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ACKNOWLEDGEMENT

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Microbial Biotechnology and Bioremediation

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Microbial biotechnology is a rapidly growing field that leverages the abilities of microorganisms for practical applications. From producing biofuels to removing pollutants, microorganisms play a crucial role in shaping our world. Bioremediation is one of the most promising applications of microbial biotechnology, using microorganisms to clean up contaminated sites and restore ecosystems. The impact of human activities has led to widespread pollution, and traditional methods of remediation are often expensive, time-consuming, and not environmentally friendly. Microbial biotechnology offers a sustainable and cost-effective alternative. Bioremediation can be performed using indigenous microorganisms or by introducing specific strains of microorganisms that are capable of breaking down contaminants. These microorganisms use the pollutants as a source of energy and nutrients, transforming them into harmless substances. This process not only cleans up the site, but it also helps to restore the natural ecosystem and biodiversity. The field of microbial biotechnology has made significant progress in recent years, with advancements in molecular biology and genomics enabling a deeper understanding of microorganisms and their capabilities. The development of new bioremediation techniques has allowed for the efficient removal of a wide range of contaminants, including heavy metals, oil and other petroleum products, and toxic organic compounds. These techniques have proven to be effective in treating contaminated soil, groundwater, and surface water [1]. However, the implementation of bioremediation techniques is not without its challenges. One of the biggest challenge is identifying the most effective strains of microorganisms for a specific contamination site. The environmental conditions, such as pH, temperature, and nutrient level can have a significant impact on the efficacy of bioremediation. Furthermore, the presence of multiple contaminants can complicate the process, requiring the use of multiple strains of microorganisms. Despite these challenges, the benefits of bioremediation, including sustainability and cost-effectiveness, make it a promising solution for cleaning up contaminated sites. The impact of microbial biotechnology and bioremediation goes beyond just environmental remediation. By leveraging the abilities of microorganisms, we can also contribute to the development of new and innovative technologies. For example, biorefineries are being developed to produce biofuels, bioplastics, and other bioproducts using sustainable processes. This not only helps to reduce the reliance on fossil fuels, but it also helps to reduce greenhouse gas emissions and promote sustainable development. Microbial biotechnology and bioremediation are critical fields that offer solutions to some of the biggest environmental challenges facing our world. With continued investment and research, we can develop and implement more effective and sustainable bioremediation techniques. These techniques will play a key role in cleaning up contaminated sites, restoring ecosystems, and promoting sustainable development. It is our hope that the benefits of microbial biotechnology and bioremediation will be recognized and supported by governments, organizations, and the public, allowing us to make a positive impact on our world.

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Original Article

Clinical Implication of Hepatic Phosphatases in Hyperthyroidism

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INTRODUCTION

Hypothalamus is the part of brain which controls the production of thyroid hormones and also involved in the regulation of pituitary gland. Thyrotropin releasing hormone (TRH), produced by hypothalamus sends a signal to the pituitary gland that activates the thyroid stimulating hormone (TSH) which upon activation releases the thyroid hormones [1, 2]. Around 80% of the tetraiodothyronine (T4) is converted to its ten times more active form, triiodothyronine(T3)by peripheral organs. Thyroxine(T4)is synthesized by the protein of thyroid residue, thyroglobulin and follicular cells [3]. The susceptibility of thyroid disorder increases with the age and the women are seven times more prone to get thyroid diseases as compared to men [4]. Incidence and prevalence rate of thyroid disorders also depend on the genetic, geographical and environmental predisposition factors and also on the abundance or

deficiency of available iodine. Overproduction and secretion of thyroid hormones manifests increased metabolic rate of the body and wide range of clinical signs [5]. Graves' disease, thyrotoxicosis, toxic multinodular goiter(TMNG) are some conditions with clinical depiction of hyperthyroidism. Certain medications also increase the risk of hyperactivity. Amiodarone is an example which is prescribed during cardiac issues, contains a large amount of iodine which ultimately leads to thyroid abnormalities. Hepatic variations in hyperthyroid patients have been studied widely. Sometimes these changes are linked with the use of anti-thyroid drugs [6-8]. Propylthiouracil (PTU), mostly used drug in hyperthyroidism, is not good for the liver as it has been reported with bad profiles of hepatic functions [9-11]. The other parameters which are reportedly associated with anti-thyroid drugs are anemia,



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Thyroid hormones significantly affect the proper growth, development and functioning of liver. It has been seen that the drugs indicated for thyroid abnormalities also cause troubles in liver function. **Objective:** To check the effect of thyroid abnormality on liver function. **Methods:** Patients were recruited from Center of Nuclear Medicine and examined for their thyroid status and liver functions. **Results:** The results obtained through biochemical tests for potential biomarkers were further explored through statistical analysis which showed the strong correlation between disturbed function of thyroid gland and liver working. Any variation in thyroid function brings change in liver functioning. **Conclusions:** Therefore, while treating thyroid patients, combination therapy must be recommended to effectively treat the associated disorder and increasing the better social and psychological status of the patient. Furthermore, patients coming with thyroid dysfunction must also be examined for liver abnormalities so that culprit cause of this thyroid abnormality could be treated from the root if present.

tachycardia, hypercalcemia, granulocytosis and higher levels of alkaline phosphatases and transaminases [12]. Pakistan is that country in which the deficiency of iodine is common and mostly the occurrence is seen more in northern areas. Due to the hyperactivity of the thyroid gland almost every tissue is affected. The reported signs are anxiety, nervousness, thinning of skin, hand tremor, increased heart rate, increased perspiration, muscular weakness and disturbed bowl movements. Hyperthyroidism varies with the patient age, diet, excessive hormonal secretion and the duration of illness. The older patients exhibit some specific signs and symptoms which make the evaluation of hyperthyroidism even more difficult. Shen et al., described a case of 24year-old patient diagnosed with hyperthyroidism who later on developed liver failure due to treatment with thiamazole. Liver functions were not evaluated at the time of diagnosis [13]. Behroozi-Lak et al., worked on a case of women of 89 years of age. The symptoms were constipation, weakness, fatigue, indigestion, and urinary leakage. Biochemical tests showed the increased levels of calcium, ALP and PTH, and the primary hyperthyroidism was diagnosed. After that they also see the higher levels of ALP which cause the renal complications. ALP involved in the alkaline hydrolysis of a large number of compounds which are naturally occurring and made synthetically [14]. ALP found in the cell membrane and all tissues of the body. In the intestinal epithelium, kidney tubules, bones, liver and placenta have higher values of ALP. ALP is also involved in the transportation of the lipid and as well as bone calcification. The activity of ALP is non-functional at the 56 °C but it is functional at low temperature. Serum ALK phosphatase should be measured in hepatobiliary and bone diseases. When tissues secrete bile it effects on the liver ALP that enhanced the serum enzyme action [15, 16]. In hepatobiliary disease enzyme is failed to secret the bile. In the condition of hepatobiliary disease, the liver synthesizes more ALP. The newly synthesized enzymes enter in the circulation and due to this the ALP level becomes high in the blood serum. Some drugs like chlorpromazine increases the level of ALP up to 2.5 times than the normal limit. Levels of ALP are also increased in infectious hepatitis. In osteomalcia the ALP is moderately higher which is usually decreased with vitamin D therapy. In Fanconni's syndrome the level of ALP is moderately elevated. The level of ALP is higher in primary and secondary hyperthyroidism due to the involvement of the skeleton. In osteogenic bone cancer the level of the ALP in highly elevated. In the children due to the bone growth the level of ALP is 1.5 to 2.5 times higher than the normal adults. In first trimester 2 to 3 folds increase has been seen. Causes may be attributed to some complications in

pregnancy like gestational hypertension and preeclempsia.

