetes mellitus (T2DM) [32]. The administration of BCE resulted in a significant decrease in fasting blood glucose levels and improved oral glucose tolerance in T2DM rats. These findings highlight the excellent hypoglycemic

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effects of BCE in rats with type 2 diabetes [33, 34].

Anti-Obesity Activity

Bombax ceiba Linn. has a rich traditional history of being used to address various ailments, including diarrhea, dysentery, digestive disorders, diabetes, and imbalances of the three doshas (tridoshas). It is recognized for its positive effects on digestion and its ability to modulate insulin, leptin, and integrin signaling by stimulating PTP-1B. This stimulation results in increased fatty acid synthase (FAS) activity, which can contribute to obesity [33]. The powdered root of *B. ceiba* has demonstrated significant effects in modifying coronary risk factors, such as atherogenic lipids, fibrinogen, and oxidative stress, in individuals with ischemic heart disease. Its antioxidant activity is attributed to its high phenolics and tannins content [33]. In a study conducted on male Wistar albino rats, the anti-obesity efficacy of *Bombax ceiba* Linn. was investigated using a high-fat diet-induced obesity model. After ten weeks of being fed a high-fat diet, the rats developed experimental obesity. From the 7th to the 10th week, the rats were orally administered a dose of 100 mg/kg of *B. ceiba* extract and 50 mg/kg of gemfibrozil. Significant result shows in this study. However, treatment with *B. ceiba* extract and gemfibrozil effectively reduced these obesity-induced alterations. Notably, *B. ceiba* extract at doses of 200 and 400 mg/kg exhibited stronger effects compared to the conventional medicine. The study suggests that the methanolic extract of *Bombax ceiba* Linn. stem bark may have anti-obesity potential against high-fat diet-induced obesity in rats by modulating FAS and PTP-1B signaling pathways [34].

Gastrointestinal Effects

The antidiarrheal properties of the methanolic extract derived from *Bombax buonopozense* leaves were investigated. The extract was evaluated for its effects on diarrhea, enteropooling, and intestinal transit in rats. *Bombax buonopozense* demonstrated a reduction in diarrhea, enteropooling, and intestinal motility in the tested rats. The estimated oral LD50 (median lethal dose) in mice was approximately 5000 mg/kg. These results suggest that the methanolic extract of *B. buonopozense* leaves contains active compounds traditionally used in Nigerian herbal medicine for the treatment of diarrhea [35, 36]. *Bombax ceiba* is renowned for its beneficial effects on gastrointestinal and urogenital disorders. Both the leaf and stem branches of *B. ceiba* exhibit ACE inhibitor, antifungal, and anticholinesterase activities [37].

Anti-Acne Effect

Bombax ceiba Linn. is widely utilized in the formulation of various cosmetics and skin preparations, finding its place in skincare products targeting skin issues like acne, pimples, and skin infections. Among its various parts, the thorns of the plant are particularly valued for their ability to combat acne and are employed in numerous acne-specific skincare formulations. An example of such a formulation is the "Himalayas" anti-acne cream, where *Bombax ceiba* Linn. plays a prominent role as a key ingredient (Jain and Verma). In an ethnopharmacological study conducted among tribal groups in Pakistan's North-West Frontier Province, the use of *B. ceiba* was recorded for treating skin ailments and traditional cosmetics. The bark of *B. ceiba* was ground and topically applied to address concerns like pimples, carbuncles, and boils [38]. *Salamalia malabarica* Schott. and Endl, commonly known as the thorn of *B. ceiba*, have also been employed in the treatment of facial acne. The alcoholic extract derived from the bark and thorns of *S. malabarica* demonstrated potent anti-acne activity against Propionibacterium acne, with a minimum inhibitory concentration (MIC) of 250 µg/ml, surpassing the MIC of the standard clindamycin. The leaf extract showed an MIC value of 500 µg/ml. Furthermore, all three extracts exhibited a reduction in P. acne-induced granulomatous inflammation in rats. The thorns of *S. malabarica* are an essential component of the polyherbal formulation "Acne-N-Pimple Cream" by Himalaya, recommended for managing acne vulgaris. Clinical observations of the cream revealed a significant decrease in the number of blackheads, whiteheads, inflamed pustules, and overall inflammation, indicating its effectiveness and safety in managing acne vulgaris [39].

CONCLUSIONS

Bombax ceiba, commonly known as *B. ceiba*, has a diverse and extensive traditional history of medicinal use. This traditional knowledge has been validated by scientific investigations, further supporting the plant's therapeutic potential. Moreover, considering its ecological and economic significance, it is crucial to emphasize the importance of conserving *B. ceiba* from an ecological standpoint. Throughout history, *B. ceiba* has been utilized in the treatment of a wide range of ailments. It has been employed for conditions such as dysentery, menorrhagia (excessive menstrual bleeding), various skin problems, hemorrhoids, snakebites, scorpion stings, boils, leucorrhoea (vaginal discharge), internal bleeding, calculus affections (stones in the body), chronic inflammation, ulcers in the bladder and kidneys, gonorrhea, hemoptysis (coughing up blood), influenza, enteritis (intestinal inflammation), pulmonary tuberculosis, cystitis (bladder inflammation), and bleeding piles.

Authors Contribution

Conceptualization: HMJ, BR, SS
Writing-review and editing: SN, NK, ZI, SAI HMJ

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Conflicts of Interest

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Original Article

Knowledge of Nurses Regarding Kidney Donation in Tertiary Care Hospital Lahore

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ABSTRACT

Globally, the majority of demographic groups are experiencing an increase in mortality rates owing to renal illness and failure. Those who agree to donate a kidney undergo the transplantation procedure. In order to lengthen life and improve quality of life, a healthy organ is transplanted into a recipient with a damaged, failing, or dysfunctional organ. **Objective:** To assess knowledge of nurses regarding kidney donation in tertiary care hospital. **Methods:** The study was a descriptive cross-sectional study. For collecting data, convenient sampling technique was used. A questionnaire was used to test nurses' knowledge about kidney donation and data were analyzed using SPSS software. **Results:** Most of the people who took the survey, 55%, said they knew little or nothing about kidney donation. 53 % of the patients had negative feelings about kidney organ donation, and there was no link between their knowledge and their feelings in this area. 36.9% of the people who took part in the research said that the fact that the recipient was a family member was the most important thing, and 68.6% said they would rather give their organ to a family member if they needed one. **Conclusions:** This study showed that nurses aren't aware of kidney donation enough and have a negative view of it. Urgent steps must be taken to change the current situation.

INTRODUCTION

All organ transplants in the United States are performed in small quantities, with the kidney being the most commonly transplanted organ. Donating organs has now become a popular way to save lives and improve the lives of people with a chance of survival. Concerns were raised regarding patient death rates. A family member's donation of one of their kidneys could have saved their lives. Consequently, it became necessary to analyze and describe the kidney donation awareness of the study participants. All organ transplants in the United States are performed in small quantities, with the kidney being the most commonly transplanted organ. Donating organs has now become a popular way to save lives and improve the lives of people with a chance of survival. Concerns were raised regarding patient death rates. A family member's donation of one of their kidneys could have saved their lives. Consequently, it became necessary to analyze and describe the kidney donation awareness of the study participants [1]. Chronic renal failure is characterized by a loss of the kidneys' ability to regulate endocrine and excretory activities, as well as a reduction in their ability to filter blood [2]. Peritoneal dialysis and hemodialysis are used to treat chronic renal insufficiency while the patient awaits a kidney transplant from a living or deceased donor. However, kidney donation is a popular treatment option for a variety of diseases. More individuals are on the waiting list than there are organs

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available for donation [3]. This surgery saves the lives of millions of individuals throughout the world by grafting an organ or part of an organ onto another individual. The process of kidney donation is contingent on the manner in which it is presented to families and the willingness of those families to consent [4]. A number of different factors contribute to the formation of the family's opinion regarding this matter. The donation of organs is met with a positive attitude and views. Positive beliefs and attitudes, for instance, might include the fact that the deceased person's family was aware of their wishes regarding kidney donation. Donating a kidney, being in possession of a donor card, and demonstrating an interest in this field [5]. Studies have shown that a person's attitude toward kidney donation can have an effect on whether or not they are willing to donate a kidney. This, along with the fact that they are knowledgeable, and the fact that they are religious, all contribute to their desire to donate organs after they pass away [6]. It is essential for medical teams to have adequate knowledge and a positive attitude toward kidney donation in order to accelerate the rate of organ donation [7]. Religious beliefs are another factor that can shape a person's perspective on organ donation. Several different faiths have put up resistance [8]. In England, the majority of kidney donations are overseen by registered nurses, and finding donors who are a good match is also considered to be a nursing responsibility. As a result, registered nurses are required to be compensated for the significant role that they play in promoting organ donation. Instruction required to comprehend the process in order to contribute to the satisfaction of the donors and their families [9]. On the other hand, claimed that a lack of understanding of the concept is one of the issues that prevents kidney transplantation. Donating a kidney is a practice that is discouraged among people. Many people, for instance, are led astray into believing that donating money will help them that their kidney will be taken out of their bodies entirely [10]. Although there are some people who are worried that their doctors will remove their kidney too soon, giving the impression that being a donor has no direct benefits, there are others who do not have these concerns [11]. In addition to all of these factors to take into account, the process is also heavily impacted by religious principles and beliefs. The act of determining whether or not one will donate an organ to a recipient. It is necessary for the nurses to acquire knowledge regarding the experiences of potential donors. The donor receives important care and treatment before, during, and after the donation process. A strong emphasis should also be placed on educational and communication components. The purpose of this research is to gauge nurses' knowledge regarding kidney transplantation. The purpose of this study is to analyses nurses' awareness, toward kidney donation, as well as to look into the factors that influence their desire or opposition to donate a kidney. The students' responses will be compared to those of the general population, which is another goal that will be achieved.

METHODS

This research was conducted in a descriptive cross-sectional study design at the University of Lahore Teaching Hospital, Lahore. The study population for this research was staff nurses working in the Medical ICU, Surgical ICU, and Dialysis wards, who were willing to participate. The data was collected using a convenient sampling technique, and the sample size was calculated to be 109 using the solvin's formula. Nurses present during the data collection were given questionnaires, which took approximately 10-15 minutes to fill. IBM SPSS Statistics version 22 was used to analyze the data, and frequencies and %ages were used to present the variables. Nurses with an MSN degree or who had already taken training related to kidney transplantation and dialysis were excluded from the study.

RESULTS

The data was analyzed by questionnaire and results are interpreted with the frequency and percentages. Table 1 depict that according to the sex majority of nurses were female 82 (74%) and 28 (25.5%) males. Age of the nurses 20-25 have 49 (44.5%) and 26-30 have 31 (28.2%) and 31-35 have 10 (9.1%) and 36-40 have 20 (18.2%). Then most of nurses were qualified as general nursing 41 (37.3%) and BScN 48 (43.6%) with clinical experience 1 to 5 year 44 (40.0%) and 6 to 11 year 45 (40.9%) and 11 to 15 year 21 (19.1%). Nurses working in surgical ICU 22 (20.0%), medical ICU 34 (30.9%), dialysis 54 (49.1%) nurses and work in other special areas of hospital wards 99 (73.9%).

