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وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا

وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي

فَضَّلَنَا عَلَى كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ

النمل15

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Editorial

"It's been a long,
A long timecomin',
But I know,
A change gonna' come!
Oh yes it will!"

A song by Sam Cooke

Dear SMLJ readers;

Many troubled waters passed below the bridge, flushing the good and the bad, altogether, to drain hope and despair. Perhaps that's, the way life goes! And that was our case.

Honestly, we schemed to surprise our audience by introducing this issue, of SMLJ, much earlier, yet the contributions were so scanty and meager to publish. For quite a time, we yearned, besought and entreated for your contributions. Everybody was busy somewhere!

Then, the change comes! We received a flood, call it, a streaming progression of papers, requesting urgent publishing services. Our sudden enthusiasm and exuberance were not good enough to suffice. That is why, we are late.
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webpage: http://www.journal.oiu.edu.sd/ojs/index.php/mlj/submissions and till we hear from you, we remain,

Yours

Ali Suleiman Elwakeel

Executive Editor

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Counsels to contributors

Manuscript submission: We only request the authors to fulfill certain prerequisites on submitting their research work papers. The directives mentioned are meant to ensure security, to facilitate and accelerate the publishing process as well as to eliminate inhospitable conflicts that might probably erupt.

The manuscripts, in the form of software copies, in CDs, flash, or any removable medium, are to be, either manually handed over to the Journal's authorized staff or sent via the journal's e-mail address, accompanied by a covering letter expressing the wish to publish the article in question. The authors are bound to fill, beforehand, an *undersigned* **Transfer of Copyright Agreement** form to be submitted as a scanner image.

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Two authors	(Lamb & Kling 2003)	Lamb, R. & Kling, R., 2003, 'Reconceptualizing users as social actors in information systems research', *MIS Quarterly* 27(2), 197.

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OriginalArticle

Sudan Medical Laboratory Journal

Frequency of Methyletetrahydrofolate Reductase Enzyme Mutation among Sudanese Patients with Sickle Cell Anemia in Khartoum State

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Abstract

Background: Sickle cell disease is an autosomal recessive and chronic hemolytic anemia whose clinical manifestations arise from the tendency of the hemoglobin (HbS or sickle hemoglobin) to polymerize and deform red blood cells into the characteristic sickle shape. The homozygous state (HbSS or sickle cell anemia) is the most common form of sickle cell disease,

Methylenetetrahydrofolate reductase (MTHFR) has a major impact on the regulation of the folic acid pathway due to the conversion of 5, 10-methylenetetrahydrofolate (methylene-THF) to 5-methyl-THF.

Objective: This study aimed to determine the frequency of the mutation of MTHFR in patients with sickle cell anaemia and to measure the prevalence of MTHFR mutation among the study population .

Methods: A total of 125 patients less than 17 years with sickle cell anaemia were examined for the mutation in the (MTHFR) gene.In this study we used Chelex method to extract of DNA and used Gel Electrophoresis to explain the band of homozygous or heterozygous mutation in MTHFR in locus A1298C.

Result: This study found that the frequency of mutation in MTHFR in A1298C was 19% in SCD patient (homozygous was 11.4%, while heterozygous was 7.6 %). significant relationship between mutation in MTHFR and SCD patient (P=001).

Conclusion: This study revealed that the three is high frequency of mutation of MTHFR enzyme among Sudanese patients with SCA (19%), 11.85% had heterozygous allele and 7.8% had homozygous allele.

Keywords: Sickle cell disease, Methylenetetrahydrofolate reductase enzyme (MTHFR), single nucleotide polymorphisms (SNP), DNA extraction, PCR.

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Introduction:

Sickle cell disease is an autosomal recessive and chronic hemolytic anemia whose clinical manifestations arise from the tendency of the hemoglobin (HbS or sickle hemoglobin) to polymerize and distort red blood cells into the characteristic sickle shape. The homozygous state (HbSS or sickle cell anemia) is the most common form of sickle cell disease, and the heterogonous stat (Hb AS) is refer as sickle cell trait.This property is due to a point mutation in single nucleotide change in the ß-globin gene leading to substitution of valine for glutamic acid at position 6 of the -globin chain (ß 6glu→val or ß s).[1]

persons who are affected with sickle cell anemia have two copies of this variant (Hb SS), and the primary hemoglobin present in their red blood cells is sickle hemoglobin. Individuals affected with other types of sickle cell disease are compound heterozygotes. They possess one copy of the Hb S variant plus one copy of another β-globin gene variant, such as Hb C or Hb β-thalassemia. These individuals produce a mixture of variant hemoglobin's. Carrier individuals have one copy of the sickle variant and one copy of the normal β-globin gene (Hb AS), producing a mixture of sickle hemoglobin and normal hemoglobin. The carrier state for sickle cell disease is often referred to as "sickle cell trait." Although individuals with sickle cell trait do not express sickle cell disease, one study found that sickle cell trait may be a risk factor for sudden death during physical training. [2] In addition, individuals with sickle cell trait are protected from malaria infection. [1] The high frequency of the Hb S variant is believed to be a result of this protective effect.

The distribution of sickle cell anaemia provides evidence for origin of the mutation in several locations within Africa (the Senegal, Benin and Bantu haplotypes) and Asia (the Arab–Indian haplotype), The Sudanese haplotype is camerion haplotype. [3]

Inheritances:

Sickle-cell conditions are inherited as autosomal recessive pattern .The types of haemoglobin a person makes in the red blood cells depend on what haemoglobin genes are inherited from his parents. If one parent has sickle-cell anaemia (SS) and the other has sickle-cell trait (AS), there is a 50% chance of a child's having sickle-cell disease (SS) and a 50% chance of a child's having sickle-cell trait (AS). When both parents have sickle-cell trait (AS), a child has a 25% chance (1 of 4) of sickle-cell disease (SS). [3]

Molecular biology of Sickle cell anemia:

Sickle-cell gene mutation probably arose spontaneously in different geographic areas, as suggested by restriction endonuclease analysis. These variants are known as Cameroon, Senegal, Benin, Bantu and Saudi-Asian. Their clinical importance springs from the fact that some of them are associated with higher HbF levels, e.g., Senegal and Saudi-Asian variants, and tend to have milder disease.In people heterozygous for HbS (carriers of sickling haemoglobin), the polymerisation problems are minor, because the normal allele is able to produce over 50% of the haemoglobin. In people homozygous for HbS, the presence of long-chain polymers of HbS distort the shape of the red

blood cell from a smooth doughnut-like shape to ragged and full of spikes, making it fragile and susceptible to breaking within capillaries. Carriers have symptoms only if they are deprived of oxygen (for example, while climbing a mountain) or while severely dehydrated. Under normal circumstances, these painful crises occur about 0.8 times per year per patient. The sickle-cell disease occurs when the seventh amino acid (if the initial methionine is counted), glutamic acid, is replaced by valine to change its structure and function.

The gene defect is a known mutation of a single nucleotide (single-nucleotide polymorphism - SNP) (A to T) of the β-globin gene, which results in glutamic acid being substituted by valine at position 6. Haemoglobin S with this mutation is referred to as HbS, as opposed to the normal adult HbA. The genetic disorder is due to the mutation of a single nucleotide, from a GAG to GTG codon mutation, becoming a GUG codon by transcription. This is normally a benign mutation, causing no apparent effects on the secondary, tertiary, or quaternary structure of hemoglobin in conditions of normal oxygen concentration. What it does allow for, under conditions of low oxygen concentration, is the polymerization of the HbS itself. The deoxy form of hemoglobin exposes a hydrophobic patch on the protein between the E and F helices. The hydrophobic residues of the valine at position 6 of the beta chain in hemoglobin are able to associate with the hydrophobic patch, causing hemoglobin S molecules to aggregate and form fibrous precipitates.