METHODS

From the CENUM (Center for Nuclear Medicine) Mayo Hospital Lahore, 115 patients of both sexes, ranging in age from 40 to 60 years, were selected as freshly referred patients for thyroid evaluation. According to the referring doctor's advice, these patients underwent clinical evaluation, thyroid scanning, and FT4 and TSH concentration determination. The skilled doctor carefully recorded the patient's medical history. The thyroid gland was physically inspected, and a clinical evaluation of its functioning status was performed. Name, age, gender, medication use, surgery, prior instances of thyroid dysfunction, use of iodized salt, the presence of a goitre and its size, and family history of thyroid problems were all part of the patient's medical history. There were additional symptoms and signs of thyroid dysfunction observed. Serum was isolated from blood by low-speed centrifugation (2000 g) for five minutes at room temperature. Until analysis, serum samples were kept at -20°C. For FT4, ALK, and TSH, serum samples were examined. Using commercial kits from Immunotech Inc., FT4 and ALK were determined using radioimmunoassay (RIA), while TSH was determined using IRMA methods (Beckman, Czech Republic). ELISA was used to measure the serum TPO-Ab titer in a subset of patients using a commercial kit from IMMCO Diagnostics, Inc. in New York, USA.

RESULTS

Figure 1 showed mean, standard deviation and standard error of mean of control and hyperthyroid population for free tetraiodothyronine 4 were 16.901, 2.10205 and + 0.28344; 38.8593, 13.23540 and + 1.72310 respectively. The standard deviation and standard error of mean were found to be highly changed among controlled and diseased population. Statistical values of this parameter for both study groups i.e., control and diseased, showed high difference. Significance or non-significance of data were estimated using analysis of variance test among study population for all designed study parameters. p- values more than 0.01 were set to be non-significant and below 0.01 were considered significant. All study parameters i.e., free tetraiodothyronine 4, thyroid stimulating hormone and alkaline phosphatase appeared highly significant. The results appeared with statistical analysis show that there is a strong correlation between disturbed function of thyroid gland and liver function. A variation in thyroid function brings change in liver function.



Means and SEMs variations in FT4 levels of Control and

Figure 1: FT4 level in control and hyperthyroid patients The mean, standard deviation and standard error of mean of control and hyperthyroid population for thyroid stimulating hormone were 1.5195, 0.69794 and + 0.09411; 0.0500, 0.00000 and + 0.00000 respectively (Figure 2).











DISCUSSION

Hyperthyroidism is a clinical condition that is characterized by the overproduction and secretion of thyroid hormones, leading to an increased metabolic rate and a wide range of clinical signs. The thyroid hormones are

Hepatic Phosphatases in Hyperthyroidism DOI: https://doi.org/10.54393/fbt.v2i02.20

regulated by the hypothalamus-pituitary-thyroid axis, and any disruption in this axis can lead to the development of hyperthyroidism. The clinical manifestations of hyperthyroidism can vary depending on the age of the patient, diet, excessive hormonal secretion, and the duration of the illness [17]. In patients with hyperthyroidism, liver function tests may reveal increased levels of alkaline phosphatases (ALP). ALP is a group of enzymes that are present in various tissues, including the liver, bone, and intestine. ALP is produced by osteoblasts in bone and cholangiocytes in the liver, and it plays an important role in bone mineralization and the transport of bile acids. In hyperthyroidism, the increased metabolic rate leads to an increase in bone turnover, which in turn increases the production of ALP. Additionally, hyperthyroidism can lead to an increase in the size of the liver, which can also contribute to an increase in ALP levels. Several studies have reported an association between hyperthyroidism and elevated levels of ALP. Boulanger et al., measured the levels of ALP in two groups of hyperthyroid patients, one group receiving treatment and the other group not receiving treatment [18]. They found that the total, liver, and bone ALP activities were significantly higher in the untreated hyperthyroid group than in the treated hyperthyroid group. They also found that bone ALP was higher than intestinal and liver ALP. Einollahi et al., reported a case of a 36-year-old woman with hyperthyroidism who was prescribed Diazepam for anxiety and palpitations. She experienced skin pigmentation and weight loss and was subsequently prescribed Carbimazole. After a month of therapy, clinical improvements were observed, and it was concluded that thyroxin acts as an inducer for different enzymes, including ALP, which triggers osteoblastic activity [19]. Shen et al., reported a case of a 24-year-old patient diagnosed with hyperthyroidism who later developed liver failure due to treatment with thiamazole [13]. During the course of treatment, high levels of ALP were seen, and liver biopsy showed intrahepatic cholestasis, mild inflammatory infiltrates, and a high degree of fibrosis. The authors suggested that regular liver function monitoring during thiamazole therapy is imperative in such patients [20]. In conclusion, elevated levels of ALP are commonly seen in hyperthyroidism, and it is important to monitor liver function in patients receiving treatment for hyperthyroidism. While the increase in ALP may be due to the increased metabolic rate and bone turnover associated with hyperthyroidism, drug-induced liver injury is also a potential complication of treatment. Therefore, regular liver function monitoring is essential to ensure early detection and management of any hepatic dysfunction.

CONCLUSIONS

While treating thyroid patients, liver markers must be assessed, and, in case of any parallel liver abnormality combination therapy must be recommended to effectively treat the associated disorder. Furthermore, during the course of ongoing thyroid therapy, patients must also be re-evaluated for other potential biochemical markers so that culprit cause of this thyroid abnormality could be treated from the root if present.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Assessment of TNF-a Gene Expression in Type 2 Diabetes Patients with Nephropathy

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INTRODUCTION

Type 2 diabetes (T2D) is the most well-known type of diabetes and an increasingly pervasive metabolic infection. It is related with microvascular and macrovascular complications and is viewed as one of the significant reasons for dismalness and mortality[1-3]. Diabetes can be ordered into four clinical classifications. Type 1 diabetes mellitus, it is safe intervened diabetes which involves 5-10% instances of diabetes. It is named as insulin subordinate diabetes mellitus (IDDM) or adolescent diabetes results from cell interceded resistance [4, 5]. Type 2 diabetes mellitus, it is additionally called grown-up beginning diabetes or non-insulin subordinate diabetes (NIDDM). It involves 90-95% instances of DM. Patients of NIDDM have insulin obstruction because of which there is a family member (when contrasted with supreme) insufficiency of insulin. Gestational diabetes mellitus, it is a

ABSTRACT

Type 2 diabetes mellitus (T2DM) and its complications, including nephropathy, are a significant public health concern worldwide. Tumor necrosis factor-alpha (TNF- α) is a proinflammatory cytokine that plays a crucial role in the development and progression of chronic inflammatory diseases, including T2DM and diabetic nephropathy. **Objective:** To evaluate of TNF- α gene expression in patients with T2DM and nephropathy compared to healthy individuals. **Methods:** The cross-sectional study conducted on 120 individuals divided into three groups: healthy individuals, type 2 diabetes mellitus (T2DM) patients without diabetic nephropathy, and T2DM patients with nephropathy. RNA was extracted and TNF- α gene expression was evaluated using PCR and statistical analysis was done using SPSS software. **Results:** The results showed almost 4.2-fold induced expression of TNF- α in T2DM patients without nephropathy compared to the normal group. **Conclusion:** The study reports that in diabetic nephropathy patients, Gene expression of gene TNF- α shows increases in cases when compared with healthy subjects.

brief condition that happens during pregnancy. It influences around of 2-4% everything being equal and includes an expanded danger of creating diabetes for both mother and youngster [6, 7]. A few realities validate the significance of heritability in T2DM: the more noteworthy concordance between monozygotic twins than among dizygotic twins and a wide variety in the pervasiveness of T2DM in epidemiological investigations with various ethnic gatherings just as positive outcomes in various other hereditary examinations [8, 9]. In such manner, it ought to be noticed that even as in excess of 30 qualities related with T2DM have been distinguished, the commitment of every individual quality in the ailment weakness is little. Furthermore, a large portion of these qualities distinguished are identified with brokenness of pancreatic β cells [10-12]. TNF alpha is so important in DM and



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nephropathy so in this study we will analyze gene expression of TNF alpha on PBMC in Diabetes with and without nephropathy as compared to healthy.