Table 1: Basic Demographics

Demographic profile	Frequency (%)
Gender	
Male	28 (25.5)
Female	82 (74.5)
Professional Qualification	
General nursing	52 (47.3)
Bachelor in nursing	48 (43.6)
Master in nursing	15 (12.5)
Age	
20-25 years	49 (44.5)
26-30 years	31 (28.2)
31-35 years	20 (18.2)
36-40 years	10 (9.1)
Total years of experience in a critical area	
1-5 year	44 (40)
6-10 year	45 (40.9)
11-15 year	21 (19.1)

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Demographic profile	Frequency (%)
Working unit	
Surgical ICU	22 (20)
Medical ICU	34 (30.9)
Dialysis	54 (49.1)

The mean of the sum of attitudes scores determined attitudes: numbers below the mean were negative, while scores above the mean were positive. 84% of participants wanted to donate organs after death. Although most participants wanted to donate their organs, 43% had an unfavorable opinion toward organ donation, 39.1% had a positive attitude, and the nurses had moderate knowledge (53%). Results show that most participants dislike kidney donation(63%).

Table 2: Knowledge related questions among nurses regarding kidney donation

Description	True	False
Kidney can be donated from brain-dead patients.	48(43.6%)	62(56.4%)
Hepatitis B and C carriers can donate all of their solid organs.	50(45.5%)	60(54.5%)
Failure kidneys can be replaced by another healthy kidney.	39(35.5%)	71(64.5%)
The kidney is the most commonly donated organ.	47(42.7%)	63(57.3%)
A person can live with one kidney.	44(40.0%)	66(60.0%)
The only organs that can be donated by a live donor are kidney, pancreas and liver.	41(37.3%)	69(62.7%)
Kidney donor must be of the same ethnicity as the recipient.	48(43.6%)	62(56.4%)
Sixty-year-old male should kidney donate with malignant lung cancer and hypertension.	43(39.1%)	67(60.9%)
Only young people can donate kidney after death.	73(66.4%)	37(33.6%)
It is not necessary for person that must be on dialysis while waiting for renal donation	43(39.1%)	67(60.9%)

Table 3: Student responses on kidney donation

Questions	Strongly Agree	Agree	Neutral	Strongly Disagree	Disagree
I am willing to donate a kidney	45 (40.9%)	29 (26.4%)	11 (10.0%)	10 (9.1%)	15 (13.6%)
Renal donation might change my body after transplant	39 (35.5%)	32 (29.1%)	16 (14.5%)	11 (10.0%)	12 (10.9%)
Should kidney donation be promoted	44 (40.0%)	21 (19.1%)	20 (18.2%)	7 (6.4%)	18 (16.4%)
I feel uncomfortable to think or talk about kidney donation	43 (39.1%)	17 (15.5%)	23 (20.9%)	14 (12.7%)	13 (11.8%)
Problems that may occur after transplant prevent me from donating	32 (29%)	20 (18.2%)	15 (13.6%)	24 (21.8%)	19 (17.3%)
A person from one race can donate a kidney to another race	31 (28.2%)	22 (20.0%)	20 (18.2%)	11 (10.0%)	26 (23.6%)
I am competent and have adequate knowledge in counseling patients on issues related to kidney donation	34 (30.9%)	22 (20.0%)	23 (20.9%)	14 (12.7%)	17 (15.5%)
I believe I have learnt enough about kidney donation from the educational curriculum	26 (23%)	29 (26.4%)	24 (21.8%)	10 (9.1%)	21 (19.1%)
I think donating one's organs adds meaning to one's life	30 (27.3%)	23 (20.9%)	26 (23.6%)	16 (14.5%)	15 (13.6%)
The donor's and recipient's blood group MUST be identical	34 (30.9%)	18 (16.4%)	32 (29.1%)	9 (8.2%)	17 (15.5%)

DISCUSSION

Lahore Teaching Hospital nurses participated in this study. The study assessed nurses' kidney donation knowledge and attitudes. 44.5% of hospital patients were 26–30 years old. 65% were married, and 52.3% had a general nursing certificate. 39% of nurses had over ten years of experience, and 49.1% worked in dialysis units. 14.5% of nurses had good kidney donation awareness, 35.5% had moderate knowledge, and 50.0% had inadequate knowledge. 51% of individuals were unaware about kidney organ donation. In Africa, 44.8% of 409 medical students had sufficient understanding of organ donation, while 40.1% had insufficient knowledge [12]. Nurses' kidney donation opinions were 14.7% positive, 53.2% moderate, and 43.1% negative. These studies found that 64% of nurses oppose brain-dead kidney donation, while 48.6% support it. 84% of members would give their organs after death. These findings are consistent with a study on 30 volunteers at a health fair at the University of California, San Francisco (UCSF) Health Science campus in Africa to assess and compare recruitment barriers to deceased donor registration efforts, which found a high willingness to donate an organ after death (83%) [11]. Nurses' kidney donation opinions were 14.7% positive, 53.2% moderate, and 43.1% negative. These studies found that 64 % of nurses oppose brain-dead kidney donation, while 48.6 % support it. 84% of members would give their organs after death. These findings are consistent with a study on 30 volunteers at a health fair at the University of California, San Francisco (UCSF) Health Science campus in Africa to assess and compare recruitment barriers to deceased donor registration efforts, which found a high willingness to donate an organ after death (83 %) [13]. The overall positive attitude was only present in 37% of the students in this study. This is significantly lower than reports from Italy (91%), Brazil (69%), Germany (55-70%), Pakistan (62%), Turkey (59%), and China (50%). Only 36% of medical students had adequate knowledge about kidney donation, which is also low when compared to other countries such as Pakistan (65%), and Nigeria (65%) [14]. This specifies an appropriate state for identifying various factors contributing to nurses' negative attitudes and lack of knowledge. There was no significant improvement in senior medical students' attitudes toward kidney donation, despite the fact that knowledge improved with seniority and was significantly associated with a better attitude. This suggests that other psychosocial and demographic factors influence attitudes toward organ donation [15]. Nurses' attitudes toward kidney donation are 40.9% negative, 26.4% moderate, and 22.6% negative. 36.9% of individuals and 68.6% of family members were willing to donate a kidney to relatives, although 57.8% were concerned about health risks. These data support the link between organ donation and deceased organ donor families [16]. However, 63% of participants are against organ donation. This is important because future nurses must encourage kidney

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donation. According to religion, nurses have a sufficient attitude toward kidney donation, with 26.4% having a positive attitude, 32.6% having a moderate attitude, and 42.9% having a negative attitude. The impact of religion on attitudes toward kidney donation is debatable. Belief in God and the afterlife has been linked to a negative attitude toward kidney and organ donation. A survey of Swiss-Italian young adults, on the other hand, found that faith in God had a positive impact on their attitude toward kidney donation. According to a report from the United Kingdom (UK), there is no significant relationship between religion and attitude toward kidney donation. Some authors reported that different religious beliefs had different effects on attitudes toward kidney donation [17]. The kidney is the most commonly donated organ in our study. The positive attitude of nurses was (47%) and the negative attitude of nurses was (47%). Similarly, in another study conducted by Ali *et al*. on medical students' knowledge and ethical perceptions of organ donation, it was discovered that the kidney was the most commonly donated organ, followed by blood, cornea, and heart [18]. In our study, knowledge of kidney donation was highest (94%), followed by heart (82%), liver (78%), cornea (59%), lungs (57%), skin (34%), and pancreas (34%). Females participated significantly more than males in our study. Females account for 74.5% of participants, while males account for 25%. This finding is consistent with studies on European medical students conducted by Burra *et al*. and Mekahli *et al*. which found that females had higher positive attitudes than males due to higher emotional values [19, 20].

CONCLUSIONS

The study found that nursing students at the University of Lahore had a good knowledge and positive attitude towards kidney donation, while practicing nurses had limited knowledge and a negative attitude. The study emphasized the importance of structured teaching programs to increase knowledge and promote kidney donation in the community.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Comparative Analysis of R and Mathematica Package for Differential Gene Expression Analysis Using Microarray Dataset on Pancreatic Cancer

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ABSTRACT

Microarrays produces enormous amounts of information requiring a series of repeated analyses to condense data. To analyze this data several computational software is used. **Objective:** To compare the analysis of R and Mathematica package for differential gene expression analysis using microarray dataset. **Methods:** Microarray Data were collected from an online database GEO (gene expression omnibus). Mathematica and R software was used for comparative analysis. In R software, Robust Multi-Array Average (RMA), was used for data normalization. While Limma package was used for DGE analysis. In Mathematica software, AffyDGED was used for normalization and DGE analysis of dataset. **Results:** 3,426 non-differentially expressed genes and 14936 genes with differential expression were separated from R. The thresholds for identifying "up" and "down" gene expression were estimated to be 0.98 and -0.19, respectively, using the RMA method to analyze this dataset. AffyDGED from Mathematica detected 1,832 genes as differentially expressed; of them, 1,591 genes overlap with the real and 1,944 differently expressed genes, giving the true positive rate of (1591/1944) =0.818. This indicates that 18% of the genuine list of differentially expressed genes could not be reliably identified by AffyDGED. **Conclusions:** R programming is one of the most popular and recommendable tools for microarrays to perform different analysis, and along with Bioconductor it makes one of the best analysis algorithms for DGE analysis. On the other hand, AffyDGED brings a contemporary algorithm useful in the real world to the Mathematica user.

INTRODUCTION

Pancreatic cancer is the fourth top reason of demise caused by cancer [1]. Lack of early detection technology for pancreatic cancer invariably leads to a typical clinical appearance of incurable disease at initial diagnosis [2]. Pancreatic cancer originates when glandular organ behind stomach starts an abnormal growth in pancreatic cells and goes out of control to form a mass structure [3]. Microarray is a technology that concurrently estimates the quantitative measurements for the expression of thousands of genes [4]. A microarray is a complex commonly known as a lab-on-a-chip [5]. A (2 Dimensional) array on a solid substrate like glass slide or silicon thin cell which appraises excessive amounts of biological material using high-throughput screening reduced for parallel dispensation and detection methods [6]. To evaluate gene expression between clusters of cells of different organs or individual's DNA microarrays is used [7]. Gene expression analysis is a method in which information from gene is used to synthesize a valuable gene product. In most cases, these products are functional proteins but, in some cases non-protein coding genes such as transfer RNA (tRNA) or small nuclear RNA (snRNA) genes are synthesized in form of functional RNA [8]. The process consists of few steps which includes, transcription, RNA splicing, translation, post translational modification and gene regulation. Gene regulation is operated by a cell regulator for structure and function which originates from cellular differentiation, morphogenesis, versatility, and adaptability of any

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organism [9]. It also assists as a substrate for evolutionary change [10]. Bioinformatics become a progressively significant tool for molecular biologists, specifically for the analysis of microarray data. Bioinformatics collaborates with different computational tools which incorporates with analysis of biological and medical data [11]. Microarrays produces enormous amounts of information requiring a series of repeated analyses to condense data [12]. Through output of microarrays direct interpretation is not possible to show differences in conditions of samples, or time points. To create microarray experiments interpretable, it requires a sequence of algorithms and methods to applied [13]. After normalization of generated data, which is required to make a contrast possible, significance analysis, clustering of samples and biological composites of interest and visualization are usually achieved. Microarray experiments deals with several bioinformatics challenges [14]. R is free and open-source Platform not only for computational analysis, but also very useful in the field of bioinformatics and their related analysis such as, Gene expression analysis, Gene knockout findings, Microarray data analysis [15]. Mathematica is a computer algebra system or program, used mostly in calculating fields of applied mathematics professionals [16]. Major Objective of this study was to analyze microarray data of pancreatic cancers created by using HG-U133_Plus_2 Affymetrix Human Genome U133 Plus 2.0 Array platform. By using this data with different programming languages and their acquired packages a comparison of expression analysis was done and different genes was discovered on the basis of their regulatory effect. This research will further enlighten a path for many different statistical studies in the field of biological and computational biological data sciences. It will also enable new ways to look forward towards the fields of personalized and predictive medicine which will encourage scientists to develop new therapeutic advancements to control chronic genetic diseases.