The allele responsible for sickle-cell anaemia is autosomal recessive and can be found on the short arm of chromosome 11. A person that receives the defective gene from both father and mother develops the disease; a person that receives one defective and one healthy allele remains healthy, but can pass on the disease and is known as a carrier. If two parents who are carriers have a child, there is a 1in 4 chance of their child developing the disease and a 1in 2 chance of their child's being just a carrier. Since the gene is incompletely recessive, carriers can produce a few sickled red blood cells, not enough to cause symptoms, but enough to give resistance to malaria. Because of this, heterozygotes have a higher fitness than either of the homozygotes. This is known as heterozygote advantage.

Due to the adaptive advantage of the heterozygote, the disease is still prevalent, especially among people with recent ancestry in malaria-stricken areas, such as Africa, the Mediterranean, India and the Middle East [4]. Malaria was historically endemic to southern Europe, but it was declared eradicated in the mid-20th century, with the exception of rare sporadic cases. [5]

Methylenetetrahydrofolate reductase(MTHFR)

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that in humans is encoded by the MTHFR gene. [6] also has a main impact on the regulation of the folic acid pathway due to the conversion of 5, 10-methylenetetrahydrofolate (methylene-THF) to 5-methyl-THF. Two common nonsynonymous coding region polymorphisms (677C>T and 1298A>C) in the MTHFR gene were

shown to confer reduced enzyme activity in vitro assays leading to a reduced pool of methyl-THF and the MTHFR variant 677C>T was related with an increased risk of hyperhomocysteinemia, particularly in folate-deficient states [7]. With regard to the MTHFR 677C>T polymorphism, results from in vitro] assays showed a reduction in enzyme activity to 65% for the heterozygous and to 30% for the homozygous state of the 677T variant[8]. For the 1298A>C polymorphism, enzyme activity in vitro is diminished in homozygous variants and, to a lesser extent, in heterozygotes compared with those homozygous for the wild-type allele. [9] ,Genetic variation in this gene influences susceptibility to occlusive vascular disease, neural tube defects, colon cancer and acute leukemia, and mutations in this gene are associated with methylenetetrahydrofolate reductase deficiency. [10]

Genetics:

The enzyme is coded by the gene with the symbol MTHFR on chromosome 1 location p36.3 in humans.[4] There is DNA sequence variants (genetic polymorphisms) associated with this gene. In 2000 a report brought the number of polymorphisms up to 24.[11]Two of the most investigated are C677T (rs1801133) and A1298C (rs1801131) single nucleotide polymorphisms (SNP).

C677T SNP (Ala222Val)

The MTHFR nucleotide at position 677 in the gene has two possibilities: C (cytosine) or T (thymine). C at position 677 (leading to an alanine at amino acid 222) is the normal allele. The 677T allele (leading to a valine substitution at amino acid 222) encodes a thermolabile enzyme with reduced activity.

Individual with two copies of 677C (677CC) have the "normal" or "wild type" genotype. 677TT individuals (homozygous) are said to have mild MTHFR deficiency. 677CT individuals (heterozygous) are almost the same as normal individuals because the normal MTHFR can make up for the thermolabile MTHFR. About ten percent of the North American population are T-homozygous for this polymorphism. There is ethnic variability in the frequency of the T allele frequency in Mediterranean/Hispanics > Caucasians > Africans/African-Americans).

The degree of enzyme thermolability (assessed as residual activity after heat inactivation) is much greater in 677TT individuals (18-22%) compared with 677CT (56%) and 677CC (66-67%). [12]

Individuals of 677TT are predisposed to mild hyperhomocysteinemia (high blood homocysteine levels), because they have less active MTHFR available to produce 5-methyltetrahydrofolate (which is used to decrease homocysteine). Low dietary intake of the vitamin folic acid can also cause mild hyperhomocysteinemia.

Low folate intake affects individuals with the 677TT genotype to a greater extent than those with the 677CC/CT genotypes. 677TT (but not 677CC/CT) individuals with lower plasma folate levels are at risk for elevated plasma homocysteine levels.[13] In studies of human recombinant MTHFR, the protein encoded by 677T loses its FAD cofactor three times faster than the wild-type

protein , [14] 5-Methyl-THF slows the rate of FAD release in both the wild-type and mutant enzymes, although it is to a much greater extent in the mutant enzyme,[15].677TT individuals are at a decreased risk for certain leukemias, [16]and colon cancer.

Mutations in the MTHFR gene could be one of the factors leading to increased risk of developing schizophrenia [17]. Schizophrenic patients having the risk allele (T\T) show more deficiencies in executive function tasks.

A1298C SNP (Glu429Ala):

At nucleotide 1298 of the MTHFR, there are two possibilities: A or C. 1298A (leading to a Glu at amino acid 429) is the most common while 1298C (leading to an Ala substitution at amino acid 429) is less common. 1298AA is the "normal" homozygous, 1298AC the heterozygous, and 1298CC the homozygous for the "variant". In studies of human recombinant MTHFR, the protein encoded by 1298C cannot be distinguished from 1298A in terms of activity, thermolability, FAD release, or the protective effect of 5-methyl-THF. [18] The C mutation does not appear to affect the MTHFR protein. It does not result in thermolabile MTHFR and does not appear to affect homocysteine levels.

Compound Heterozygotes:

Mutations at 677 and 1298 are both in the same gene, MTHFR. They are at different locations in the same gene. Some studies have shown that the MTHFR protein in people with the genotype 677CT 1298AC does its job a bit less well than the normal MTHFR[2]

Severe MTHFR deficiency:

Severe MTHFR deficiency is rare (about 50 cases worldwide) and caused by mutations resulting in 0-20% residual enzyme activity.[11] Patients exhibit developmental delay, motor and gait dysfunction, seizures, and neurological impairment and have extremely high levels of homocysteine in their plasma and urine as well as low to normal plasma methionine levels[19].

Methods:

Ethical approval and consent to participant:

Approval of This study was obtained from hematology department of medical laboratory science (MLS), Omdurman Islamic University, and ministry of health issued by the local ethical committee, Khartoum State, Sudan. Written consent was taken from each member of the study.

Study Location:

A case control study. With total samples were 125, which include 79 sample from patient with sickle cell anaemia and 46 as normal control.

The study was conducted in Khartoum State in different haemglobinopathy laboratory centres (Khartoum Teaching Hospital, Ministry of Health Biochemistry department, faculty of Medicine University of Khartoum, Haematology Department, National Health Laboratory, and Federal Ministry of Health).

Study populations:

The study population was children of less than 17 years diagnosed with sickle cell disease based on their Hb electrophoresis on cellulose acetate paper at alkaline pH, who attend one of the laboratory

centres either for their regular chick up or for diagnosis and evaluation.

Sample collections:

5ml EDTA blood was collected under sterile condition, store at 4ºC for 7 days till Buffy coat preparation, and haemolysed sample for Hb electrophoresis were also collected and store for DNA extraction.

DNA extractions by Chelex method:

DNA was extracted from all samples using th Chlex method according to manufactures instructions.

Polymerase Chain Reactions (PCR):

Extracted DNA was brought from-20 ºC, thawed and centrifuged again to pellet down any remaining Chelex and kept on ice cro-rack for processing , at the same time primers, dNTPs, and buffer were brought at room Temperature and kept on ice cro-rack for thawing and sterile PCR water was brought out and aliquoted on 1.5 ml tube and kept as above.