METHODS

It was a case-control cross-sectional research. The study was conducted at the Immunology and Resource lab of the University of Health Sciences in Lahore. The sample size for each group is computed to be 40. This research examined a total of 80 participants, who were separated into two groups of 40 persons each. Group I has 40 healthy patients. Group II consists of 40 T2DM patients without of diabetic nephropathy. DN was diagnosed based on the presence of microalbuminuria, which was defined as 30 to 300 mg albumin/24 hours or an albumin to creatinine ratio (ACR) of 30 to 300 mg/g, or macroalbuminuria, which was defined as >300 mg albumin/24 hours or an ACR >300 mg/g (American Diabetes Association., 2014). Diagnosis of diabetes was made when fasting blood glucose was 126 mg/dl or HbA1c was 6.5%. (ADA., 2014). 5ml venous blood was collected in EDTA coated vacationers from T2DM patients with and without nephropathy and was brought to the Resource lab within 4 hours of the sample collection to avoid genomic RNA degradation. The primers were suspended using low TAE buffer in a calculated amount to achieve concentration 1µg/µl as stock. A working solution of 10pm/µl diluted from stock were used for all further PCR experiments. Primers were optimized for reaction conditions of annealing temperature, Mg concentration, amount of buffer and dNTPs. These optimum conditions were in further experimentation. The following primers was used.

Gene	Primer	GC content (%)	Product Size
TNFα-F	5' CGAGTGACAAGCCTGTAGC 3'	45	//EZ
TNFα-R	5' GGTGTGGGTGAGGAGCACAT 3'	50	400

Table 1: Primer used for PCR

RNA was extracted from blood samples within 6 hours of sample collection. Samples were stored in trizol if extraction is delayed. RNA quality and quantity were assessed using nanodrop technology. PCR reaction was followed by gel electrophoresis. The statistical programme SPSS was used for all calculations (version 20.0).

RESULTS



Figure 1: Distribution of male and female in both group



Figure 2: GAPDH PCR Results of cDNA Primer optimization (TNFa)

Correlation between AST, ALT and expression levels of genes TNF-a was carried out through Pearson correlation test. There was a significant negative correlation between levels of ALT and AST and expression levels of TNF-a. Correlation was also computed between two genes and it was found that there is no correlation between in TNF-a cases as well as in controls (Table 2).

	TNF-a	ALT	AST
TNF-a	1	-	-
ALT	0.1693	1	-
AST	0.0786	0.6592	1

Table 2: Correlations between AST, ALT, TNF-a

DISCUSSION

TNF- is a proinflammatory cytokine that plays a key role in the development and progression of chronic inflammatory illnesses, such as type 2 diabetes mellitus (T2DM) and diabetic nephropathy (DN) [13-15]. T2DM is a metabolic illness defined by elevated blood glucose levels owing to insulin resistance, while DN is a long-term consequence of diabetes that compromises the function of the kidneys. The study of TNF-gene expression in T2DM and DN patients as compared to healthy individuals might give useful insights into the molecular processes underlying the pathophysiology of these illnesses and prospective treatment targets. For quantitative real-time polymerase chain reaction (gRT-PCR) analysis, researchers took blood or tissue samples from participants and extracted RNA, which is subsequently reverse-transcribed into complementary DNA (cDNA). gRT-PCR measures the quantity of messenger RNA (mRNA), which corresponds to the level of gene expression. qRT-PCR has been utilised in several studies to analyse TNF- gene expression in T2DM and DN patients. Reidy et al., did a research comparing the gene expression of TNF- in peripheral blood mononuclear cells (PBMCs) between T2DM patients with and without DN and healthy controls [16, 17]. TNF- a gene expression was considerably greater in T2DM patients with DN compared to those without DN and healthy controls, as shown by the findings. Kalantarinia et al., investigated the expression of the TNF- gene in renal biopsies from patients with DN and

healthy controls [18, 19]. TNF- gene expression was considerably greater in renal biopsies from patients with DN compared to healthy controls, according to the research. TNF- a gene expression is elevated in T2DM patients with DN relative to those without DN and healthy controls, suggesting a possible role for TNF- in the formation and progression of DN. It is essential to highlight, however, that these studies have limitations, such as limited sample sizes and the possibility of confounding variables, which may alter the interpretation of the findings [20]. Therefore, comparing the TNF- a gene expression of patients with T2DM and DN to that of healthy individuals might provide light on the molecular processes underlying the development of these disorders. To validate these results and further understand the function of TNF- a in T2DM and DN, however, more investigations with bigger sample numbers and more stringent study designs are required.

CONCLUSIONS

The study reports that in diabetic nephropathy patients, Gene expression of gene TNF-a shows increases in cases when compared with healthy subjects.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

VEGF Genotype and Allele Frequency of Diabetes Mellitus and Diabetic Retinopathy in Lahore, Pakistan

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ABSTRACT

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INTRODUCTION

Diabetic retinopathy is characterized as basement membrane (BM) thickening, pericyte loss, endothelial cell (EC) dysfunction, microaneurysms, microvascular infarcts and neovascularization in a patient with diabetic retinopathy. **Objectives:** To determine the VEGF

renovascularization in a patient with diabetic retinopathy. **Objectives:** To determine the VEGF genotype and allele frequency of diabetes mellitus and diabetic retinopathy in Lahore, Pakistan. **Methods:** A total of 100 blood samples were taken including diabetes mellitus (50) and diabetic retinopathy patients (50). Diseased and control subjects were selected for blood sampling. Demographic and clinical characteristics was evaluated. The BMI, HbA1c and the blood pressure of both groups were also examined. The VEGF genotype and allele frequency of diabetes mellitus and diabetic retinopathy was done. The statistical analysis was done by chi-square test and SPSS to study significant differences in control and diabetic retinopathy subjects. **Results:** The normal estimation of DM group was 46.18 ± 1.23 years while of DR class was 52.86 ± 1.36 years. The mean value of BMI of DM group was 26.0 ± 0.62 while of DR group was 26.57 ± 0.70 . DD genetic mutation was substantially higher in diabetic retinopathy bunch (p<0.05) relative to the II gene mutation, the huge contrasts (p<0.05) were seen in diabetic group. **Conclusions:** This study concluded that Vascular endothelial growth factor gene was detected in both group of diabetes. In retinopathy people with T2DM the substantial elevated VEGF DD genetic variation was seen relative to retinopathy people without diabetes.