METHODS

This study analyzed the microarray data of healthy vs pancreatic cancer patient trough R language and Mathematica software. Total 24 samples of 24 different patients were used in this study from which 12 samples were taken from pancreatic cancer patients and 12 from normal healthy patient. Microarray Data were collected from an online database GEO (gene expression omnibus). The dataset which we took from GEO had Accession number GSE14245. This poised dataset was built on transcriptomic approach that profiled the saliva samples from 12 pancreatic cancer patients and 12 healthy control subjects using the Affymetrix Human Genome U133 Plus 2.0 Array platform [17]. R Language and Mathematica was used for the analysis. First step was data normalization followed by differential gene expression (DGE) analysis and extraction of up and down regulated genes. Data normalization is done to remove any zero or negative counts to make data less contaminated and easy to use for further analysis. In this research, our major focus was on the comparison of algorithms used for normalization and DGE analysis in both platform (R and Mathematica). In R software, Robust Multi-array Average (RMA), was used for data normalization. While Limma package was used for DGE analysis. In Mathematica software, AffyDGED was used for normalization and DGE analysis of dataset. Detailed methodology is shown in Figure 1.

Figure 1: Overall methodology of the study

RESULTS

In Figure 1, we can see the difference between boxplots of log-intensity distribution which are plotted to check the difference between distribution. After RMA normalization we can easily see a comparable difference. Specially if we see sample no. 21 in both figures, there is some visible difference that means several zero counts has been

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removed from this dataset after normalization.

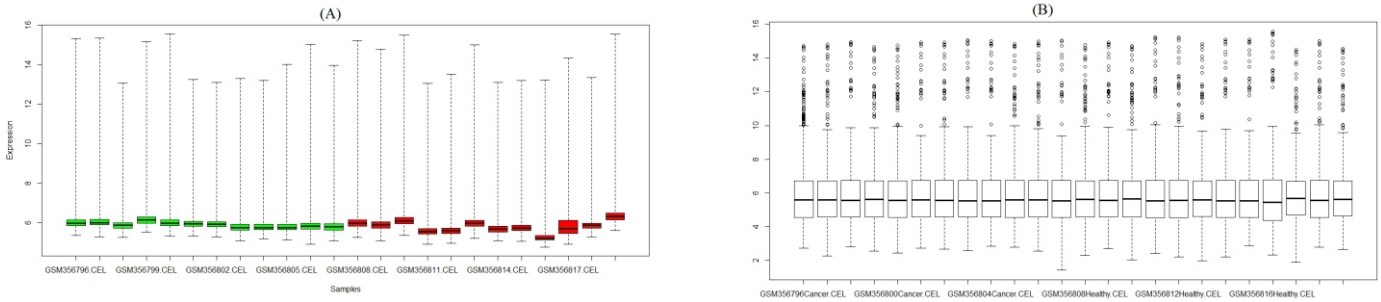


Figure 2: (A) Box plot showing probe intensity of different genes present in microarray dataset (B) Microarray data variation after normalization using RMA method

After normalization, DGE analysis was performed by R software using Limma package. The data of up and down regulated gene extracted from dataset is mentioned in Table 1. Almost 6.2% of genes in this data did not show any regulatory effects in any case and almost 67% genes were down regulated which means that the effect of genes having low expression values doesn't show much possibility to trigger disease (Table 1).

Table 1: Results of UP and DOWN regulated genes

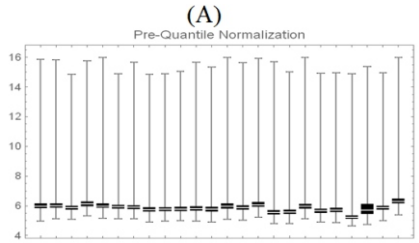
Trend	Genes
UP-Regulated genes 0 < -1	14936
Not-affected genes 0	3426
Down-Regulated genes > 1	36313

Table 2 shows a list of 8 up and down regulated genes based on the highest logFC and p-values. The estimated logFC for multiple treatment conditions compared back to the same control group will be positively correlated even in the absence of any biological effect. Maximum values in up regulated genes estimated by R were 4.8 and minimum value in up regulated expression was 0.98. In down regulation, maximum value estimated was -11.23 and minimum value was -0.19.

Table 2: Top 8 values from up and down regulated differential gene expressions

ID	logFC	Avg. Expression	T	p-value	adj. p-value	B	Fold Change Cancer/Normal	Gene symbol
241174_at	11.44353	11.52234	48.80763	5.02E-27	2.75E-22	40.76645	2785.1382	AP4E1
1553088_a_at	10.40041	10.43073	44.01448	7.09E-26	7.73E-22	39.72194	1351.55932	BCL2L11
1552899_at	9.726866	9.696013	43.70573	8.48E-26	7.73E-22	39.64606	847.3806098	LINC01312
1557866_at	10.1436	10.33103	43.18777	1.15E-25	8.99E-22	39.51616	1131.169839	CFAP157
243269_s_at	9.989396	10.03627	42.43105	1.81E-25	1.15E-21	39.32031	1016.501089	FAM205BP///FAM205A
230092_at	9.465539	9.446353	42.25046	2.02E-25	1.15E-21	39.27247	706.9864018	UBXN10
1564662_at	9.252544	9.308898	41.59133	3.01E-25	1.37E-21	39.09416	609.9485222	ZNF852
208191_x_at	10.6127	10.64971	41.23602	3.75E-25	1.58E-21	38.99557	1565.812265	PSG4

Figure 3 shows pre- and post-quantile normalization done by mathematics software. In pre-quantile plot we use raw data as an input to make these boxplots. Here we clearly see variation among samples because there is no elimination of perfect match probe intensities are applied on it. Form sample no.14 up till sample no. 24 all these samples are from normal patients but shows a large amount of variation amongst all these.



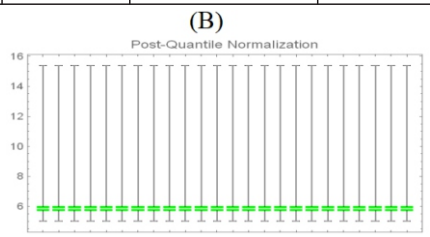


Figure 3: (A) A box-and-whisker comparison before quantile normalization performed by Mathematica (B) After quantile normalization results

In Figure 4 below, X-axis show threshold data of DE that is total number of genes present in an entire dataset which was almost 54130 genes as per Mathematica AffyDGED algorithm analyzed. While on Y-axis values of up and down regulated threshold is presented. In this case, our minimum

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cutoff threshold value of up regulated genes is 0.18 and maximum value is 0.49 whereas minimum value of down regulated genes is -0.155 and maximum value is -0.78.

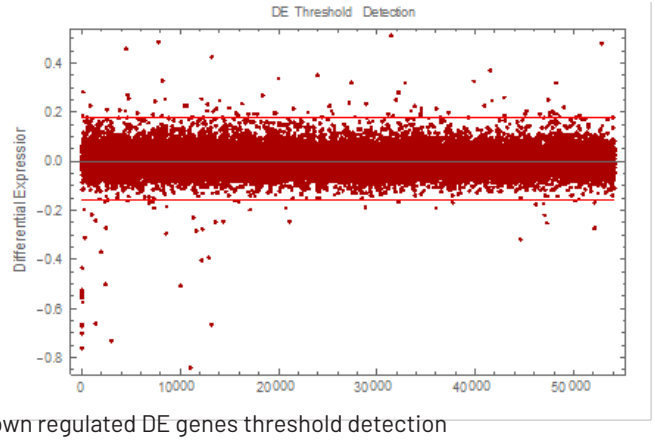

Figure 4: Image showing UP and Down regulated DE genes threshold detection

Table 3 shows top 15 up regulated genes extracted using Mathematica, the best part in AffyDGED algorithm is that it also gives a very detail view of comparison between control and experimental group and also give the values for how genes are differentially expressed in experimental group which is known as cutoff value.

Table 3: Up regulated genes extracted by Mathematica

Affymetrix probe set name	fluorescence intensity of GE in the experimental group	fluorescence intensity of GE in the control group	p-value	Cutoff values of differential gene expression	Gene symbol	GenBank. Accession
241174_at	11.44353	11.52234	5.02E-27	2.75E-22	AP4E1	AV647279
1553088_a_at	10.40041	10.43073	7.09E-26	7.73E-22	BCL2	NM_138626
1552899_at	9.726866	9.696013	8.48E-26	7.73E-22	L11LIN	-
1557866_at	10.1436	10.33103	1.15E-25	8.99E-22	C01312C	AK094948
243269_s_at	9.989396	10.03627	1.81E-25	1.15E-21	FAP157FAM205BP///FAM205A	AL040346
230092_at	9.465539	9.446353	2.02E-25	1.15E-21	UBXN10	AA135547
1564662_at	9.252544	9.308898	3.01E-25	1.37E-21	ZNF852	BC014381
208191_x_at	10.6127	10.64971	3.75E-25	1.58E-21	PSG4	NM_002780
215856_at	9.105225	9.173238	4.30E-25	1.68E-21	SIGLEC15	AK025833
242316_at	9.097469	9.181627	6.84E-25	1.82E-21	TMOD3	AI810103
208257_x_at	10.64889	10.67999	7.48E-25	1.82E-21	PSG1SHI	NM_006905
1556619_at	9.284751	9.340856	7.75E-25	1.82E-21	SA9	CA413715
226611_s_at	9.76453	9.899672	7.94E-25	1.82E-21	CENPV	AA722878
208469_s_at	9.338312	9.495589	8.01E-25	1.82E-21	PPT2-EGFL8///EGFL8///PPT2	NM_030652