Master Mix preparation:

Samples and reagents were brought out from the freezer and kept on ice in a frozen cryo-rack during assembly procedure. A4 worksheet with PCR samples data were recorded for each samples to be tested. Master mix (MM) was prepared using forward and reversed primer ,the amount of each reagents were calculated to go into the MM in 1.5 µl sterile tube, according to the number of samples to be processed with an extra one more samples than actually being tested to compensate for retention of solution in pipette tips and tube. PCR reagents, except for samples DNA, were added in the order listed on the worksheet, adding water first and Taq polymerase last. The specified volume of MM was added into the each tube, all reagents were kept in a frozen-cryo-rack during mixing and returned to the freezer immediately after use, caps were closed tightly and the PCR tubes were moved to samples loading area.

In the samples preparation area specified volume of sample was loaded into an appropriately labeled PCR tube. To avoid contamination, the tips were always changed and the avoidance of touching the side tube and capped was recommended.

PCR amplification of MTHFR gene:

Genomic DNA was amplified with primer flanking Codon A1298C of MTHFR gene with flowing forwardprimers:5’CAAGGAGGAGCTGCTGAA GA3, and reverse primer: 5’CCACTCCAGCATC AC TCACT3’

PCR optimizations:

Small fraction of sample was first subjected to PCR amplification, after successful amplification the rest of the samples were analyzed in batches.

DNA visualization:

Agarose gel by electrophoresis.,5 µl from each PCR product were mixed with 2 µl Brmoephenol blue dye and loaded into 1.5% Agarose gel dissolved in TBE (Tris-borate Ph 8.0 and 0.002 M EDTA Ph 8.0). The gel was stained with ethidium bromide (0.5mg/ml), run for 30 minutes at 60 volt/cm for conventional PCR.

Results:

Study subjects comprised 79 SCD patients (39males and 40 females; mean age 4 month -17 years) and 46 controls (28 males and 18 females; mean age 4 -14 years).The DNA were extracted

todetect the incidence of the mutation in the (MTHFR) gene, among the diagnosed SCD patients and normal control in Khartoum State.

In this study we used Chelex method to extract of DNA and used Gel Electrophoresis to explain the band of homozygous or heterozygous mutation in MTHFR in locus A1298C.

The incidence of mutation in MTHFR in A1298C was 19% in SCD patient (homozygous was 11.4%, while heterozygous was 7.6 %).

The significant relationship between mutation in MTHFR and SCD patient compared with control (P=000).

Table 1: Shows mean and range of age /years and six among patients and controls:

	Patients	Controls
No (125)	79	46
Age in years mean, (range)	7.3(0.4 -17 years)	28.63(4-84)
Sex Male (45)	22(42.3%)	28(60.9%)
Female (59)	30(57.69%)	18(39.1%)

Table 2: Shows the mean and range of some hematological parameter among patient and control:

hematological parameter	Patients	Controls
Hb gm/dl	7.9(3.7-14.9)	12.8(9.9-16.0)
Hct% mean (range)	24.6 (13.5-44)	38.3%(29.7-49)
MCV/fl	85.8(8-102)	90.6(80-100)
MCHC gm/dl	32.7 (24-41)	32 (30-36)
WBC 10x3 cell/L	14.7(4.5-46.6	8(4-13)

Table 3: Shows the differentiation between Patients and Control:

Characteristics		Patients	Controls
Contgenousity:	**Yes** **No**	62% 34%	69.6% 31.4%
Hb variants	**AA** **AS** **SS**	00% 31.6% 68.4%	100% 00% 00%
MTHFR	**AA**	79.7%	97.8%
Homozygous		11.4%	0.0%
Heterozygous		7.6%	2.2.0%

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Table 4: Showed Main characteristics of the MTHFR (1298A>C) genetic variants

			Gene		
Polymorphism	Allele variant	Amino Acid change	Location	Position	NCBIdbSNP rs
	1298A>C	E429A	1p36.3	Exon 8	rs1801131

Table 5: showed the Comparison of allele and genotype frequency of 1298 normal/abnormal single nucleotide polymorphism of MTHFR in Sickle cell patients of males and females:

Gender	Male	Female	Total
No	39	40	79
Normal	34	29	63
Heterozygous	3	6	9
Homozygous	2	4	6
Normal	36	32	68
Abnormal	3	8	11

Table 6: Shows genotype frequency of the 1298 normal/abnormal single nucleotide polymorphism of MTHFR in Sickle cell patients and controls:

Genotype	Sickle cell disease(n=79)	Controls (n=46)
Normal	63(79.7%)	45(97.9%)
Heterozygous	9(11.4%)	0(00%)
Homozygous	6(7.6%)	1(2.1%)
P=000		

Table 7: shows allele frequency of the 1298 normal/abnormal single nucleotide polymorphism of MTHFR in Sickle cell patients and controls:

Allele	Sickle cell disease(n=79)	Controls (n=46)
Normal	135 (86.5%)	90 (97.8)
Abnormal	21(13.5%)	2 (2.2%)
	P=003	

Table 8: shows the relationship of Contgenousity marriage and MTHFR alleles:

		1298 genotype			Total
		AA allele	**AC allele**	**CC allele**	
Contgenousity	**Yes**	**27**	**4**	**3**	**34**
	No	**16**	**5**	**3**	**24**
Total		**43**	**9**	**6**	**58**
P=001					

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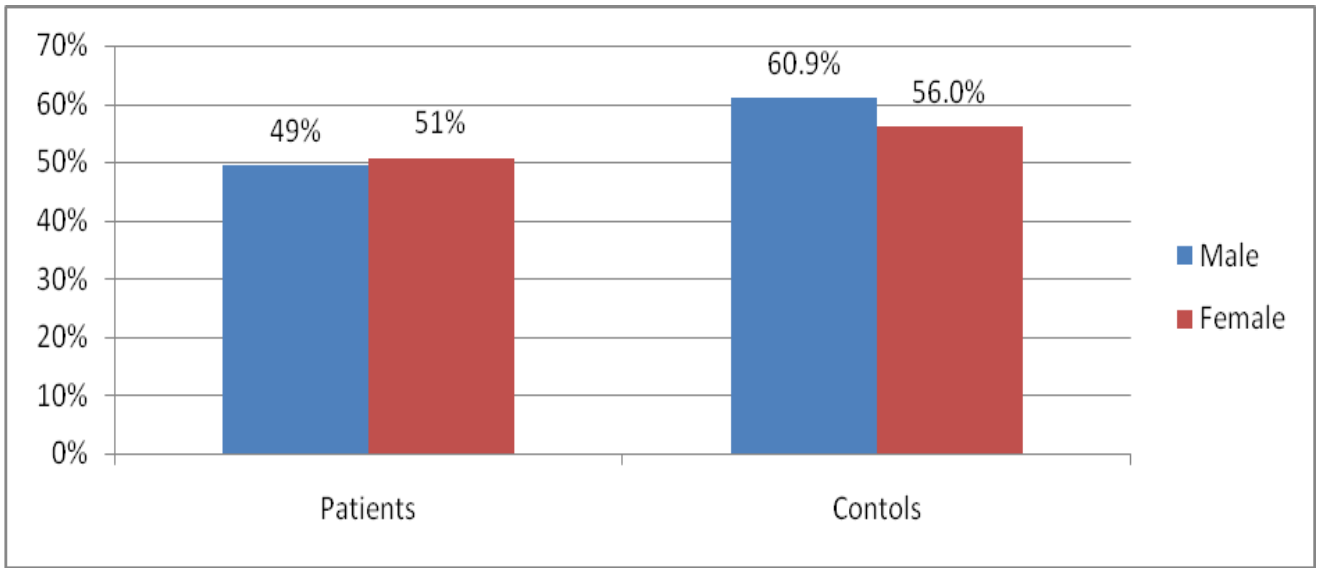