Diabetes mellitus (DM) is a set of metabolic issues categorized by prolonged high blood glucose level made by deficits in insulin function. The significance of insulin as an anabolic hormone causes metabolic anomalies in carbohydrates, lipids and proteins [1]. The standard diabetes grouping suggested by the American Diabetes Association as T1D, T2D and other forms announce in 1997 [2]. Type 1 diabetes is 5%-10% of patients identified with diabetes, and is affected by the damage of β cells of pancreas. Type 1 diabetes constitutes for 80% -90% diabetes for teenager and adolescents [3-5]. According to the International Diabetes Federation (2013), the overall frequency of diabetes in individuals (>20-79 years of age) a study released by IDF in 2013 was 8.3%, with 14 million greater number of men than women, the bulk aged between 40 and 59, and the figure is expected to

increase above 592 million by 2035 with worldwide incidence of 10.1%. The maximum incidence of diabetes has been reported in Middle East and North Africa (10.9%), while, maximum adults were identified with diabetes (138.2 millions) in Western Pacific area and there is maximum incidence of diabetes in many states. According to the National Health Survey the frequency of diminished glucose tolerance and diabetes in Pakistan is present amongst the people of age >25 years, is 22.4% [6]. Diabetes Mellitus is one of the greatest hazardous sophisticated ailments that affects health and give rise to societal difficulties. It is recognized as a universal epidemic of the 21st century. The occurrence of this disorder is still growing and is frequently related to critical problems that causes the bigger danger and death in diabetic patients[7]. There are many patients who do not experience any



problem for several years like DR, diabetic neuropathy and DN. Lifelong consequences of hyperglycemia in the first years after the identification of type 2 diabetes mellitus are believed to rely on epigenetic conditioned metabolic memory [8]. The predictive variability between all people with diabetes designates a possibly heterogenous existence of many diabetic complications that may be correlated with genetic predisposition [9]. Genetic influences assume a significant part in the pathological process of T2DM which defines the clinical course of type 2 diabetes mellitus and associated abnormalities are determined by the environmental conditions [10]. Some microvascular abnormalities correlate unregulated glycemic rates in diabetic subjects [11]. In people of age 20-74 years the primary reason of blindness is DR [12]. From past reports that were done in 1990 to 2010, diabetic retinopathy was considered as the fifth usual reason of unnecessary vision loss and is the source of mild to extreme vision disorders [13].

METHODS

For this research, total 100 individuals (n=100) were selected and were equally divided in two groups i.e. control group and diseased group. Individuals with type 2 diabetes mellitus (n=50) were considered as control group whereas individuals having diabetic retinopathy (DR) (n=50) were considered as diseased group. All the individuals were selected from District Lahore, Punjab, Pakistan. The sampling was done from private sector hospital i.e. Mughal Eye Hospital (Trust), Lahore. Patients were examined by ophthalmologist Doctors. The experimental work was carried out in hi-tech equipment in University of Health Science Lahore, Lahore. Before participation of diseased group in research, each individual was undergone for complete ophthalmological examination i.e. best corrected visual acuity, slit lamp examination and retinal examination. Fluorescein angiography was done for confirming the presence or absence of diabetic retinopathy of diseased group.Whereas, severity level of retinopathy was determined by diabetic retinopathy severity scale. The following individuals were included for diseased group: Type 2 diabetes patients. Age at onset from above 20 to 75 years. Duration of disease \geq 5 years. Diabetic retinopathy patients without macula edema condition. The individuals having ocular trauma, nephropathy or any other blood borne disease were excluded from this research. A questionnaire performa was designed to take general information (age, gender, family history, BMI, smoking, diabetic duration, lifestyle etc.) from individuals of control group and diseased group. Before sampling, all the individuals of control and diseased groups signed a consent form. Blood samples of all the individuals were collected in labeled falcon tubes having (200µI) 0.5M EDTA solution. All the falcon tubes were vortexed to mix the blood and EDTA solution and after vertexing, tubes were stored in freezer at -20°C. Organic standard chloroform and ethanol precipitation method was used for DNA extraction. Oligonucleotide primers were used for the amplification of needed gene fragment or polymerase chain reaction (PCR). A particular set of forward and reverse primers were used for VEGF gene through published work which was used in past studies. The analysis of data was done with the help of SPSS software (version 20) to determine the significance and its degree between demographic characteristics of different groups by using Chi-square test.

RESULTS

The present research was intended to discover the relationship of diabetic retinopathy (DR) with VEGF gene and with various clinical and demographical boundaries in the number of inhabitants in Lahore. Within 100 study subjects, 50 subjects having DR considered as unhealthy group and 50 were control having DM. Among the ailing group, 52% were women and 48% were men whereas in examination with control, 56% were women and 44% were men. During sampling, sexual orientation was chosen arbitrarily. Table 1 indicates DR participants average age was 52.85 ± 1.36 whereas in healthy subjects the average age was 46.18 ± 1.23. The normal estimation of DM group was 46.18 ± 1.23 years while of DR class was 52.86 ± 1.36 years. Table 4.1 demonstrates the p estimation of this variable. The information about age was categorized into four classes. Class I (31-40), class II (41-50), class III (51-60), class IV (61-70). The mean value of BMI of DM group was 26.0±0.62 while of DR group was 26.57±0.70. The p value of BMI of two groups was 0.55. A large portion of people were extremely obese. The average length Of DM group was 7.38±0.38 and 11.12±0.75 of DR group (table 1). The average value of systolic blood pressure of DM group was 132.70±1.850 (mmHg) whereas of DR group was 135.32±1.564. The average value of diastolic pulse was 84.84±0.941 for DM group whereas 82.64±0.895was for DR group.

Mean <u>+</u> S.D age (years)	46.18±1.23	52.86±1.36	0.000*
Gender (female/male)	49/51	52/48	
Duration (years)	7.38±0.38	11.12±0.75	0.000*
BMI (Kg/m2)	26.0±0.62	26.57±0.708	0.556**
HbA1c level	7.4±0.053	7.6±0.063	0.082***
Diastolic Blood Pressure (mmHg)	84.84±0.941	85.64±0.895	0.539**
Systolic Blood Pressure (mmHg)	132.70±1.850	135.32±1.564	0.282*
Family history (%)	40/50(80%)	42/50(84%)	81% (average)

Table 1: Clinical and demographical factors of DM and DR groups

 * Significant, ** Significant, *** Highly significant

Vascular endothelial growth factor gene sequencing was financially conducted. In retinopathy people with T2DM the substantial elevated VEGF DD genetic variation was seen relative to retinopathy people without diabetes. Table 2 demonstrates the genetic mutation of vascular endothelial growth factor and rates of alleles in all examination disciplines.

Study Groups	Genotype Frequency			Allele frequency	
otday oroups	11	ID	DD	- I	D
T2DM with DR	12%	36%	52%	0.30	0.70
T2DM without DR	26%	34%	40%	0.43	0.57

Table 2: VEGF genotype and allele frequencies

DD genetic mutation was substantially higher in diabetic retinopathy bunch (p<0.05) relative to the II gene mutation as demonstrated in table 3 the huge contrasts (p<0.05) were seen in diabetic group. These correlations indicate that huge contrasts were present among gene variation of diabetic retinopathy and diabetes mellitus groups.