DISCUSSION

This thesis was basically focused on using bioinformatics techniques i.e., statistical computing and algorithms to analyze datasets and perform differential expression analysis on it. The Pancreatic cancer Microarray dataset (GSE14245) was used, which was extracted by using the transcriptomic approach profiled the saliva supernatant samples from 12 pancreatic cancer patients and 12 healthy control subjects using the Affymetrix Human Genome U133 Plus 2.0 Array platform. There are very few research articles are available which shows the difference between both of these tools. Not only in tools and platform our interest is also towards the efficiency of algorithms used by these platforms to preform differential expression analysis. RMA is one of the most commonly used algorithms which give normalized data after eliminating the mismatch probe values so it gives a good quality of normalized values moreover, it also has a quantile normalization method which compare background correction within each probeset ratio. Irizarry *et al.*, also performed similar analysis on dataset generated from Affymetrix GeneChip system. The dataset was of high-density oligonucleotide array data. They explained why there is a need to examine and normalize microarrays datasets using probe level densities. They also used RMA algorithm for normalization, and they concluded that there was no shortcoming for using RMA for normalization of microarray data [18]. Mathematica used AffyDGED algorithm for DE analysis which is somewhat similar to RMA but have a lot of differences as well, so we can say that AffyDGED is a mixture of both mas5 and RMA. When results were

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retrieved from Mathematica, our next step was comparison. For this we take average scores of up and down regulated gene expression and also check how many genes are up and down regulated and also compare the amount of not effected or overlapped genes. 3,426 non-differentially expressed genes and 14936 genes with differential expression were separated from R. The thresholds for identifying "up" and "down" gene expression were estimated to be 0.98 and -0.19, respectively, using the RMA method to analyze this dataset. A plot of the processed data always reveals a tight clustering of data about the line y=0. This observation was used to develop code that scans vertically up and down in small increments and establishes a breakpoint in each direction any time the density of data at a vertical position is 50% less than it was at the previous increment. These breakpoints become the thresholds for determining differentially expressed up and down genes. Gregory Alvord *et al.*, performed DEG analysis of microarray data from Soybean genome. They also used Rand Bioconductor for DEG analysis and RMA algorithm for normalization. These programs successfully identified differential gene expression results from soybean genome data [19]. AffyDGED from Mathematica detected 1,832 genes as differentially expressed; of them, 1,591 genes overlap with the real and 1,944 differently expressed genes, giving the true positive rate of (1591/1944) =0.818. This indicates that 18% of the genuine list of differentially expressed genes could not be reliably identified by AffyDGED. Allen also studied differential gene expression using Affymetrix microarrays. He also used AffyDGED algorithm of Mathematica for analysis. AffyDGED algorithm performed very well and took very less time for analysis [20].

CONCLUSIONS

Microarray technology continues to be heavily used by the biomedical and basic science research communities throughout the world. R programming is one of the most popular and recommendable tools for microarrays to preform different analysis, and along with Bioconductor it makes one of the best analysis algorithms for DGE analysis. On the other hand, AffyDGED brings a contemporary algorithm useful in the real world to the Mathematica user, but this is not much familiar to every researcher so, it is much needed to explore this software by those who have interest in exploring fundamental biology questions with their favorite computational tool chest.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Determine the Curing Effects of *Silybum marianum* (milk thistle) Administered Orally to Non-Alcoholic Fatty Liver Disease (NAFLD) Patients for Six Months

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ABSTRACT

Silybum marianum is the scientific name of milk thistle. For centuries, it is used to treat hepatitis, cirrhosis, jaundice, diabetes, and indigestion. The bioactive agents of milk thistle contain Apigenin, silybin, betaine, free fatty acids, silybin, silychristin, and silidianin. **Objective:** To determine the potential of *Silybum marianum* (milk thistle) in non-alcoholic fatty liver disease patients. **Methods:** It was a cross-sectional and experimental based study with NAFLD patients. A significant age range of 30 to 60 years was chosen for the 40 patients (20 men and 20 women). Screening period after liver biopsies (before and after the use of one capsule of *S. marianum* (Silymarin Standardize milk Thistle 300mg/day metabolic maintenance). Ultrasound technology assessed the liver condition at the start of the study and after the herbal 6-months therapy. **Results:** Out of 40 patients, 10 (25%) had hyperlipidemia and 5 (12.5%) were diabetics. The results of the 6-month therapy research study showed that the blood AST and ALT levels of NAFLD patients had significantly decreased. The average ALT and AST baseline concentrations were 85 and 65.9IU/ml, respectively. AST value (before and after therapy) showed a statistically significant difference in 80% of the NAFLD patients treated with *S. marianum* (32 out of 40; p=0.007). With a p-value of 0.05, alanine transaminase (ALT) normalization of disease severity was accomplished in 65% of patients (26 out of 40 patients). **Conclusions:** The therapy of NAFLD via *S. marianum* is successful in terms of biochemical improvement, especially when other medications have failed or when used in conjunction with other therapeutic techniques.

INTRODUCTION

Milk thistle seeds (*Silybum marianum*) has been used for more than 2000 years as a curing agent for many illnesses exclusively liver diseases. The milk thistle originated from Southern Europe, Asia Minor, and North Africa. It has now become a common weed and cultivated plant in Europe, Africa, the Americas, and Australia [1, 2]. The white patterns (variegation) on the leaves, which have been used as a vegetable, are the reason of giving milk thistle as its name [3]. Roasted seeds have been used as a replacement for coffee. The mature, untreated seeds of milk thistle have been used in traditional medicine for 2000 years to cure depression, headaches, digestive and liver issues, detoxification, and encourage breastfeeding. A modified form of milk thistle is Silymarin, also known as silydianin as a whole, which is a standardized extract that is sold and used commercially. Milk thistle seed powder components, especially silibinin, function as antioxidants and hepatoprotectives [1, 4, 5]. It is found successful in treating liver cirrhosis, fibrosis, gallbladder disease, and toxin poisoning. They also promote liver regeneration [6, 7]. Nonalcoholic fatty liver disease (NAFLD) is a clinic-pathologic illness characterized by considerable lipid

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deposition i.e., more than 5% of the liver weight is deposited as triglycerides in the liver parenchyma of NAFLD patients [8]. It is estimated that 20–30% of the Western population possessed [9]. The pathophysiology of NAFLD is yet unclear, and empirical treatment is used to treat this illness. Even if there hasn't always been a benefit to losing weight, NAFLD may get better with it. Human research on the treatment of alcoholic cirrhosis and hepatitis is conflicting, nevertheless. The lipid, biliary, immunomodulatory, and anti-inflammatory properties of milk thistle seed. Other therapeutic qualities include antiviral, anticancer, and others. Milk thistle medicines are risk-free, well-tolerated, and have no significant adverse effects [4, 10, 11]. The current study compared the effectiveness and safety of *Silybum marianum* when given orally to NAFLD patients for three months.

METHODS

It was a cross-sectional research-based study conducted among patients with NAFLD visited Sheikh Zaid Hospital in the region of Lahore. 40 patients (n=20 males and 20 females) were selected having a considerable age range of 30-60 years. Liver biopsies during the screening period were carried out to rule out alternate causes of liver diseases and to prove the histologic diagnosis of NAFLD. The primary indicator was the persistent elevation of ALT and AST. After an attempt to control the metabolic circumstances and after six or more months of follow-up which indicated fatty liver biopsies during the screening period via the intake of one capsule of *S. marianum* (Silymarin Standardize milk Thistle 300mg/day metabolic maintenance). At the beginning of the trial and the conclusion of the treatment, the liver was evaluated using ultrasound technology. The updated criteria were used in the ultra-sonographic examination to identify fatty liver. The patients were followed up with a weight-reducing diet for at least three months and reported a higher amount of alanine aminotransferase (ALT) values were included in this group. Patients who used more than 20g of ethanol per day were disqualified. By monitoring alanine aminotransferase (ALT) readings, the patients were monitored while on a weight-reduction regimen for at least three months. Raw data regarding socio-demographic aspects of NAFLD patients obtained from the survey of 40 patients was carried out by using SPSS software (version 22.0). ALT and AST levels were found by using 95% accuracy using a two-sided p-value of 0.05. baseline and population comparisons were undertaken using student t-tests and chi-squared tests for equal proportions. A p-value of 0.05 was considered significant.

RESULTS

40 candidates (n=20 males and n=20 females) with a mean age value of 48.2 5 ± 12.3 were selected for this current study. No patient had reported clinical cirrhosis or malignant liver. In our study, there were no patients with a BMI higher than 40 were included. Results showed that out of 40 patients, 5 (12.5%) were diabetics and 10 (25%) reported hyperlipidemia (Table 1).

Table 1: Socio-demographic aspects of NAFLD patients

Parameters	S. marianum
Age	35.9 ± 10
Gender (F/M)	20/20
BMI	44.8 ± 9.3
Diabetics	5 (12.5%)
Hyperlipidemia	10 (25%)

The research study of this 6-month's therapy session demonstrated a notable decline in the serum AST and ALT concentrations in NAFLD patients. In the study groups, the average baseline serum ALT and AST concentrations were 85µ 10IU/ml and 65.9µ 10IU/ml, respectively. Alanine transaminase (ALT) normalization was achieved in 65% of patients (26 out of 40 patients) with a p-value of 0.05. 80% of NAFLD patients (32 out of 40) treated with *S. marianum*, showed statistically significant difference in AST value (before and after therapy)(p=0.007)(Table 2).

Table 2: Clinical values before and after 6 months of therapy with one capsule of *S. marianum* (Silymarin Standardize Milk Thistle 300mg/day metabolic maintenance)

Parameter	Baseline ALT level (IU/ml) (Initial)	ALT normalization N (%)	Baseline AST level (IU/ml) (After 6 months)	AST normalization n (%)
S. marianum	85µ 10IU/ml	26/40 (65%) (p=0.05)	65.9µ 10IU/ml	32/40 (80%) (p<0.007)

DISCUSSION

The presence of fatty acids in human blood is produced by dietary lipid intake, lipolytic adipose tissue activity, and fatty acid synthesis [12]. According to the current study's findings, a 7% weight loss (average 6.6 kg) is associated with a considerable reduction in the degree of liver steatosis and an improvement in biochemical indicators. There was a substantial drop in AST, ALT, and insulin levels, as well as insulin resistance among those people [13]. Many studies have demonstrated the connections between NAFLD and *Silybum marianum* and highlighted the fact that insulin resistance, and metabolic syndrome, a loss in physical activity, an increase in weight, and changes in eating habits might all be triggering the progression of NAFLD [14-17]. Oxidative stress is considered to be responsible for the majority of hepatocyte lipid accumulation, hepatic inflammation, and fibrosis. Similar findings were also reported by. For lowering fibrosis and inflammation or delaying the course of NAFLD, there is not enough solid data to support any treatment interventions [4]. However, to delay the progression of the illness, weight

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loss, and anti-oxidant medications may be necessary [18, 19]. Our current 6-month treatment research study revealed that there was a noticeable decrease in the blood AST and ALT values of NAFLD patients. The average baseline levels of ALT and AST in the study groups were 85 and 65.9 IU/ml, respectively. 80% of NAFLD patients (32 out of 40) treated with *S. marianum* exhibited a statistically significant difference in AST value (before and after therapy) (p=0.007). Alanine transaminase (ALT) normalization was achieved in 65% of patients (26 out of 40 patients) with a p-value of 0.05 [20]. Another research study suggested the treatment of NAFLD patients with *S. marianum* and vitamin E is well-tolerated and safe. Each instance finished the research, and patient medication adherence was good in both groups [21]. Other research studies also have employed the use of vitamin E and *Silybum marianum* as antioxidants to defend the liver against toxins [22]. They have been researched for use of anticarcinogen as a supportive therapy for liver damage brought on by poisoning with *Amanita phalloides*. The active component of the *S. marianum*, silybin, has a variety of distinct hypothesized mechanisms of action, although the main one is still unknown [23]. *S. marianum* is thought to have antioxidant properties since it increases the activity of superoxide dismutase in lymphocytes and erythrocytes. It is also considered to note that *S. marianum* also helps in the development of the hepatocyte membrane, preventing toxins from entering the cell through enterohepatic recirculation, and aiding in liver regeneration by boosting ribosomal protein synthesis and activating nucleolar polymerase A. Previous study by Del Ben *et al.*, suggested *S. marianum* would be useful in treating NAFLD were either uncontrolled or conducted on a wide range of people with fatty liver. There are rare research studies available for NAFLD management. The bulk of studies on the treatment of NAFLD emphasizes the improvement of concomitant diseases such as obesity, diabetes mellitus, and hyperlipidemia [24].