Figure 1: Shows Frequency of distribution of males and females

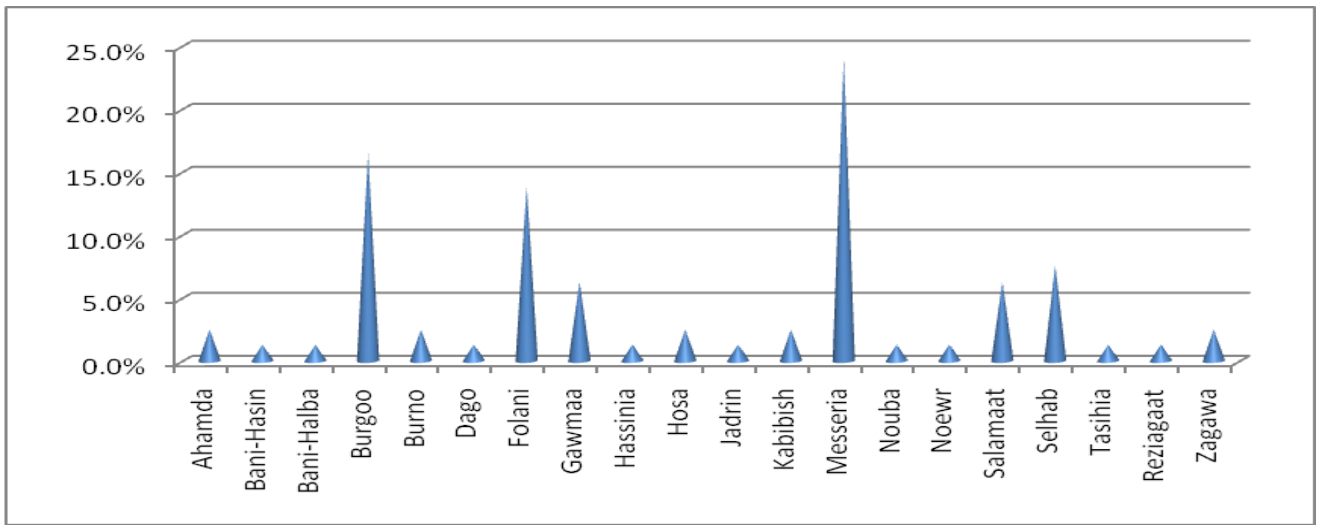

Figure 2: Shows Frequency of the different tribes with sickle cell disease:

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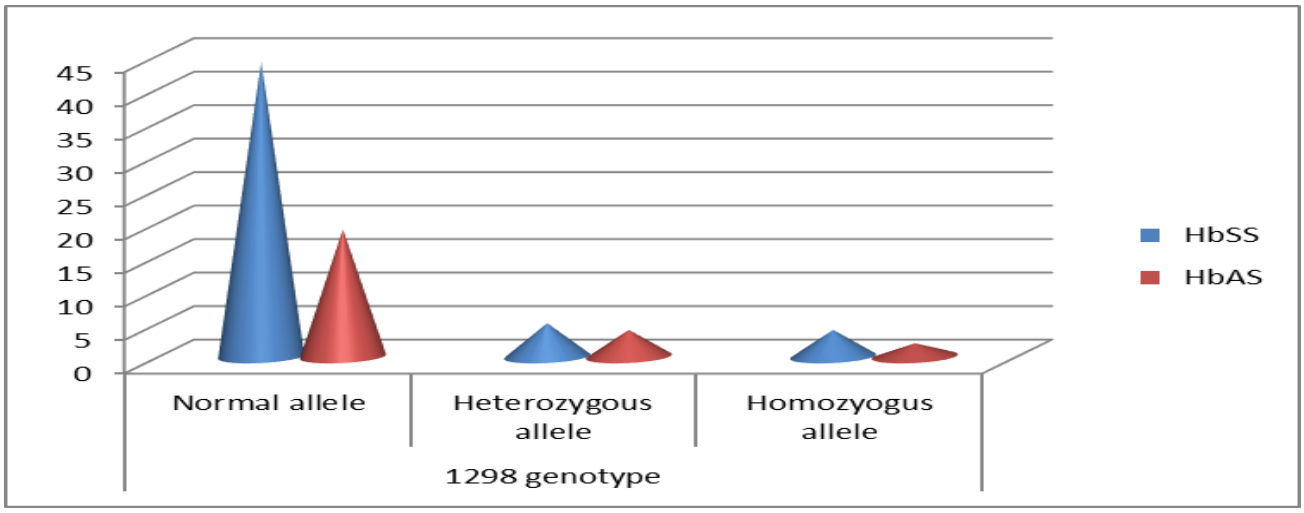

Figure 3: Shows Association of 1298 MTHFR genotype and Hb variants

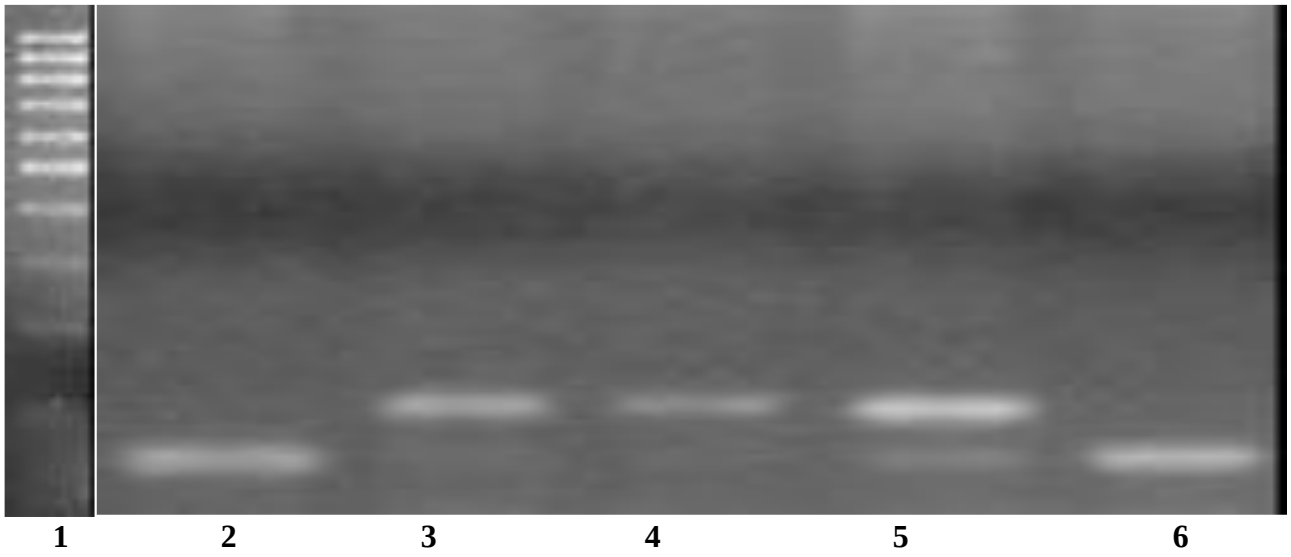

Figure 4: Agarose gel electrophoresis image showing the sizes of RFLP using *MboII* digestion, lane 1 represents the molecular weight marker 100 (bp), lane 2 and 6 is 72 bp (wild type), lane 3 and 4 were homozygous, lane 5 (heterozygous)

Discussion

In this study the populations were 46 normal as control and 79 patients with sickle cell disease to detect mutation in MTHFR gene. The study was carried out in Tropical Medicine Research Institute in National Health Laboratory in Khartoum State, from March 2011 to July 2011.

In this study we found that, frequency of MTHFR gene mutation among patients with sickle cell disease 19%, compare with normal control 2.1%

68.4% had haemoglobin SS and 31.6% had haemoglobin AS and the only one case of MTHFR gene mutation was from haemglobin AA and his origin was from Hausa tribe.

Female represent (50.6%) of the studied patient while male represent (39.3%) and their age range from 4 month to 17 years.