Study subjects	Chi-square value	Degree of freedom	p-value	95% Confidence Interval	
DM vs. DR	9.490	2	<0.05(0.009)	0.009-0.013	

Table 3: Comparison of (I/D) VEGF genotypes using Chi-square



Figure 1: 2 % agarose gel showing the optimization of PCR product $D \ I \ S \ C \ U \ S \ S \ I \ O \ N$

Diabetes Mellitus (DM) is not only a disorder that involves ailment yet in addition results in financial issues that likewise influences the estimation of life, therefore it is one of the greatest disturbing illness of general public. It is additionally perceived as highly predominant disorder of 21^{st} epoch worldwide[7]. Whereas diabetic retinopathy(DR) because of microvascular complexity of uncontrolled and life-long diabetes mellitus which results in visual deficiency in people. The common frequency of diabetic retinopathy was 34.6% whereas occurrence of extreme phases of this aliment was 10.2%[14]. The joined frequency was 28.78% in diabetic patients of Pakistan with contrast from 10% to 90% [15]. The expanded ischemical states of the tissue and hyperporouness in retina define DR. VEGF is a glycoprotein because of its attributes of formation of new

blood vessels formation appeared to be a conceivable applicant quality of diabetic retinopathy. Vascular endothelial growth factor guideline for the formation of new blood vessels is enacted when variables lead towards low oxygen that causes hypoxia are attached to the segment of hypoxia response and starts the VEGF release that consequently starts formation of new blood vessels [16]. The mechanism of formation of new blood vessels formation is strongly regulated by non-developmental and encouraging stimuli [17]. A latest analysis showed that vascular endothelial growth factor grafting is changed from non developmental to a pro-developmental state in the eyes of patients with diabetes and 460 allele may be a crucial element in the control of isoforms of VEGF [18]. In this investigation, we likewise surveyed demographical and clinical factors such as, sex, age, HbA1c percentage, BMI, financial status as a hazardous aspect for expectation of diabetic retinopathy. In this present investigation, women are more predominant when contrasted with men. Yang et al. (2011) saw similar consequences of sex in people of China. Sexual orientation as such does not make a difference in diabetic retinopathy [19]. However, different components for example HbA1c percentage, body mass index and period of diabetes with retinopathy with diabetes and associated with the commencement and progression of diabetic retinopathy. Body mass index and hemoglobin percentage were non-critical in this investigation. However, a few investigations indicated contradictory effects about relationship of body mass index with diabetic retinopathy [20]. Diabetes mellitus span is significant indicator of diabetic retinopathy. There will be multiplied the frequency of diabetic retinopathy as the people experiencing ten to nineteen years of diabetes yet with over twenty years of diabetes have right around multiple times more risks of diabetic retinopathy [21]. Smoking additionally watched hazard predictor for some infections. In this investigation, the level of smoking for diabetic retinopathy was 2%, whereas 7% was for diabetes bunch. It is determined that smoking does not impact diabetic retinopathy positively. Some past investigations clarified that smoking did not establish a fundamental aspect for diabetic retinopathy. Solid family ancestry was additionally go about as danger aspect in the progression of numerous ailments. Particularly the disorder like DM indicates the significance of family ancestory. Particularly more than half considered groups had acquired this issue from fatherly or maternal foundation.

CONCLUSIONS

This study concluded that Vascular endothelial growth factor gene was detected in both group of diabetes. In retinopathy people with T2DM the substantial elevated VEGF DD genetic variation was seen relative to retinopathy people without diabetes

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Seroprevalence of Crimean Congo Hemorrhagic Fever Virus in Livestock, Pakistan

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INTRODUCTION

Crimean The Crimean Congo Hemorrhagic fever virus belongs to the family *Nairoviridae* and genus *Orthonairovirus* and is one of the most prevalent tick-borne viruses [1]. CCHFV is the causative agent of severe haemorrhagic disease in humans with a high fatality rate of up to 30% [2]. Several tick genera can become infected with CCHFV however ticks of the genus *Hyalomma* are the principal vector [3]. Many vertebrate hosts like dogs, cattle, sheep, hares etc. get infected by the bite of infected ticks and the virus remains in their bloodstream for about one week after infection, allowing the tick-animal-tick cycle to continue when another tick bites. Humans acquire this

ABSTRACT

Crimean-Congo Haemorrhagic Fever Virus (CCHFV) is among the deadly human pathogens which cause a highly lethal haemorrhagic fever. CCHFV, a high-priority zoonotic pathogen is distributed widely and is transmitted in a vertical transmission cycle through these animals. Humans get infected by an infected tick bite, contact with viremic livestock blood, and through nosocomial route. Several CCHFV outbreaks have been reported for the past 2 decades in Pakistan and the virus has emerged in previously non-endemic regions as well. It is important to screen animals for CCHFV through an efficient diagnostic assay to prevent the viral zoonotic spill over to humans. **Objectives:** To screen the presence of CCHFV in sera collected from cattle, goat, and sheep in various regions in Punjab, Khyber Pakhtunkhwa (KP) and Sindh through a preestablished IgG ELISA assay. Methods: A recombinant nucleoprotein (rNP) of CCHFV was used to capture the anti CCFHV IgG antibodies in the animal sera. Results: Among 164 animals tested, 65% (103/164) showed the presence of IgG CCHFV antibodies. From the total 103 animals tested positive, 14.5 % (CI 0-28.2%) were cattle, 63.7% (CI 38.5-60.3%) were goats and 42% (CI 24.4-48.8%) were sheep. Conclusions: High seroprevalence of the CCHFV was expected from these areas as numerous cases of CCHFV have been reported previously. Since no commercial tests are available for the detection of CCHFV-specific antibodies in animals, this IgG ELISA test can be used to screen the animals in areas at risk such as those that have the presence of permissive ticks.

> infection from bite of an infected tick or by close contact of blood from an infected animal. Moreover, human to human transmission is also common in nosocomial setting. In animals, CCHFV infection shows mild or no clinical symptoms, but they do develop viremia that can last around 15 days [4] however in humans, the infection shows severe symptoms with high fatality rates if left untreated [5]. In humans, CCHF is manifested by fever, headache, vomiting, diarrhea, and muscular pain and bleeding diathesis with multiorgan dysfunction is observed in more severe cases [6]. Of the epidemic-prone diseases prioritized by the WHO R&D Blueprint, CCHF is the most widespread and is found in



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around 30 countries. CCHFV is distributed throughout the world and several outbreaks have been reported worldwide [7-8]. The incidence of this infection is consistent with the presence of the permissive ticks, Hyalomma, present in the geographical location [9]. The presence of Hyalomma ticks have been reported in many areas throughout Pakistan as well. A licenced vaccine or specific treatments are still not available for CCHFV immunization. Ribavirin is used as a general treatment, but it only helps alleviate the infection if it is given at an early stage hence it is important to diagnose the infection at an early stage. Pakistan is mainly an agricultural country and hence livestock forms an important part of the economy. Close contact of animals with humans is a major cause of spread of CCHFV infection. People in rural areas are particularly at risk because they are mostly employed with animal husbandry. Moreover, there is an influx of animals throughout the country during Eid ul Adha so the risk of infection is higher during that time. Furthermore, the recent political unrest in the country has also played a role in the spread of CCHFV as there has been an influx of refugees from Afghanistan which is a CCHFV endemic area [10]. Regular screening of ruminants and domestic animals helps identify the geographical areas where CCHFV circulates and can also prevent the spread of infection to unsuspecting human [11]. The first CCHF case was reported in Pakistan in 1976 and since then many mini outbreaks have happened especially in Baluchistan [12]. In this study animal blood samples from various regions in Punjab (Islamabad, Rawalpindi), KPK (Kurram agency), Sindh (Tando Allahyar, Karachi, Larkana, Hyderabad) were tested using enzyme linked immunosorbent assay (ELISA) reported previously to have high sensitivity and specificity for the detection of CCHFV directed IgG antibodies [12]. A recombinant nucleoprotein of the CCHFV belonging to a Chinese strain 8402 sourced from Department of Veterinary Science, National Institute of Infectious Diseases, Japan was used to capture the IgG antibodies directed against CCFHV. This nucleoprotein is relatively conserved and can be used to detect many strains of the CCHFV. This assay is cost effective and can be replicated easily in labs with limited facilities such as those in rural areas. Assays available commercially are largely intended for use in human so this assay will help with serological screening of animals which is very important in the onehealth approach.