CONCLUSIONS

The therapy of NAFLD via S. marianum is successful in terms of biochemical improvement, especially when natural medications have prioritized or when used in conjunction with other liver therapeutic techniques. The cost of milk thistle seed S. marianum therapy is the lowest of any therapy, and its side effects are hardly noticeable. Our findings are need to be verified in more extensive research with pre-and post-treatment biopsies in the future.

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Original Article

Computational Exploration of Functional and Structural Impact of Single Nucleotide Changes in DNMT3A Gene among Acute Myeloid Leukemia Patients

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ABSTRACT

Acute myeloid leukemia (AML) is a blood cell malignancy of the myeloid line, characterized by fast proliferation of aberrant cells that build up in the bone marrow and blood, interfering with normal blood cell synthesis. DNMT3A is a DNA methyltransferase that plays a role in DNA methylation, an epigenetic modification associated with gene expression regulation. DNMT3A mutations are frequently found in AML and are associated with poor prognosis. **Objective:** To evaluate the impact of DNMT3A mutations on protein structure and function, specifically in the context of AML. **Methods:** SNPs of DNMT3A gene reported in AML (R882P, R882L, R882S, R882G, and R882C) were retrieved from National Centre for Biotechnology Information (NCBI) database and different *in silico* approaches were used to investigate how these mutations affect protein structure and function. **Results:** Prediction tools indicated that mutations are pathogenic affecting DNMT3A function and were found in evolutionarily conserved regions. Protein stability analysis showed that mutations reduce DNMT3A's structural stability, alter secondary structure of the protein, particularly helices, interacts with other proteins and reduce protein-protein affinity. RNA folding analysis revealed abnormal folding patterns caused by mutant, affecting protein translation. DNMT3A expression was reported to be considerably greater in AML compared to normal tissues, and mutations were associated with poor overall survival in AML patients. Methylation levels and post-translational modification sites of DNMT3A were also investigated. **Conclusions:** Overall, this research highlighted the negative impact of DNMT3A mutations on protein structure and function, emphasizing their importance in the development and prognosis of AML.

INTRODUCTION

Leukemia is a kind of blood and bone marrow malignancy characterized by the fast development of abnormal white blood cells [1]. Based on the kind of stem cell involved and whether the leukemia is acute or chronic, there are four primary subtypes of leukemia [2]. Acute myeloid leukemia (AML) is the most frequent kind of leukemia in adults and is associated with a high number of annual deaths in the United States [3]. It is distinguished by an overabundance of undifferentiated myeloid cells in the bone marrow, resulting in a lack in normal blood cell synthesis [4]. The symptoms may include fever, weight loss, fatigue, breathlessness, frequent infections, and abnormal bleeding [5]. The causes of AML are diverse and can include exposure to therapeutic or environmental agents that damage DNA, although the exact etiology is often unclear. AML is caused by a combination of hereditary and ecological factors, such as genetic mutations, age, radiation exposure, chemical exposure, and previous treatments [6]. Genomic profiling advances have thrown some insight on the function of genetics in AML, but further research is needed to fully understand the mechanisms involved. Diagnosing AML involves analyzing peripheral blood or bone marrow samples for the presence of abnormal myeloid cells. It should be diagnosed when marrow or blood has > 20% blasts of myeloid lineage [7]. The prognosis of AML depends on factors such as the patient's age and subtype of the disease. It is mostly diagnosed at the median age of 68 years. Treatment

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typically involves chemotherapy, sometimes combined with targeted therapy drugs, and may be followed by a stem cell transplant. Recent studies have focused on the molecular pathogenesis of AML, identifying genetic variations that influence the prognosis and altering the classification of the disease. One of the commonly altered genes in AML is DNMT3A (~20%), which plays a role in DNA methylation [8]. It consists of 35 exons and encodes a protein of 912 amino acid. It was mapped to chromosome 2p23.3. Mutations in DNMT3A are linked to cytogenetically normal acute myeloid leukemia (CN-AML). These mutations impair the enzyme's ability to methylate DNA fully, leading to changes in gene activity and the production of abnormal white blood cells [9]. In the field of research, bioinformatics methods and in silico techniques have revolutionized the study of life sciences. These computational approaches help categorize proteins based on structure and function and assist in the development of servers for molecular sorting using machine learning methods [10]. In silico methods also facilitate screening potential therapeutics against molecular targets, reducing the need for extensive laboratory work and conserving resources [11].

METHODS

To perform in silico analysis, 18 computational tools were employed (Table 1).

Table 1: Tools applied for analysis

In silico tools	Function
NCBI	For retrieval of SNPs
SIFT, Align GVGD, FATHMM, PANTHER	For the identification of deleterious SNPs
MUpro, I-Mutant Suite, mCSM	For protein stability analysis
ConSurf	For estimation of the conservation profile
HOPE Project	For analysis of structural effects of DNMT3A gene mutation
SWISS-MODEL	For protein modeling
SOPMA	For secondary structure analysis
STRING	In order to anticipate protein-protein interactions
Vienna package	For the prediction of effect of mutations on RNA secondary structure
GEPIA, UALCAN, cBioPortal, Cytoscape	For analysis of post-translational modifications

RESULTS

From NCBI, five SNPs were reported to be found in AML. To identify the damaging and deleterious effects of SNPs that could interfere with the structure and function of DNMT3A gene, four in silico tools (SIFT, Align GVGD, FATHMM, and PANTHER) were used. All the SNPs were predicted as damaging and deleterious by these computational algorithms (Table 2).

Table 2: Analysis of damaging effects of DNMT3A mutations on structure and function of gene using in silico tools

DBSNP RS#	SNPs	SIFT	Align GVGD	FATHMM	PANTHER
rs147001633	R882P	Affect protein	C65	Damaging	Probably damaging
rs147001633	R882L	Affect protein	C65	Damaging	Probably damaging
rs377577594	R882S	Affect protein	C65	Damaging	Probably damaging
rs377577594	R882G	Affect protein	C65	Damaging	Probably damaging
rs377577594	R882C	Affect protein	C65	Damaging	Probably damaging

To analyze the effects of point mutations on the stability of protein structure, three software (MUpro, mCSM, and I-Mutant) were used. The SNPs were shown to reduce the protein structural stability (Table 3).

Table 3: Effects of SNPs on the structural stability of DNMT3A protein

	Mupro		mCSM		I-Mutant	
SNPs	Delta Delta G value	Prediction	ΔΔG Kcal/mol	Prediction	DDG value (kcal/mol)	Prediction
R882P	-0.94342015	Decrease stability	-0.335	Destabilizing	-0.78	Decrease
R882L	0.073176169	Increase stability	-0.301	Destabilizing	-0.62	Decrease
R882S	-0.64353902	Decrease stability	-0.228	Destabilizing	-1.46	Decrease
R882G	-1.2625806	Decrease stability	-0.302	Destabilizing	-1.65	Decrease
R882C	-0.47408111	Decrease stability	0.096	Stabilizing	-1.14	Decrease

To analyze the evolutionary conservation, ConSurf interpret the results, according to which all the SNPs were predicted as highly conserved (Figure 1).

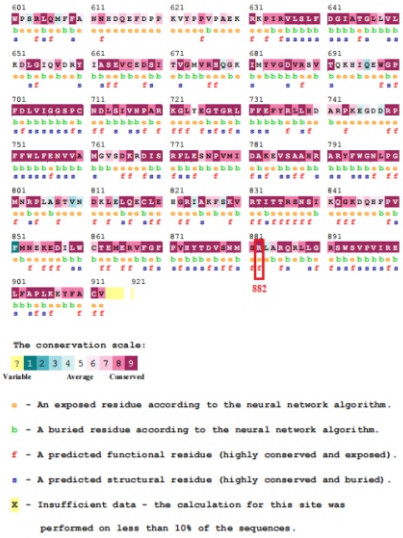

Figure 1: ConSurf results of DNMT3A gene amino acid sequence on a multi-colored bar sheet with conservation scale below

To assess the consequences of amino acid changes on physical and chemical characteristics, spatial organization, hydrophobicity, size, charge and function of the protein, HOPE was used. It predicted that all the mutant

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residues were smaller than the wild-type residue (Table 4 and Figure 2). External interactions will be lost by the smaller size.

Table 4: HOPE's interpretation of the effect of amino acid changes on DNMT3A protein structure and stability

SNPs	Size	Change of Charge
R882P	W>M	+ve to neutral
R882L	W>M	+ve to neutral
R882S	W>M	+ve to neutral
R882G	W>M	+ve to neutral
R882C	W>M	+ve to neutral

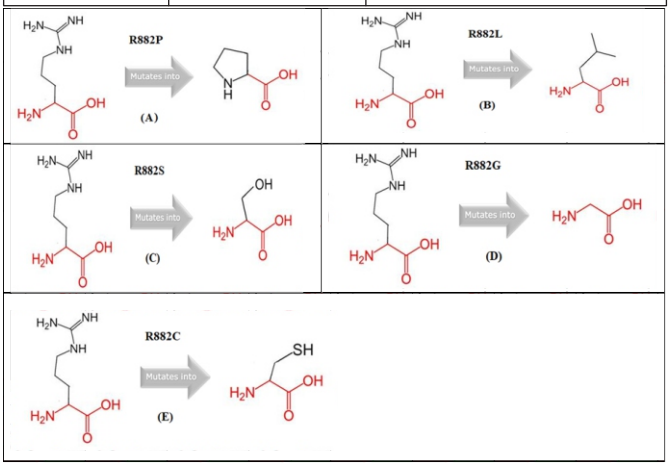
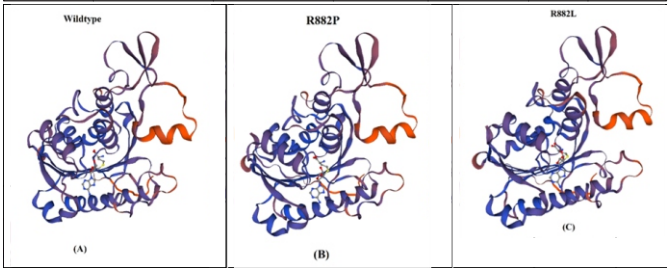


Figure 2: The native (left) and mutant (right) amino acid residues of DNMT3A gene mutations are shown in schematic form

For the homology modeling of the wild and mutant variants, SWISS MODEL was used. It is designated to construct 3D structure of protein (Figure 3). Different parameters (GMQE, QMEAN) were obtained using this tool (Table 5).