The frequency of homozygous mutation in MTHFR in female is 4% and male is 2%, while the frequency of heterozygous mutation in MTHFR in female is 6% and male 3%.

The significant relationship between SCD patient and the mutation in MTHFR was (P=000) as genotype.

The significant relationship between SCD patient and the mutation in MTHFR was (P=003) as allele.

We observed frequency of Contgenousity marriage in the patients group 62% and this could be a risk factor for genetic disease as sickle cell anaemia and mutation of MTHFR as shown in this study.

In El-Bahrain Al Jishi et al. (2005) described, for the first time, a 41-year-old married woman with a combination of Takayasu arteritis (TA) and primary antiphospholipid antibodies (aPL) syndrome who underwent carotid stenting. She had single nucleotide polymorphism (SNP) double homozygosity for methylenetetrahydrofolate reductase (MTHFR); C677T (T/T genotype) and A1298C (C/C genotype). Serum homocysteine was elevated and that might be attributed to C677T SNP. It was thought that MTHFR 1298C/C genotype would be an independent risk factor for ischemic stroke. In our study we agree with them in mutation of MTHFR A1298C, but we disagree with them in association between MTHFR and the disease.

The previous study in Saudia Arabia Fawaz et al (2004) found that there is role T/T (and C/T) at the MTHFR 677 (41.38%) in the patients with sickle cell anaemia this study agree with our study which we found mutation in the MTHFR in (19%) of patient with Sickle cell anaemia, but we disagree with it because in our study locus of MTHFR was A1298C.

The MTHFR 677TT genotype was detected in 1.8% of SCD patients in Brazil, in the patients with sickle cell anaemia this study agree with our study which we found mutation in the MTHFR in (19%) of patient with Sickle cell anaemia, but we disagree with it because in our study locus of MTHFR was A1298C.

The previous study in USA They studied the frequency of the thermolabile methylene tetrahydro-folate reductase (MTHFR) variant (C677T) in adult sickle cell patients with and without AVN. The frequency of the MTHFR mutation was 35.6% in patients with AVN and 12.9% in those without AVN (p=0.006). These data suggest that the thermolabile MTHFR variant may be a contributing risk factor for AVN in some populations with sickle cell disease. We agree with it, because in our study there is mutation in the MTHFR in (19%) of patient with Sickle cell anaemia but we disagree with it because in our study locus of MTHFR was A1298C.

To our best knowledge this is the first study in Sudan to investigate MTHFR gene polymorphism in sickle cell disease.

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Original Article **Sudan Medical Laboratory Journal**

Neutrophil-to-Lymphocyte Ratio not associated with Early Diagnosed Prostatic Carcinoma Patients

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Abstract:

Background: Prostate cancer has become the most common malignancy among men in several developed countries especially Sudan. The neutrophils lymphocytes ratio (NLR) was one of recent inflammatory biomarkers, nowadays was used as predictor and prognostic tools in different malignant disorders **Aim:** This study was aimed to assess the neutrophils to lymphocytes ratio in early diagnosed prostatic carcinoma patients.

Materials and Methods: This study is descriptive case control study, was conducted at Almoalim Medical City (AMC) Khartoum, Sudan. One hundred males, fifty were known prostatic cancer patients (early diagnosed) used as case group. Others fifty were normal healthy male matched the case group in age used as normal control group. The complete blood count (CBC) was done from EDTA anticoagulated blood samples and NLR was calculated using automated Hematology analyzer SYSMEX NX-550, prostate specific antigen was estimated using (*chemiluminescence*) e411 Cobas, all laboratory tests were made under fully quality control procedures. Data was collected by using direct structured questionnaire. Data was analyzed by SPSS version 18 and the significant value was present when the P. value less than 0.05. **Result:** This study showed that the Neutrophils Lymphocytes ratio (NLR) and absolute lymphocytes were insignificant different in the early diagnosed prostatic carcinoma patients compared with normal non prostate cancer with p value more than 0.05 while the absolute neutrophils was showed a statistically significant different between case and control with p value 0.03. **Conclusion:** This study concluded that the neutrophils lymphocytes ratio (NLR) may not applicable as predictor prognostic marker for the early diagnosed prostatic carcinoma patients.

Key word: Prostate cancer, Neutrophil-to-Lymphocyte, *chemiluminescence*

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Introduction

The prostate is the gland in the male genital tract, which after puberty helps through the secreted fluid that cleans the male's urethra before the sperm ejaculation. [1-3]

The malignancy it very complex diseases that most leading cause of death worldwide, the prostatic carcinoma it was most reported as sixth leading cause of death and ranked as second cancer in the men worldwide [4-8]. In 2017 according to world health organization, the prostate cancer was estimated as ninetieth leading cause of death in Sudan, [4]. Prostate cancer was diagnosed by detection and estimation of peripheral blood prostate specific antigen (PSA) as tumor markers, imaging modalities, and demonstration of prostatic biopsy, out of upper limit of PSA level was used for screening but the very high level of PSA biomarker in follow-up [9-10]. The complete blood count (CBC) nowadays one of the simple hematological parameters in the laboratory test, was made using different automated hematology analyzers and chancing for many parameters. Different peripheral blood biomarkers were established of the complete blood count like neutrophils lymphocytes ratio, lymphocytes monocytes ratio and thrombocytes lymphocytes ratio. [11-13]. The neutrophils lymphocytes (NLR) ratio was added to list of inflammatory biomarkers and it was calculated of the white blood cell parameters, Previous study was reported a high NLR was reported with higher inpatient mortality and NLR was valuable as prognostic marker for coronary artery bypass grafting outcome and post coronary bypass grafting atrial fibrillation. [14]. previous study that deal with NLR as predictor and prognostic tools for prostate cancer patient that conclude, the NLR can be used as indicator of poorer prognosis of patient with metastatic castration resistant prostate cancer could be used as predictor for prognostic status. [15]. Another study was stayed with NLR as prognostic value with verity of cancer and concerned with comparison of NLR and derived NLR (dNLR) were giving same result for both parameters [16]. Other study was showed the elevation of preoperative NLR is associated with poorer rates of survival in gastric carcinoma (GC) patients and play a role in GC surveillance programs. [17]

Materials and Methods:

This is a descriptive case control study, was conducted at Almoalim Medical City (AMC) Khartoum Sudan during the period of September 2019. One hundred subject were involved in this study 50 were Sudanese adult males known diagnosed by prostate carcinoma (early diagnosed); their mean age was 52 years designated as case group. Further 50 normal healthy male matched the case used as normal control group. 6 ml of vinous blood were collected from all studies group. Three ml in container contain EDTA as anticoagulant for complete blood count (CBC), which done by automated hematological analyzer SYSMEX NX-550 then the NLR was calculated. The serum was prepared from three ml; then prostate specific antigen was estimated using (*chemiluminescence*) e411 Cobas. all laboratory tests were made under fully quality control procedures. Every patient with

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the prostatic carcinoma and had inflation or under therapeutic course or under chemotherapy were excluded from this study Data were collected by using direct structured questionnaire. Data were analyzed by SPSS version 18 and the significant value was present when the P. value less than 0.05. the results were reported in the table and figures The ethical consideration was obtained from faculty of medical laboratory ethical board, and this study was authorized by Almoalim Medical City Ethical Board (AMCEB). The consent was taken from all participant before samples were gathered.

Results:

In this study the neutrophils lymphocytes ratio (NLR) were calculated from the white blood cells parameters for each participant.

The present study showed that the mean of absolute neutrophils count was statistically higher in early diagnosed prostate carcinoma patients compared with normal healthy control group with p value 0.03 table 1.

The mean of absolute lymphocytes count found that no statistically differences with p value 0.35 table 1.