METHODS

For this study, 164 animal serum samples from regions including Karachi, Islamabad, Rawalpindi, Tando Allah yar, Hyderabad, Larkana and Kurram Agency were screened for the presence of anti CCHFV IgG antibodies. The animal blood samples were collected in collaboration with Pakistan Agricultural Research Council (PARC). The

samples were heat inactivated at 60 °C prior to testing to destroy any viral particles present [13]. The ELISA was carried out as previously described [12]. Briefly, the ELISA plates were coated with 100µl per well of the antigen (1: 400 dilution) and incubated overnight at 4 °C. The plates were washed with Tween20-Phosphate Buffered Saline solution (PBS-T) and inoculated for an hour using a blocking solution (PBS containing 0.5% Tween-20, 500 µg dextran sulfate/ml and 5% heat-inactivated horse serum). Following a third PBS-T wash, test samples that had been diluted four times from 1: 100 to 1: 6,400 were added to the plates. The samples were first incubated for an hour at 37 °C, followed by three PBS-T washings. Following that, the plates were infected for an hour at 37 °C with an anti-cow/goat antibody labelled with HRPD (1: 1000 dilution, Zymed). The optical density was measured at 405 nm after the plates had been cleaned and inoculated with ABTS (2,2 -azinobis 3ethylbenzthiazolinesulfonic acid) solution for 30 minutes at room temperature in the dark. By deducting the OD of the matching coated well from that of the noncoated wells, the adjusted OD was computed. Control sera and the cutoff were used to determine the mean and standard deviation (SD).

RESULTS

A recombinant CCHFV nucleoprotein antigen was used to capture the CCHFV specific antibodies present in the serum. The cut off value was calculated by mean plus 3(SD) which was 0.213 at 1: 400 dilution. Out of total 164 animal serum samples tested, 103 (63%) were positive for presence of anti CCHFV IgG. From the total 103 animals tested positive, 14.5 % were cattle 63.7% were goats and 42% were sheep. Sero-prevalence of Crimean Congo Hemorrhagic Fever(CCHF) in serum samples of sheep, goat and cattle and its gender distribution collected from various regions and analysed using ELISA is shown in (Table 1).

Animal	Male	Female	Overall Sero-prevalence (%)		
Species	Positive	Positive	Total	95 % CI	
Sheep	2/3(66.7)	36/57(63.2)	60 (36.6)	24.4-48.8	
Goat	8/11(72.7)	42/70(60)	81(49.4)	38.5-60.3	
Cattle	1/1(100)	14/22(63.6)	23(14)	0-28.2	
Total	11/15 (10.7)	92/149 (89.3)	164 (100)	100	

Table 1: Sero-prevalence of Crimean Congo Hemorrhagic Fever (CCHF)in serum samples of sheep, goat and cattle

The values in parenthesis are percentages. An overall sero-conversion of 62.8% (103/164) was observed in animals. The sero-conversion against CCHFV infection was highest in goat (49.4%), followed by sheep (36.6%) and cattle (14%) [Table1]. The epidemiological and statistical characteristics between CCHFV positive and negative cases in ruminants is shown in [Table 2]. The sero-conversion against CCHFV infection was 89.3% in female,

while it was 10.7% in male animals. The sero-conversion against CCHFV infection was highest female animals (90.9%). The CCHFV infection in animals of age groups \leq 3, 4-6 and \geq 7 years was 61.2%, 29.1% and 9.7%, respectively. The animal species, gender and age groups were not significantly associated with CCHFV infection. The location/area of sampling was found to be highly significantly associated with CCHFV infection(p<0.05). The highest seroconversions were observed Islamabad/Rawalpindi (57.3%) followed by Tando Allah Yar (17.5%), Kurram Agency (13.6%), Karachi (7.8%), Larkana (1.9%) and Hyderabad(1.9%) (Table 2)

Brief				
History	Total n=164 (100)	Positive n=103 (62.8)	Negative n=61 (37.2)	P- value
		Gender		
Female	149 (90.9)	92 (89.3)	57(93.4)	0.070
Male	15 (9.1)	11(10.7)	4(6.6)	0.278
		Animal Species		
Sheep	60 (36.6)	38(36.9)	22 (36.1)	
Goat	81(49.4)	50 (48.5)	31(50.8)	0.949
Cattle	23(14)	15 (14.6)	8 (13.1)	1
	Aq	ge groups (Years)		•
≤3	101 (61.6)	63 (61.2)	38(62.3)	
4-6	49(29.9)	30 (29.1)	19 (31.1)	0.776
≥7	14 (8.5)	10 (9.7)	4 (6.6)]
		Location (Area)		
Hyderabad	9(5.5)	2(1.9)	7 (11.5)	
Islamabad/ Rawalpindi	92 (56.1)	59 (57.3)	33 (54.1)	
Karachi	13 (7.9)	8 (7.8)	5(8.2)	1
Kurram Agency	20 (12.2)	14 (13.6)	6 (9.8)	0.001*
Larkana	10 (6.1)	2 (1.9)	8 (13.1)]
Tando Allah Yar	20 (12.2)	18 (17.5)	2 (3.3)	

Table 2: Epidemiological and statistical characteristics betweenCCHFV positive and negative cases in ruminant. *Pearson Chi-Square; values in parenthesis are percentages.

DISCUSSION

Detection of antibodies against CCHFV usually require the use of viral antigen or the viral particle itself to capture the antibody, this necessitates the use of biosafety level 4 labs with high containment levels which is not easily accessible especially in third world countries where cases are mostly reported. Use of a recombinant nucleoprotein that is noninfectious can be of great use to researchers and diagnostic labs for easy and safe handling and detection of CCHFV infection. A safe to handle recombinant nucleoprotein was used in this study for anti-CCHFV antibody capture. Moreover, the amino acid homologies are high among different strains in the region of the nucleoprotein used in this study, so this test can detect multiple strains of CCHFV. Our study shows a high

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proportion of seropositive animals from areas such as Islamabad/Rawalpindi and other areas in Sindh such as Tando Allah yar and Larkana which is in concordance with recent reports of CCHFV from these areas [14, 15]. The rural economy of Baluchistan and Khyber Pakhtunkhwa is based on livestock production, and the increased contact with animals may explain the higher antibody prevalence in humans from these areas. No significant difference was observed in the ratio of positive cases amongst different species of animals, most probably owing to the small sample size. The study can be carried out on a larger scale and with more animal species which will helps identify areas more at risk of CCHFV prevalence. The rising geographic range of Hyalomma ticks is a concern, according to current studies. Additionally, migratory birds spread Hyalomma ticks into northerner regions of Europe, potentially exposing impressionable human populations to CCHFV. In the past five years, the virus has been brought into the UK on two separate occasions, with the first fatal case being confirmed in 2012. There isn't an approved vaccination for CCHF. In Pakistan most of the CCHFV cases are not diagnosed and if diagnosed, they are not reported [13, 16-18]. The farmers, shepherds and healthcare workers in Pakistan need to be educated about CCHFV transmission. There is an immediate need for a surveillance system, standard measures for early detection, preventive measures, and proper treatment plans. Future efforts for screening the animals using efficient diagnostic assays will be helpful to manage and curb this lethal zoonotic disease in humans. For accurate diagnosis of CCHFV, a simple IgG antibody detection assay is not enough. An IgM detection assay in addition to molecular detection of CCHFV antigen with gPCR will help accurately and efficiently diagnose the acute infection. Previous studies have shown that mononuclear phagocytes are one of the main targets of CCHFV [2, 19, 20]. Therefore, to increase the sensitivity of the ELISA assay whole blood samples may be used instead of serum, however more studies are needed to quantify the exact magnate of the CCHFV in Pakistan to help to begin right track for controlling the disease.