Table 5: Representing the different QMEAN Z score, GMQE, Identity, and template query number of wild type and mutant proteins via SWISS-Model

SNPs	Total number of amino acids	Number of amino acids in model	Template query number	Sequence identity	GMQE	QMEAN
Wildtype	912	613-912	6pa7.1. K	100.00%	0.39	-1.14
R882P	912	613-912	6pa7.1. K	99.85%	0.39	-1.07
R882L	912	613-912	6pa7.1. K	99.85%	0.39	-1.19
R882S	912	613-912	6pa7.1. K	99.85%	0.39	-1.19
R882G	912	613-912	6pa7.1. K	99.85%	0.39	-1.26
R882C	912	613-912	6pa7.1. K	99.85%	0.39	-1.23



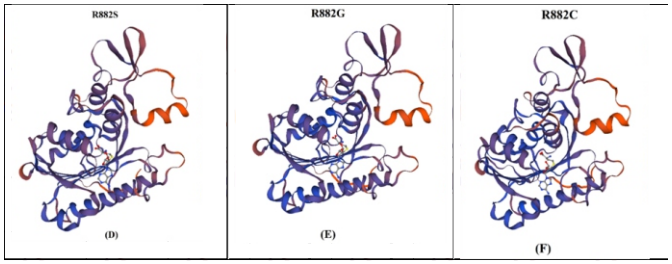


Figure 3: Protein structures of wildtype and mutants using SWISS-MODEL

Self-Optimized Prediction Method from Alignment abbreviated as SOPMA, was used to interpret the secondary structure of protein. It predicted different characteristics of secondary structure i.e., alpha helix, 310 helix, Pi helix, beta bridge, ambiguous states, and other states (Figure 4).

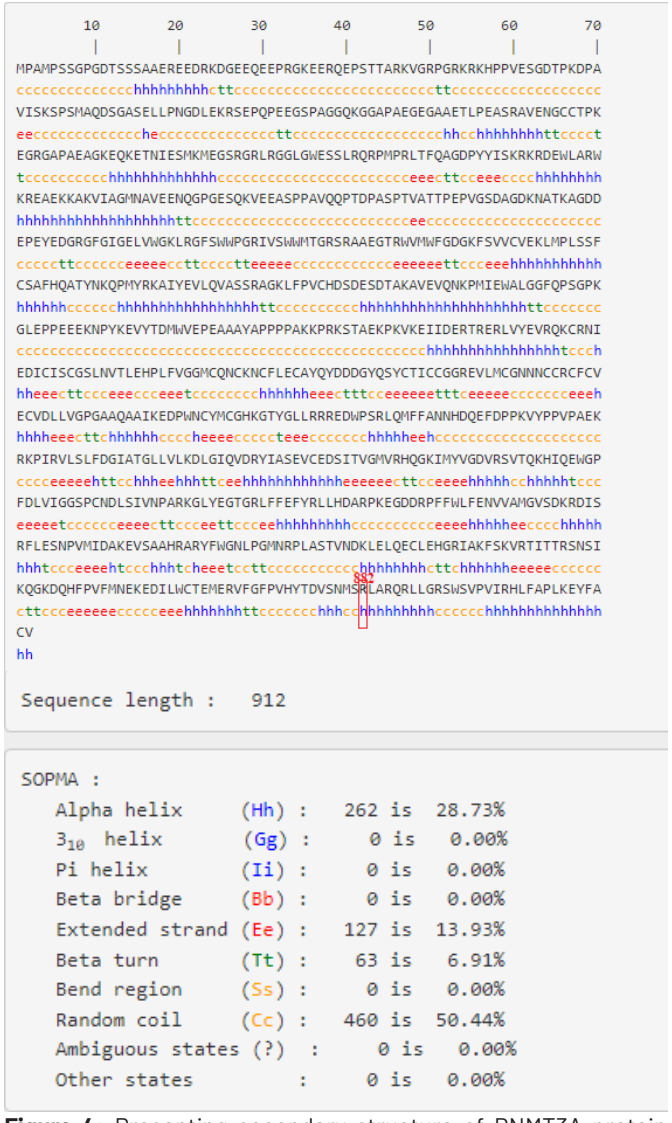


Figure 4: Presenting secondary structure of DNMT3A protein using SOPMA

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To create protein interaction network of DNMT3A protein, STRING Database was used. It predicted that DNMT3A is functionally associated with five other proteins (EZH2, DNMT3L, MYC, DNMT1 and HIST2H3PS2). Any change in the structure of DNMT3A protein can affect the functions of these proteins related to it (Figure 5).

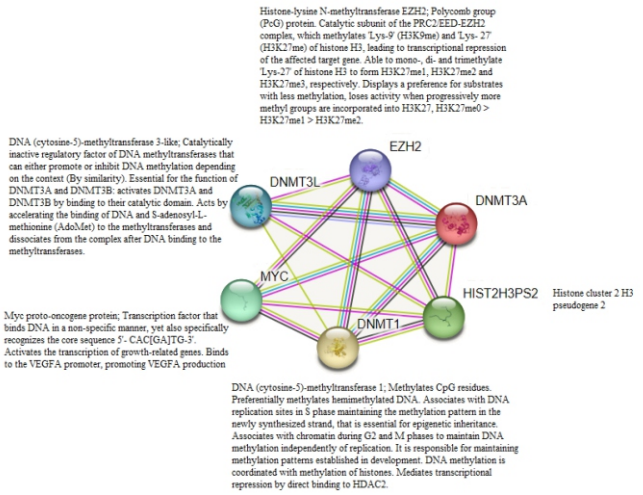

Figure 5: Network of protein-protein interactions of DNMT3A using STRING Database

For the assessment of secondary structure of mRNA, RNA fold webserver on the Vienna package was used. All the mutations resulted in anomalous RNA folding, thus affecting mRNA localization and influencing the translation of protein (Figure 6).

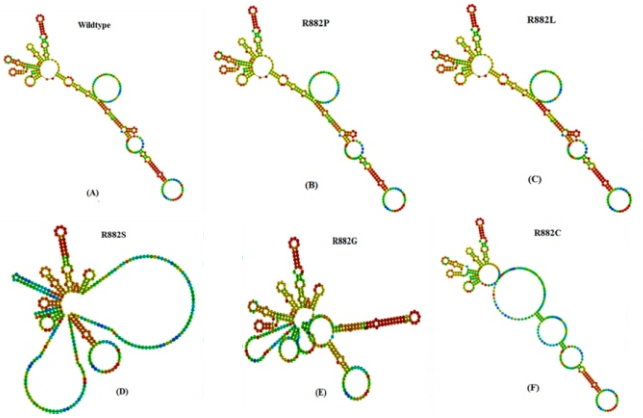

Figure 6: Impacts of SNPs on RNA secondary structure by Vienna package

To check the expression of DNMT3A in AML and overall survival analysis, GEPIA2 was used. In figure 7 (A), Transcripts per million (TPM) graph showed that mRNA expression of DNMT3A gene was significantly higher T (n=173) in LAML as compared to normal tissues N (n=70). In figure (B), the relationship between DNMT3A mRNA expression and the prognosis of patient, including overall survival (OS), was analyzed by the Log-rank test. As log-rank p value is < 0.05 (0.32), it was considered statistically significant as it was explaining that both high and low DNMT3A groups have different distribution curve.

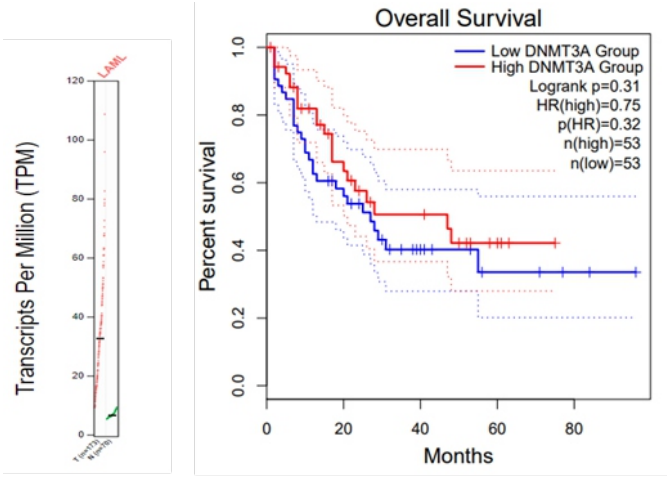

Figure 7: (A) Overview of DNMT3A mRNA expression in AML from TCGA obtained from GEPIA2 (B) Kaplan-Meier plots showing overall survival between high and low DNMT3A groups in AML

For comprehensive and interactive analysis of expression and methylation levels of DNMT3A gene in AML, UALCAN was used. In Figure 8 (A), a significant variation of DNMT3A methylation was seen between male and female. In Figure 8 (B), methylation levels in different age groups of AML patients were shown. Individuals with age of 21 to 80 years were found to have significant methylation levels.

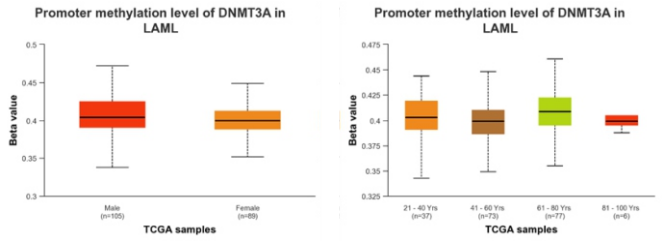

Figure 8: (A) DNMT3A promoter methylation profile based on patient's gender in LAML (B) DNMT3A promoter methylation profile based on patient's age in LAML

To check the PTM sites, cBioPortal was used. It predicted that 23% (altered / profiled = 131 / 560) of genetic alterations were reported in DNMT3A including in-frame, missense, splice and other mutations etc. It displayed all Post Translational Modifications (PTMs) available for the transcript. Different PTM types were shown with varying color codes such as green for phosphorylation, red for ubiquitination and purple for sumoylation (Figure 9).