The current study revealed there is no any statistically significant difference in the mean of neutrophils lymphocytes ratio (NLR) in early diagnosed prostate carcinoma patients compared with normal healthy control group with p value 0.86 table 1.

Table1 Compression of the Mean±SD of absolute neutrophils, lymphocytes, neutrophils lymphocytes ratio (NLR), and total prostatic antigen (TPSA)

Parameters	Study population		P. value
	Case (n=50) Mean±SD	Control (n=50) Mean±SD	
Neutrophils	3.7828 ±1.47915	3.2100 ± 1.24052	0.03
Lymphocytes	1.9046 ± 0.6458	2.0400 ± 0.80051	0.35
NLR	2.2210 ± 1.14524	2.1510 ± 2.63560	0.86
TPSA	37.1436 ± 68.32074	1.1320 ± 0.89345	0.00

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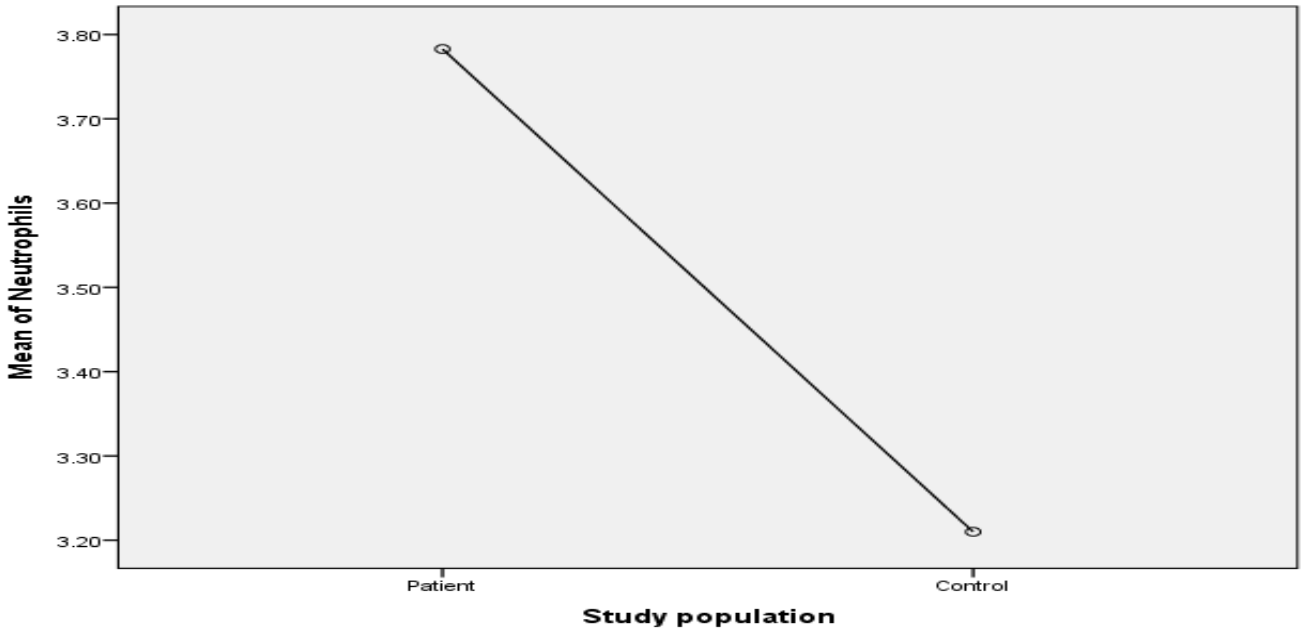

Figure 1 the distribution of neutrophils mean between case and control

Discussion:

Leukocytes comprise an integral portion of the innate, as well as of the adaptive immune system, and include granulocytes (neutrophils, basophils, eosinophils) monocytes, macrophages, dendritic cells and lymphocytes (B&T cells), which can exert immune-stimulating or immune-suppressive functions [18]. In cancer patients, several pathways can be activated to suppress the effective adaptive immune response, triggered to avoid the destruction of the tumor by immune cells [19].The neutrophils lymphocytes ratio recently was used as predictor to helping of different prognosis status and biological stress in the different malignant disorders [15]. The present study there was in significant association between neutrophils lymphocytes ratio and prostatic cancer in onset early diagnosed with the P. value less 0.05, this finding was in agreement with study done by Minardi et al and other done by Linton et al both studies were reported that there is no statistically significant association between prostatic cancer and neutrophils lymphocytes ratio as predictor prognostic tool in the prostatic cancer patients [18, 19],

The finding of this this study in contrast with study done by Templeton et al, who showed that the significant association between prostatic cancer and neutrophils lymphocytes ratio as predictor for poor prognosis, this variation might be due to our patients were recently diagnosed by prostatic carcinoma and not under treatments and still not developing the inflammatory status in our patients that reflect a predictor for good or poor prognosis. [20]. The interesting finding of this study found that statistically significant different of absolute neutrophils count through the early prostatic cancer patients in comparison with those normal healthy control participants with P. value < 0.05 this finding was in the same line with studies that done

by An et al and Lord et al, in which they concluded that the neutrophils play a major role in tumor growth and metastasis and can enhance the producing of many inflammatory mediators [21-22]

Regarding the mean of absolute lymphocytes count this study showed that insignificant different of absolute lymphocytes count between prostatic cancer patients and healthy control participants *P*. value more than 0.05. This finding was corresponding with study done by Mehmet et al who concluded that there in significance in absolute lymphocytes between prostatic carcinoma and benign prostatic hyperplasia [23]

Conclusion:

Based on our results, this study concluded that the neutrophils lymphocytes ratio dose not relevant as predictor in the onset diagnosed prostatic carcinoma patients, but the absolute neutrophils count may play helpfully marker in the onset diagnosed prostatic carcinoma patients.

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Original Article

Sudan Medical Laboratory Journal

Immunoinformatics Prediction of Peptide-Based Vaccine against MERS-Corona-viruses

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Abstract

MERS-Corona-viruses cause massive and pandemic outbreaks of respiratory infection in several regions of continents' and revealed a global epidemic trend. However, no effective antiviral drug or vaccine has been developed to treat coronavirus. Aim of the study to detect epitopes can be as vaccine. A total of 28 outer spike glycoprotein (s) sequences of Corona-viruses were retrieved from the National Center for Biotechnology Information database (NCBI) on them, several tests were conducted using Immune Epitope Analysis Database (IEDB) to detect the highly conserved immunogenic epitopes of B and T cells from which all possible epitopes that can be used as a therapeutic peptide vaccine to be selected. Several conserved cytotoxic T-lymphocyte epitopes, linear and conformational B cell epitopes were predicted for Corona-viruses spike glycoprotein and their antigenicity was calculated. Among B-cell epitopes 106–SQDVKQ-111 is antigenic and in the case of T cell epitopes, 279-FQFATLPVY-287 and 786-FSFGVTQEY-794 and 69-ITYQGLFPY-77 and, 924- AQYVAGYKV-938, 1271-ALNESYIDL-1285, and 1300-AGLVALALC-1314 are extremely antigenic promising for vaccination against Corona-viruses They demonstrated population coverage against the whole world **91.81%**.The study led to the discovery of various epitopes, conserved among various strains belonging to different countries. The potential antigenic epitopes can be successfully utilized in designing novel vaccines for combating and eradication of MERS Corona-viruses disease.