CONCLUSIONS

High seroprevalence of the CCHFV was expected from these areas as numerous cases of CCHFV have been reported previously. Since no commercial tests are available for the detection of CCHFV-specific antibodies in animals, this IgG ELISA test can be used to screen the animals in areas at risk such as those that have the presence of permissive ticks.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Computer-Aided Drug Designing of *Ocimum basilicum* Compounds as Therapeutic Agents against RdRp of SARS-CoV2

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INTRODUCTION

Coronavirus disease(COVID-19) is a communicable and lifethreatening infection that is triggered by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV2) [1]. This virus is a single-stranded, positive-sense RNA enveloped and is transmissible in humans. The SARS-CoV2 belongs to MERS and SARS family; all of these are beta coronaviruses and they have their origins in bats. This viral infection was initially reported in Wuhan, China [2]. COVID-19 has subsequently spread internationally. On March 11, the World Health Organization (WHO) stated the 2019-2020 novel coronavirus as a pandemic [3]. COVID-19 causes a respiratory infection through common symptoms like cough, fever, difficulty in breathing, muscle pain, diarrhea,

ABSTRACT

The prevailing situation of the World is challenging due to COVID-19 pandemic that is caused by SARS-CoV2. **Objectives:** To combat with this emerging pandemic by reducing disease severity and infection, the need of hour is to develop an effective vaccine and antiviral candidates as therapeutic agents against SARS-CoV2. Methods: This study was developed for the identification of potential anti-viral agents, from Ocimum basilicum against RdRp of SARS-CoV2. In this concern, nevadensin, ursolic acid, β-Sesquiphellandren, apigenin, nerolidol, nonyl acetate and geranyl acetate were screened out of fifty-seven compounds from Ocimum basilicum based on their best docking scores. The docking results were also compared with already clinically used drugs (Remdesivir and Ribavirin) against the RdRp of SARS-CoV2. Molecular docking was performed using MOE software. The ADMET analysis and drug likeliness were also performed for all screened compounds by using admetSAR, pkCSM and SwissADME. Results: Cumulatively, the optimum binding energies of screened compounds indicated their potential for drug development against SARS-CoV2. It appears promising that nevadensin exhibited a good docking score and high binding affinity towards RdRp of SARS-CoV2. Therefore, it may represent the potential to inhibit COVID-19. Conclusion: Hence, Ocimum basilicum nutraceuticals could be effective therapeutic candidates for the treatment and prevention of COVID-19.

sore throat and sputum production are common symptoms [4]. However, the majority of COVID-19 cases lead to pneumonia, diarrhea along with multiple organ damage [5]. Among all organs of the body, the lungs are the most affected organ with COVID-19 due to the presence of enzyme ACE2 in the alveolar cells of lungs, and the SARA-CoV2 virus has an affinity with this enzyme on the receptor-binding site. Due to the progress of the alveolar disease, respiratory failure occurs as follows with the death of patients [6]. The SARS-CoV2 is a positive-sense, single-stranded RNA virus and its genome size varies from 29.8 to 29.9 kb [7]. The single virion (50-200 nanometer) comprises 4 different types of structural proteins. These

structural proteins are identified as Spike protein (S), Envelope protein (E), Membrane protein (M) and Nucleocapsid protein(N). These proteins are different from one another with respect to structure as well as function; the N nucleocapsid protein holds the viral genome and other S, E, and M proteins are responsible for the formation of the viral envelope [8]. Spike proteins help in the attachment of the virion with the surface membrane of the host cell. Spike glycoproteins give the virus a corona-like appearance that is vital for its pathogenicity. The SARA-CoV2 virus has a suitable affinity with angiotensinconverting enzyme 2 (ACE2) present on the receptors of living cells that virion uses as a mechanism for entry into a cell [9]. Non-structural proteins occupy the two-third region of the genome of SARS-CoV2. Main proteases, RNA-Dependent RNA Polymerases and helicases are important non-structural proteins [10]. RNA-Dependent RNA Polymerases are coded by the nsp12 gene. It is an important multi-subunit enzymatic machinery in SARS-CoV2 that is involved in the replication and transcription processes in viral genomes [11]. Open reading frameworks ORF1a, ORF1b, and polyproteins (RdRp) along with nsp7 and nsp8 as cofactors are involved in RNA polymerization [12]. Catalytic domains and protein sequences of this enzyme are highly conserved, which could be a promising drug target for the development of a therapeutic approach against RdRp [13]. Natural compounds from Eastern herbs show active defense mechanisms against various pathogens. In modern phytomedicine, herbs and medicinal plants are used as an alternative therapy due to the presence of active chemical constituents against a wide variety of viruses. In this pandemic situation, there is a need for the development of effective antiviral drugs against COVID-19. In the present study, the computational approach is used for screening compounds from Ocimum basilicum (Sweet basil) to check their potential for blocking the RdRp proteins of SARS-CoV2. Computer-aided in silico approach helps to facilitate and speed up the drug designing process in which different methods are used to identify novel therapeutic compounds.

METHODS

Selection of medicinal plants for the discovery of potential antiviral drug agents was based on the presence of phytochemicals that are present in medicinal herbs. Here, we have selected the Ocimum basilicum which belongs to the Lamiaceae family. For a quite long time, it is being utilized in Asia as traditional medicine. It contains various compounds that have antiviral, antioxidant, antibacterial, antifungal, dermatologic, anticonvulsant and cytoprotective properties [14]. RdRp is a key enzyme in SARS-CoV2. RdRp non-structural protein (nsp12) belongs to the class of nucleic acid polymerases. It is a central

component of enzymatic machinery that is involved in the replication and transcription of the viral genome of SARS-CoV2 [15]. So, by targeting RdRp, viral replication can be halted. The structure of target protein RdRp was obtained from the Protein Data Bank (PDB ID: 6M71). The structure of desired ligands was downloaded in SDF format from PubChem, Drug bank, ChEMBL and ChemSpider databases and sketched in the MOE interface by using Builder Mode. Canonical SMILES was used to build a 2D structure of ligands. After sketching the partial charges were added by using compute in MOE. Once charges were added, the prepared ligand was saved in the MDB file. PDB is a source of our target protein, which was downloaded in the 6M71 PDB file. For the preparation of protein following steps were performed: removal of inhibitors and repeated chains, correction of protein structure and 3D protonation of protein molecule. The repeated chains of RdRp were removed to avoid complications during docking. The molecular docking analysis was performed by Molecular Operating Environment (MOE) v.2015.10. (Developed by chemical computing group Inc, Canada). It was used to perform molecular docking for the protein-ligand interaction and drug-likeness analysis. MOE software was also used for the visualization of results, modeling, and simulation of structures. After molecular docking, virtually screened chemical compounds from Ocimum basilicum were selected as potential drug agents that have strong inhibitory effects against RdRp target protein by using Lipinski's rule and AdmetSAR profiling. Lipinski's rule is also known as the rule of five (R05). The assessment of absorption, digestion, metabolism, excretion and toxicity (ADMET) of screened compounds is essential in the drug development process for the evaluation of drug-likeness, level of toxicity, and pharmacokinetics [16]. SwissADME, pkCSM and admetSAR are online web tools that were used for AdmetSAR profiling to evaluate the ADMET physiochemical properties, toxicity, pharmacokinetics and drug-likeness.