Figure 9: Representing genetic alterations of DNMT3A and PTM

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sites by using cBioPortal

Cytoscape was used for the visualization and analysis of network graphs of DNMT3A gene involving nodes and edges. A gene hub was identified by Cytoscape network analysis (Figure 10). Each gene represented by a node was predicting the high correlation of DNMT3A with them. The thickness of the line connecting the two nodes reflected the strength of the link favorably.

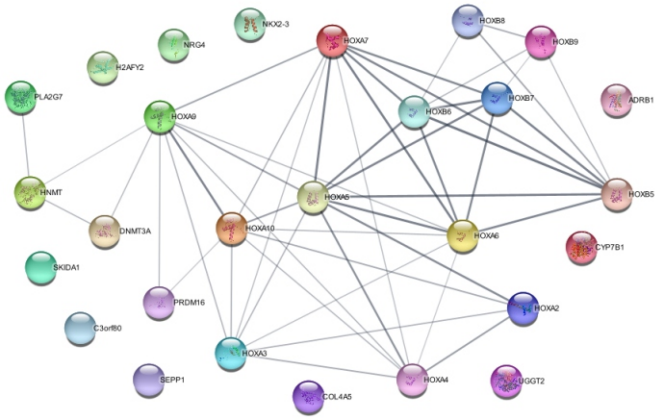

Figure 10: Gene hub presenting correlation of DNMT3A with other genes

DISCUSSION

In silico methods are preferred in many scientific disciplines because they are cost-effective, time-efficient, flexible, reduce ethical concerns, and can make predictions about complex systems so they are used to better understand how mutations might disrupt protein structure and function [12]. DNMT3A is a de novo DNA methyltransferase that has lately gained attention as a result of its common mutation in a variety of immature and mature hematologic neoplasms. DNMT3A mutations occur early in cancer formation and tend to be associated with a poor prognosis in persons with acute myeloid leukemia (AML), making this gene an intriguing target for innovative therapies [13]. Disease-causing SNPs are frequently detected in evolutionarily conserved areas. Five SNPs of DNMT3A were found to be involved in AML. All the five SNPs (R882P, R882L, R882S, R882G, R882C) were retrieved from dbSNP of NCBI database. For the assessment of function, different prediction tools (SIFT, Align GVGD, FATHMM, and PANTHER) were used. These tools predicted that all the mutations are pathogenic and affecting the function of DNMT3A. In recent studies, it was revealed that DNMT3A is required for methylation of unaltered DNA in CpG islands by converting cytosine to 5-methylcytosine, which is linked with gene silencing [14]. In vitro, enzymatic assays revealed that mutated DNMT3A reduced DNA methylation and overexpression of the two most common DNMT3A mutants (R882H and R882C) advanced proliferation in cell culture trials [15]. It strongly demonstrated a substantial pathogenetic involvement of DNMT3A mutations in AML [16]. To investigate the impact of point mutations on DNMT3A structural stability, MUpro, mCSM and I-Mutant were used. The outcome revealed that almost all the SNPs were found to diminish the structural stability of the protein. The previous studies have found that mutations in the DNMT3A protein's catalytic region were anticipated to result in function loss [17]. Protein stability regulates protein conformational structure and consequently dictates function. Any change in protein stability can result in misfolding, disintegration, or abnormal protein aggregation [18]. To evaluate if a mutation has a deleterious effect on the host, evolutionary conservation in the protein sequence is critical. The degree to which an amino acid position has been evolutionarily preserved reveals its structural and functional significance [19]. ConSurf was used for the evolutionary conservation analysis, according to which all the five substitutions were found to be present in exposed region and are highly conserved, having high conservation scores. Therefore, increases the risk of tumorigenesis [20]. HOPE was used to assess the consequences of amino acid replacements on the protein's physical and chemical characteristics, spatial structure, hydrophobicity, size, charge, and function. All of the mutant residues were anticipated to be smaller than the wild-type residue. The lower size will eliminate external interactions. The variations in hydrophobicity and size between mutant and wildtype residues may cause protein framework disruption by disrupting H-bonding connections with adjacent residues [21]. The mapping of amino acid substitutions can be accomplished using 3D protein structure analysis. To create 3D models of the mutated residues, SWISS-MODEL was used. Chakravarty explained the changes in secondary structure during the transition suggest that helices and strands are likely to be extended at the expense of turns and coils. The decrease in bounding factors of the interface residues as they transition from the unbound to the bound form reflects a loss in flexibility during complex formation [22]. For the analysis of secondary structure, SOPMA was used. This software predicted that all the mutations were present in the exposed region in the form of helix. Helices can tolerate more mutations than strands without change, because they have more inter-residue interactions [23]. Mutations that alter secondary structure inside the protein core are more likely to produce proteins that do not fold correctly, making their structures more difficult to crystallize. Hence, anomalous proteins are formed [24]. STRING database was used to analyze how DNMT3A is associated with other proteins. It showed the association of DNMT3A gene with five other genes (EZH2, DNMT3L, MYC, DNMT1 and

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HIST2H3PS2). DNMT1 maintains methylation during DNA replication. Trowbridge *et al.*, demonstrated that DNMT1 haploinsufficiency impaired leukemia stem cell (LSC) activity by depressing bivalent chromatin domains [25]. DNMT3A can reside in the nucleus as dimers, tetramers, and larger oligomeric complexes. The oligomers are made of either homo-dimeric DNMT3A molecules or heterodimeric DNMT3A–DNMT3L molecules [26]. Apart from programmed changes in oligomerization, such as those caused by developmental changes in DNMT3L expression and differential DNMT3A/3B isoform usage, a number of pathologic changes, such as mutations at DNMT3A binding interfaces have been shown to influence oligomerization and alter cell behavior [27]. For the prediction of changes in RNA secondary structure, RNA fold webserver in Vienna Package was used, according to which all the mutants of DNMT3A led to the abnormal folding of RNA, thus influencing RNA localization and affecting the protein translation. In the current study, it was elaborated that in addition to its known involvement in HSC differentiation, DNMT3A has been linked to the preservation of RNA splicing and genomic integrity, both of which are dramatically altered when DNMT3A is mutated [28]. The loss of DNMT3A resulted in the downregulation of spliceosome genes and aberrant RNA splicing. Mutations induce abnormalities of DNMT3A splicing, likely through changing exonic splicing silencers [29]. GEPIA2 was utilized to examine the expression of DNMT3A in AML and overall survival analysis. It was illustrated by GEPIA2 that mRNA expression of DNMT3A gene was significantly higher in AML as compared to normal tissues. DNMT3A expression was shown to be higher in AML in a prior study [30] and its mutations were independently linked with poor outcome in AML patients with an intermediate-risk cytogenetic profile or CN-AML [31]. UALCAN was employed for a thorough and interactive investigation of the expression and methylation levels of the DNMT3A gene in AML. A statistically significant overrepresentation of DNMT3A methylation status was reported in patients ≥ 50 years old in recent research. There was no evident relationship between DNMT3A methylation status and gender. While the prevalence of AML is increasing, no difference in frequency has been seen between males and females [32]. For prediction of post translational modification sites, cBioPortal was used. Large-scale studies have recently revealed that overlap between PTMs and SNPs results in damaged PTMs, which severely influence both gene and protein function and are linked to human cancer [33]. Radivojac *et al.*, also discovered a link between phosphorylation site disruptive variations and somatic cancer mutations [34]. Cytoscape was used to visualize molecular interaction networks of human DNMT3A protein with other associated proteins [35]. Maintaining protein interactions is critical for maintaining system homeostasis [36]. Any change in the gene leads to disruption of functioning of correlated genes. Thus, various studies have found a high relationship between DNMT3A genetic variants and prognosis in AML patients, with mutations predicting a markedly bad prognosis in AML patients [37].

CONCLUSIONS

In this study, *in silico* tools were used to analyze the impact of DNMT3A mutations, which are associated with hematologic neoplasms, particularly AML. The findings shed light on the potential mechanisms underpinning DNMT3A's function in cancer formation and emphasize its therapeutic potential. All the analyzed SNPs were found to be deleterious and damaging, destroying the DNMT3A structure and function. These SNPs may reduce the DNMT3A capacity to fully methylate DNA which abrupt its activity preventing the normal differentiation, ultimately leading to AML. Overall, this study demonstrates the importance of *in silico* methods in elucidating the complex molecular processes involved in cancer development and progression, particularly AML.

Authors Contribution

Conceptualization: AMA
Methodology: SA, KJ, AT
Formal analysis: SA, KJ, AT
Writing-review and editing: SA, AMA

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

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Determination of Physiological, Biochemical and Anti-oxidative Status in Type 1 Diabetes Mellitus Patients

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ABSTRACT

Type 1 Diabetes Mellitus (T1DM) disorganization of glucose equilibrium distinguishes by autoimmune disruption of the insulin producing pancreatic β-cell that constantly leads to insulin scarcity and resulting hyperglycemia **Objective:** To determine the physiological, biochemical, and anti-oxidant status in Type 1 Diabetes Mellitus Patients. **Methods:** It is a comparative study. 60 diabetic patients and 50 Samples of healthy individuals were taken from Nawaz Sharif Hospital. Blood samples (5.0 ml) were obtained and centrifuged at 4000 rpm for 10 minutes to separate the serum. Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), Malondialdehyde (MDA), Nitric oxide (NO), micronutrients (Vitamin A, Vitamin C and Vitamin E) and Electrolytes was determined. **Results:** MDA level is progressively higher in T1DM (14.01±0.06) as compared to control group (1.27±0.21) (P- Value 0.000). GSH status is notably reduced in diabetic patients (0.15±.05) as compared to normal (6.24±0.33). Comparable anti-oxidant catalase is reduced (2.82±.04) in affected individuals as compared to normal individuals 4.19±1.09. SOD level was remarkably marked up to (13.52±3.21) in susceptible persons as compared to normal (2.15±0.23). Vitamin A level was markedly reduced to (1.62±0.26) in patients as compared to healthy individuals (7.18±0.33). **Conclusions:** T1DM patients particularly showed reduced amounts and competency of antioxidant protections due to elevated consumption of specific anti-oxidant components such as low level of intracellular glutathione and Catalase and primarily low levels of vitamin A, vitamin E and vitamin C and exalted level of MDA, SOD and NO.