Keywords: Immunoinformatics, Vaccination, MERS Coronaviruses, Peptide.
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Introduction:

The rapid emergence and dissemination of infectious diseases have taken a heavy toll on humans since the beginning of the twenty-first century. One of the most familiar examples was the outbreak of severe acute respiratory syndrome (SARS) in the winter of 2002 and 2003, 2019 caused by a novel coronavirus (SARS-CoV) novel coronavirus disease (COVID-19) have increased not only in Wuhan, Hubei Province but also China and the world[1-3]. Since it was discovered, coronavirus was considered relatively harmless to humans until the outbreaks of SARS and MERS and COVID-19 in 2003 and 2012, 2019 respectively. SARS-CoV is a new type of coronavirus identified after the discovery of SARS-CoV, belongs to the Beta coronavirus lineage C (3, SARS-CoV that causes severe acute respiratory disease with a high fatality rate [4-6].

MERS-Coronavirus is approved and long – acquainted virus system that can be classified into four categories depend on their genome sequence:

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Alpha coronavirus, Beta coronavirus, Gamma coronavirus, and Delta coronavirus . Coronavirus is a class of enveloped RNA virus with a 27–3l kb long single-stranded positive-sense genome. The genome includes two large replicase open reading frames, ORF1a and ORF1b, encoding two viral replicase polyproteins. The region downstream of ORF1 contains at least 10 small ORFs, encoding the spike protein (S), a small envelope protein (E), membrane protein (M), nucleocapsid protein (N), and the assumed nonstructural proteins [7].

MERS -CoV belongs to the genus Betacoronavirus in the Family Coronaviridae, as SARS-CoV does. However, they do not use the same host cell receptor for infection [8]. Complete genome sequencing indicated that this new virus is the first lineage C Beta coronavirus species known to infect humans [9] MERS -CoVs are positive-strand RNA viruses. The virion includes a nucleocapsid (N) core surrounded by an envelope containing three membrane proteins: spike (S), membrane, and envelope. The S protein of MERS -CoV, a 1353 -amino-acid type I membrane glycoprotein, is known to be responsible for receptor binding [9], membrane fusion [10], and the induction of neutralizing antibodies [11]. Although the S protein of MERS -CoV shares little amino-acid identity with that of other CoVs (< 30%), it shares common structural features with the S proteins of other CoVs [12]. Its two components are S1, which contains the receptor-binding domain (RBD) [13]. Moreover, a combination of computed tomography imaging, whole-genome sequencing, and electron microscopy computed tomography (CT) and real-time reverse-transcriptase-polymerase chain reaction (rRT-PCR) and Serological assays were initially used to screen and identify SARS-CoV- 1 & 2 [14-17]. However, no effective antiviral drug or vaccine has been developed to treat MERS-Coronavirus. Traditional vaccines use completely killed viruses or weakened viruses to stimulate the immune response and create protective immunity. Nevertheless, sometimes you want to target immunity to specific parts of the virus and not to others. Alternatively, you may want to generate immunity against a protein that is not naturally immunogenic. We utilize computational protein design to place antigenic loops on the surfaces of other proteins, stabilizing them and making them polyvalent for a better immune response. Aim of the study to detect epitopes can be a vaccine.

Materials and Methods

Immunogenic Part for MERS Virus:

Protein Sequence Retrieval

The twenty-nine strains of MERS Coronaviruses spike glycoprotein were retrieved from NCBI in April 2019 (https://www.ncbi.nlm.nih.gov/protein/term = Nipah + virus + G + glycoprotein). The retrieved strains were from different parts of the world. The retrieved strains and their accession numbers were depicted in **figure**1.

Determination of MERS Coronaviruses spike glycoprotein MERS-Conserved Regions:

The retrieved sequences of MERS-Coronaviruses spike glycoprotein strains were aligned to obtain conserved regions using multiple sequence alignment (MSA). Sequences were aligned with the aid of ClustalW as implemented in the BioEdit program, version 7.2.5. Then epitopes prediction and analysis of each protein were done using

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different tools of immune epitope database IEDP software (http://www.iedb.org) (18).

Epitopes Prediction:

To detect the candidate epitopes from MERS - Coronaviruses spike glycoprotein, for B and T cells, several analysis prediction tools from Immune Epitope Database (IEDB) (http://www. iedb .org/) were used(18).

B-cell Epitope Prediction:

B cell epitope is the portion of an immunogenic which interacts with B lymphocytes. B-lymphocytes upon exposure differentiated into plasma cells and memory cells. Thus B cell epitopes are shown to being accessible and antigenic. Accordingly, the classical propensity scale methods and hidden Markov model programmed software from IEDB analysis resource were used for the following aspects:

Prediction of Linear B-cell Epitopes:

Bepipred from immune epitope database (http://toolsiedb.ofg/bcell/) was used as a linear B-cell epitopes prediction from the conserved region of MERS Coronaviruses spike glycoprotein with a default threshold value of 0.5 (19).

Prediction of Surface Accessibility:

Emini surface accessibility prediction tool of the immune epitope database (IEDB) was used (http://tools.immuneepitope.org/tools/bcell/iedbT he surface accessible epitopes were predicted from the conserved region of MERS spike glycoprotein with the default threshold value is it 1.000 (19).

Prediction of Epitopes Antigenicity:

The kolaskar and tongaonker antigenicity method was used to determine the antigenic sites with a default threshold value of 1.034 (http://tools.immuneepitope.org/bcell/) [20].

T-cell Epitopes Prediction:

MHC Class I Binding Predictions:

Analysis of peptide binding to MHC class I molecules was assessed by the IEDB MHC-I prediction tool at (http://tools.iedb.org/mhci/n). MHC-I peptide complex presentation to T-lymphocytes underwent several steps. For instance, the attachment of cleaved peptides to MHC-1 molecules was predicted by an Artificial Neural Network (ANN) [20]. Also, all of the epitope's lengths were set as 9 amino acids. Besides, all the conserved epitopes that bind to alleles at a score equal to or less than 100 half-maximal inhibitory concentrations (IC50) were selected for further analysis [21].

MHC Class II Binding Predictions:

Analysis of peptide binding to MHC class II molecules was assessed by the IEDB MHC II prediction tool at (http://tools.immuneepitope. org/mhcii/) [23-24]. For MHC-II binding prediction, human allele references set were used. MHC class II groove can bind peptides with different lengths. Therefore, for the analysis, the NN-align as prediction method from the IEDB MHC-II prediction tool was used. It allows for identification of the MHC class II binding core and epitopes binding affinity All conserved epitopes that bind to many alleles at score equal or less than 500 half-maximal inhibitory concentrations (IC50) were selected for further analysis.

Population Coverage Calculation:

For the calculation of the population coverage for all potential MHC-I and II epitopes bindings, the

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IEDB tools (http://tools.iedb.org/ tools/ population/ iedb_input) was used. The MERS virus spike glycoprotein was assessed for population coverage against the whole world and North Africa with selected MHC-I and MHC-II interacted alleles [25].

Homology Modeling:

Raptor X protein structure prediction server was used for creating the 3D structure of virus spike glycoprotein (http://raptorx.uchicago.edu/StructurePrediction/predict/) [26]. The reference sequence [NP_112027.1] was used as an input and Chimera 1.8 was used as a tool to visualize the selected epitopes belonging to B cell and T cell (MHC-I and MHC-II). Homology modeling was used for visualization of the surface accessibility of the B lymphocytes predicted candidate epitopes as well as for visualization of all predicted T cell epitopes in the structural level [27].