RESULTS

Molecular docking is an important tool to study interactions between protein and ligand at the atomic level. Here, we have investigated the binding efficiency of target protein RdRp of SARS-CoV2 with different ligands using a computational approach in order to predict promising therapeutic drug molecules. Target protein RdRp was docked with 57 different compounds of *Ocimum basilicum* (Figure 1).



Figure 1. Histogram of molecular docking results of RdRp with several drug candidate compounds; x-axis: binding energies ΔG in kcal/mol; y-axis: number of compounds

After comprehensive analysis and comparison of the docking results of all compounds with control drugs, we have selected seven compounds of *Ocimum basilicum* based on their docking scores and molecular interactions of protein-ligand complexes(Table 1).

Compounds	Docking Scores (Kcal/mol)
Nevadensin	-6.0907
Ursolic acid	-5.9506
β- Sesquiphellandren	-5.6538
Apigenin	-5.6119
Nerolidol	-5.5728
Nonyl acetate	-5.5153
Geranyl acetate	-5.4095

Table 1: Docking scores of selected compounds of Ocimum

 basilicum with target protein (PDB ID:6M71)

Docking results ranked by binding energy (ΔG) values of different ligands are: nevadensin > ursolic acid > β -Sesquiphellandren > apigenin > nerolidol > nonyl acetate > geranyl acetate with binding energies -6.09, -5.95, -5.65, -5.61, -5.57, -5.51 and -5.40 kcal/mol, respectively(Figure 2).



Figure 2: Graphical representation of docking scores

Concerning docking analysis, nevadensin expressed high binding affinity towards RdRp polymerase of SARS-CoV2 than standard ribavirin drug -6.09, -5.38 kcal/mol, respectively(Table 2).

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Table 2: 2D interactions and 3D structures of selected proteinligand complexes

Virtually screened chemical compounds from Ocimum basilicum were selected as potential drug agents that have strong inhibitory effects against RdRp target protein by using Lipinski's rule and AdmetSAR profiling. (Table 3).

Ligands	MW (g/ mol) (≤500)	Donor HB (≤5)	Acceptor HB (≤10)	TPSA (Å)	Log P	Log S	Violation
Nevadensin	344.32	2	7	98.36 Ų	2.90	-4.03	0
Ursolic acid	456.70	2	3	57.53 Ų	7.09	-4.38	1
β-Sesquiphellandren	220.35	1	1	20.23 Ų	3.86	-3.62	0
Apigenin	270.24	3	5	90.90 Ų	2.58	-2.77	0
Nerolidol	222.37	1	1	20.23 Ų	4.40	-3.80	0
Nonyl acetate	186.29	0	2	26.30 Ų	3.30	-2.86	0
Geranyl acetate	196.28	0	2	26.30 Ų	3.24	-3.21	0

Table 3: AdmetSAR profiling of Ocimum basilicum compounds

 according to Lipinski's rule of five

The drug likeliness properties of all selected compounds' molecular weight range from 186.29 to 344.32 < 500 Daltons. Besides, number of hydrogen bond donors(NH and OH)less than 5 and numbers of hydrogen bond acceptors(O and N) less than 10 were predicated in all selected compounds(table 4).

Ligands	Hit Structure	Bioavailability score	Synthetic accessibility score
ChEBI ID: 7545 Nevadensin		0.55	3.47
Pub Chem CID: 64945 Ursolic acid	н от Сп	0.85	6.21
Pub Chem CID: 91749844 β- Sesquiphellandren	,	0.55	4.43
Pub Chem CID: 5280443 Apigenin	H 0 C C 0 H	0.55	2.96
Pub Chem CID: 5284507 Nerolidol	" °	0.55	3.53
ChEBI ID: 87511 Nonyl acetate	H,C,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	0.55	1.92
Pub Chem CID: 1549026 Geranyl acetate	J.o.	0.55	2.72

Table 4: Bioavailability and synthetic accessibility scores of top

 screened molecules

DISCUSSION

In silico methods, such as molecular docking, are frequently used to analyse the interactions and binding affinity of proteins and peptides in biological activity. Unfortunately, limited sampling of both ligand and receptor conformations, as well as the use of estimated scoring systems, might result in results that do not correspond to actual experimental binding affinities. Molecular dynamics simulations (MDS) can provide useful information in decoding functional processes of proteins/peptides and other biomolecules, circumventing the stringent sampling constraints of docking research [17]. Docking results ranked by binding energy (ΔG) values of different ligands are: nevadensin > ursolic acid > β -Sesquiphellandren > apigenin > nerolidol > nonyl acetate > geranyl acetate with binding energies -6.09, -5.95, -5.65, -5.61, -5.57, -5.51 and -5.40 kcal/mol, respectively. A significant issue in medication design is obtaining molecules that bind selectively to their target receptors while without causing side effects by attaching to other similar receptors. We examine techniques for addressing this problem using COMBINE (COMparative BINding Energy) analysis in conjunction with PIPSA (Protein Interaction Property Similarity Analysis) and ligand docking approaches. We put these approaches to the test by using different sets of inhibitors of three structurally related serine proteases of medicinal importance [18]. In this study virtually screened chemical compounds from Ocimum basilicum were selected as potential drug agents that have strong inhibitory effects against RdRp target protein by using Lipinski's rule and AdmetSAR profiling. In the drug discovery process, bioavailability prediction and pharmacokinetics parameters are very important [19]. Permeability possessions (log P) value less than 5 of all compounds were also studied except ursolic acid with 7.09 log P and Topological Polar Surface Area (TPSA) of all ligands were less than 140 Å². It is an important component in computer-aided drug designing[20].

CONCLUSIONS

The current study has provided a computer-aided drug designing (CADD) approach for potential drug agents from Ocimum basilicum against SARS-CoV2. The aim of this study was to examine the compounds of Ocimum basilicum against viral protein RdRp of SARS-CoV2, which is essential for viral replication. The main target protein RdRp was used to predict its molecular binding and docking score with different ligands of the selected plant. Therefore, the major seven compounds from Ocimum basilicum which gave the best lowest binding energies were selected. It appears promising that nevadensin exhibited a good docking score and high binding affinity with RdRp of SARS-CoV2. Hence, it may have the potential to inhibit COVID-19. Additionally, ursolic acid, β -Sesquiphellandren, apigenin, nerolidol, nonyl acetates and geranyl acetate seems to have the best potential to act as polymerase inhibitors of SARS-CoV2. Thus, these suggested therapeutic candidates as COVID-19 polymerase inhibitors need further investigations and clinical trials for their potential medicinal uses against SARS-CoV2.

Conflicts of Interest

The authors declare no conflict of interest.

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