INTRODUCTION

Typically Diabetes mellitus (DM) is a conflation of multifarious derangements displaying with development of glucose intolerance and high blood glucose level, consequence deficiency of insulin and imperfect insulin functioning [1]. Complications, such as disorders in the regulatory mechanism for mobilization and storage of metabolic fuels arise in the anabolism and catabolism of lipids, proteins and carbohydrates due to insufficient insulin secretions, insulin action or both [2, 3]. Globally in 2010, approximate 285 million people in the age group 20-79 anticipated to have diabetes. By 2030, supposed this approximation is elevated to 438 million. Moreover, in adult population the magnitude of people with impaired glucose tolerance (IGT) is extrapolated to rise to 472 million by 2030. The draining impact of DM interpolates disorders of numerous organs, as a result metabolic complications such as vision impairment, nephrosis, and neuralgia [4]. Regular energy source is imperative for every cell to work in the human body. Glucose is the basic energy source for body, a mobilizable fuel source for cells which rotate in the blood [5]. Pancreatic hormone insulin is effective for blood glucose level regulation on auxiliary side of the cell membrane. The hormone coheres to its receptor sites. Across mandatory channel through glycolysis it manages entry of glucose into breathing cells and tissues. Insulin triggers catabolism, regulates lipogenesis from extreme component of cytoplasm acetyl CoA and glycogenesis from extravagant component of cytoplasm glucose. The above

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mentioned functions virulent to digestion events stimulate the hormone Glucagon, rather by entering the cells. Glucose remains in the blood when glucose level at below verge [6]. Signaling of DM are high blood glucose levels resulting insufficient or defective discharge of insulin from pancreas. Insulin conducted flow of glucose through target cells. Metabolic complications linked with DM that can eventually lead to premature death. At the time of prognosis 25% T2DM patients possess micro-vascular convolution & recommended that instant of prognosis they possessed disease for above than 5 years [7]. In 2006 World Health Organization (WHO) recommended that symptoms for a single elevated glucose level are: excessive quantity of urine, excessive thirst, excessive desire to eat, and weight loss. Furthermore, elevated volume on following incidents like fasting plasma glucose (FPG) ≥7.0mmol/L (126mg/dl), oral glucose tolerance test (OGTT), a plasma glucose ≥11.1mmol/L (200mg/dl) after two hours of the oral dose. For DM, International expert committee in July 2009 proposed the modifier prognosis ethic HbA1C raise ≥6.5%. Particular committee recommended word 'pre-diabetes' may be eliminated but describes the extent, HbA1c values ≥ 6.0% and < 6.5% to confirm those individuals that at high extent of progressing DM [8]. It is a general truth that oxygen is the vital component of life. Anyhow in some situations, when it produces reactive species that generates necrosis, this oxygen may be a killer of cells and eventually the cell death. By the production of particular mechanism Reactive Nitrogen Species (RNS) and Reactive Carbonyl Species (RCS) also stimulate oxidation that intervenes with the normal physiological process inside the cell [9]. Almost 0.1% - 0.5 % of oxygen that fall into the electron transport chain is transferred to superoxide Reactive Oxygen Species (ROS) and the remains are used in metabolic procedures under normal physiological conditions. Other than electron transport chain of mitochondria ROS can also be originated from other sources, like cytochrome P450 [10]. Immoderate levels of molecular oxygen or ROS could result by ineffectual removal of ROS or ROS arising from endogenous or exogenous sources, thus eventual elevated oxidative stress. Oxygen is extremely reactive specie that has the competency to become part of basically dangerous and detrimental molecules. Glutathione, vitamin C and E, cysteine etc. are the different types of biological antioxidants [11]. Acclivity of ROS level due to decrease in demolition or inflation in the generation of catalase, superoxide dismutase and glutathione peroxide antioxidants. The inequality in the levels of above mentioned enzymes make the tissues vulnerable to oxidative stress proceeding progress of diabetic ramification [12]. Mitochondria is key source of oxidative stress in diabetes. Utilized oxygen factor is converted to water and the rest of oxygen is converted to oxygen free radical which is a major ROS that transforms into other RS for instance, ONOO, OH, and H_2O_2 during oxidative metabolism in mitochondria [13]. On insulin alarming ROS and RNS have got negative codification, particularly a risk factor for T2DM [14].

METHODS

It is a comparative, cross-sectional study. The whole experimental work was conducted in the Biochemistry Lab, School of Biochemistry and Medical Lab Technology, Minhaj University, Lahore after the acceptance of ethical and Research committee, Minhaj University Lahore. 5.0 ml blood samples of 60 diabetic patients and 50 samples of healthy individuals were taken in clotted gel vials from Nawaz Sharif Hospital. For the estimation of Reduce Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), Malondialdehyde (MDA), Estimation of Nitric oxide (NO), Estimation of micronutrients (Vitamin A, Vitamin C and Vitamin E) and Electrolytes concentration by flame photometer (Na^+ and K^+) blood samples were further processed. Centrifugation of blood samples was conducted at 4000 rpm for 10 minutes and serum was separated. Blood samples were collected in EDTA tubes. Superoxide Dismutase (SOD) was determined by spectrophotometric method [15]. Determination of Thiobarbituric Acid Reactive Substances (TBARS) in Tissues was conducted for the measurement of MDA by spectrophotometric method [16]. Estimation of Catalase (CAT) was estimated by spectrophotometric method [17]. Estimation of Glutathione (GSH) was estimated by the mechanism of Moron [18]. Determination of Nitric Oxide (NO) was done by a well-recognized method of colorimetric Griess assay [19]. Estimation of Vitamin C (VIT C) or ascorbic acid was analyzed by the method of Roe and Keuther [20]. Estimation of Vitamin A (VIT A) or Tocopherol was analyzed in the plant samples by the Emmutir-Engel reaction as reported by Rosenberg *et al.*, 1992 [21]. Statistical analysis was done by using SPSS (Version 17).

RESULTS

The MDA level is progressively higher in T1DM patients 14±.06 as compared to control group 1.27±0.21 (Table 1). GSH status is notably reduced to 0.15±.05 from normal value 6.24±0.33 in contrast to healthy individuals. Comparable anti-oxidant catalase is reduced to 2.82±.04 in affected individuals as compared to normal individuals 4.19±1.09. SOD level is remarkably marked up to13.52±3.21 in patients as compared to normal group 2.15±0.23 (Table 1).

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Table 1: Anti-oxidative status profile of Type 1 diabetes mellitus patients

Variables	Control (n=50)	Subjects (n=60)
MDA	1.27±0.21	14.0±.06
GSH	6.24±0.33	0.15±.05
Catalase	4.19±1.09	2.82±.04
SOD	2.15±0.23	13.52±3.21

Table 2 demonstrates that vitamin A level is markedly reduced to 1.62±.26 in patients as compared to control group 7.18±0.33. Vitamin C value is also reduced in patient's 0.45±.07 in comparison to control group 6.23±1.08. Vitamin E status is also higher 4.44±0.82 in control group and considerably reduced 2.04±0.54 in patients.

Table 2: Vitamin profile of Type 1 Diabetes mellitus patients

Variables	Control (n=50)	Subjects (n=60)
Vitamin A	7.18±0.33	1.62±.26
Vitamin C	6.23±1.08	0.45±.07
Vitamin E	4.44±0.82	2.04±.54

Results illustrated in Table 3 depicts that the control group has reduced quantity of advanced oxidation protein products (AOPPs) 3.28±0.49 and T1DM patients have eminent quantity of advanced oxidation protein products (AOPPs) 77.29±2.41 . Nitric Oxide value is also noticeably elevated in patients 9.14±0.77 and low level of parameter in control group 2.05±0.35.

Table 3: Different biomarkers of Type 1 diabetes mellitus patients

Variables	Control (n=50)	Subjects (n=60)
AOPPs	3.28±0.49	77.29±2.41
Nitric Oxide	2.05±0.35	9.14±0.77

Table 4 depicts a higher quantity of sodium in T1DM patients 161.19±18.09 as compared to control group (132.23±11.26). Potassium levels are significantly raised in T1DM group (12.63±1.33) as compared to control group (6.29±0.11).

Table 4: Electrolyte profile of Type 1 Diabetes mellitus patients

Variables	Control (n=50)	Subjects (n=60)
Sodium	132.23±11.26	161.19±18.09
Potassium	6.29±0.11	12.63±1.33

DISCUSSION

Diabetes is multifarious metabolic disarray indicated by hyperglycemia developing from inadequate insulin discharge, insulin reluctance activity [22]. T1DM results in an immune-mediated deterioration of β-cells of pancreas, governing to insulin insufficiency. Insulin is required for survival. T2DM commonly develops in obese persons and is linked with high blood pressure and elevation of lipids. Nutrients ability to provoke insulin discharge from β-cell of pancreas, revert their ability progress oxidative fluctuation in islet cells [23]. Oxidation stress is also linked to insulin reluctance, furthermore, participates to poor insulin activity [24]. Hence, the medication goals to decrease insulin reluctance and to activate insulin discharge. T1DM reports 5-10% analyzed cases of diabetes and illustrates hyperglycemia as its indication. Type 1 Diabetes autoimmune disarray causes auto- reactive T cells including immune-mediated recurrence of β-cells [25]. It consequently precedes release of pro-inflammatory cytokines with reactive oxygen components. Marked demolition of pancreatic β-cells in islets of Langerhans along with deficiency of insulin discharge [26]. Free radicals are produced by glucose oxidation, non-enzymatic glycation of proteins moreover, from consequent oxidative deterioration of glycated proteins. Adversely high level of free radicals coetaneous drop of antioxidant protection system leads to degradation of cellular organelles and enzymes, enhanced lipid peroxidation, and progress of insulin reluctance [27]. Destruction in the antioxidant balance develops oxidative stress state. Here complicated association between antioxidant and oxidants for instance ROS, regulates production of oxidative stress. When production of reactive species enhances oxidative stress arises in cellular system, body's antioxidant ability and protection devastates. If free radicals are not ejected by cellular antioxidants, particularly irrupt and destroy lipids, carbohydrates, proteins and nucleic acids. There is increasing confirmation that have linked with pathological diversity of oxidative stress states, involving cancer, cardiovascular diseases, inflammatory incisive disease , deficient supply of blood to a body part and joints pain [28]. Elevated MDA level of plasma, serum and other tissues are particularly documented in diabetic patients. AOPPs that accumulate in aging patient with diabetes known as pro-inflammatory and pro-oxidative compounds may play a significant part in elevating incidence of endothelial impairment and consequent cardiovascular diseases. Various records indicate decreased GSH level in diabetes. Aberrant GSH condition included β-cell disarray moreover pathogenesis of inexhaustible aggravations of diabetes. The irregulation extensively involved during disease states. Catalase is an anti-oxidative enzyme approximately exists in all living organisms [29]. The defalcation enzyme proceeds in β-cell, cumulating in oxidative stress and approximately breakdown cell. Beta cell full in mitochondria, this organelle thought a cause of ROS. By hydrogen peroxide catalase secures pancreatic β-cells from impairment. Poor catalase capacities may induce blood disorder with hemolytic anemia which is associated either to defalcation of glucose-6-phosphate dehydrogenase or obscure conditions and also deteriorate heme proteins, induce cell death also combine with redox active metal ions, generate notably harmful hydroxyl

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radicals[30].

CONCLUSIONS

T1DM patients particularly exhibited reduced amounts and competency of antioxidant protections due to elevated consumption of specific anti-oxidant components e.g. low level of intracellular glutathione and Catalase and primarily low levels of vitamin A, vitamin E and vitamin C and exalted level of MDA, SOD and NO. Hence, prolonged exploration of correlation between ROS, T1DM and its complications in direction to interpret molecular mechanisms by which elevated oxidative stress stimulates progress of diabetes problems may be explored.

Authors Contribution

Conceptualization: HMA
Methodology: GK
Formal analysis: AAA, MA
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All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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