Results

Multiple sequence alignment of the Retrieved Strains

All retrieved sequences of MERS Coronaviruses spike glycoprotein strains were aligned to obtain conserved regions using multiple sequence alignment (MSA) with accession number which revealed a few discrepancies in amino acid A instead of V and S in position 26 and 226 respectively Figure1

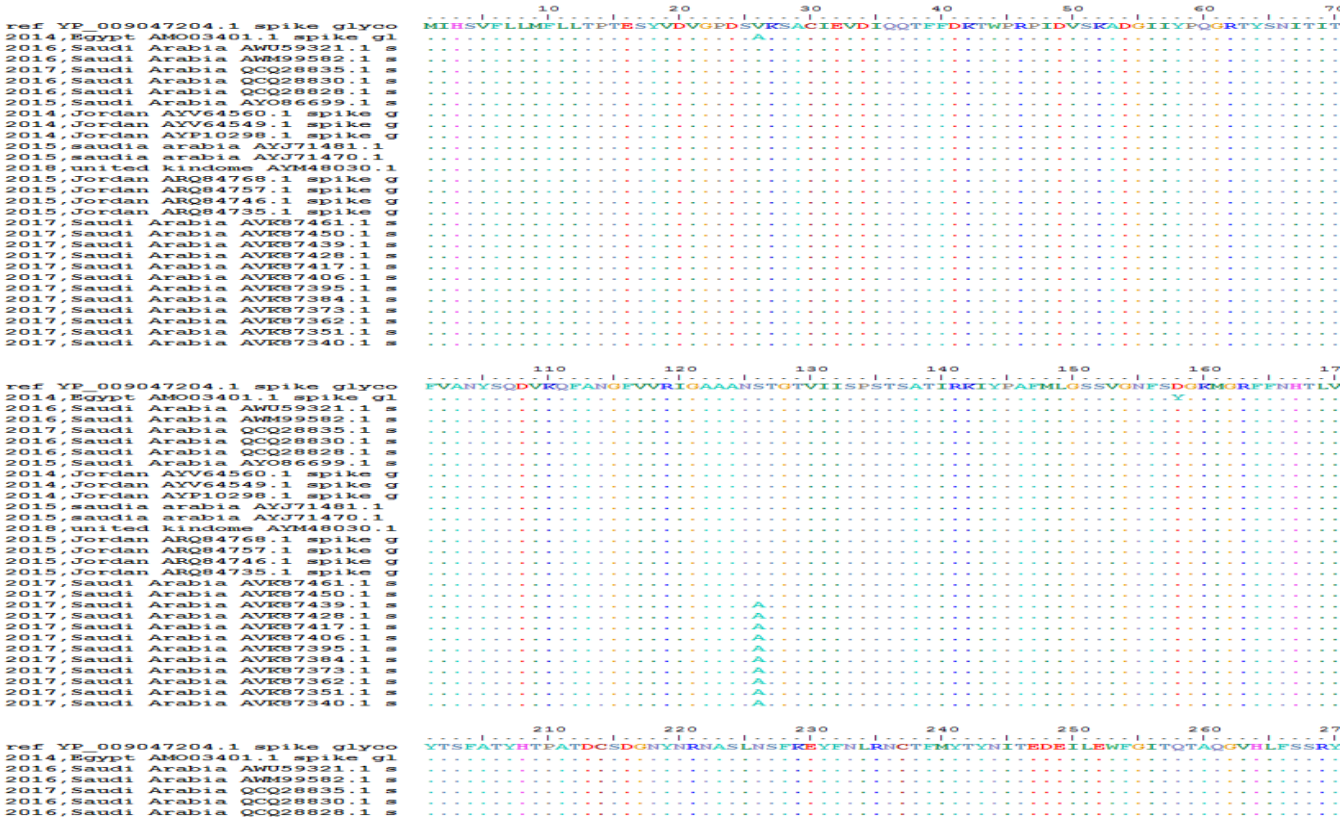

Figure 1 Sequence alignment showed that some regions were mutated region, and dots show the conservancy between different retrieved sequences

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Prediction of B-cell Epitope

The reference sequence of MERS-Coronavirus glycoprotein was subjected to Bepipred linear epitope, Emini surface accessibility, Kolaskar, and Tongaonkar antigenicity methods in IEDB to predict the likelihood of specific regions in the protein that bind to B cell receptor, being in the surface and immunogenic respectively. The thresholds of Bepipred linear epitope, Emini surface accessibility, and Kolaskar and Tongaonkar antigenicity were shown in Figures 2 & 3 and Table 1. For Bepipred linear epitope prediction method, the average binding score of viral protein to B cell was predicted as a linear epitope and Emini surface accessibility provided epitopes that were potentially predicted on the surface bypassing the default threshold 1.000. Kolaskar and Tongaonkar antigenicity provided epitopes that gave a score above the default threshold 1.045. The epitope predicted by these different tools against B cell were provided in Table 1. Accordingly, one conserved epitope was successfully predicted to elicit the B cell lymphocytes since they were conserved among all retrieved strains, got higher score values in Emini surface accessibility, and Kolaskar and Tongaonkar antigenicity prediction methods. These epitopes were **106- SQDVKQ -111**. The three-dimension structural (3D) level of this epitope was shown in Figure 4.

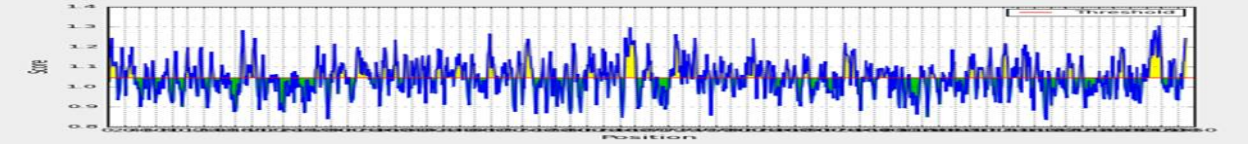

Figure 2 surface accessibility analyses using the Emini surface accessibility scale

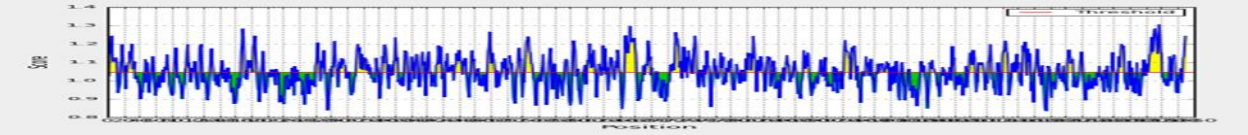

Figure 3 Kolaskar and Tongaonker antigenicity

Table 1: B-cell Epitopes Prediction, the Position of Peptides is According to the Position of Amino Acids in the Spike Glycoprotein of the MERS- Corona Viruses.

No.	Start	End	Peptide	Length	a.emini score	b.koloskare score
6	**106**	**111**	**SQDVKQ**	**6**	**2.651**	**1.037**

a: default threshold value 1.000

b: default threshold value 1.034

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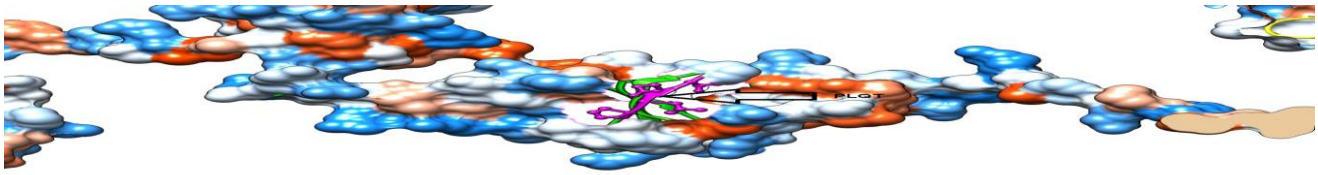

Figure 4. ***Position of Proposed Conserved B Cell Epitopes in Structural Level of spike Glycoprotein of*** MERS -***Coronavirus*****.**

T lymphocytes Epitopes Binding Prediction:

MHC-I Binding Predictions:

The reference structural protein (spike glycoprotein) was analyzed using the IEDB MHC-1 binding prediction tool to predict T cell epitopes interacting with different types of MHC-I alleles. Conserved peptides were predicted to interact with different MHC-1 alleles. The peptide **279-FQFATLPVY-287** and **786-FSFGVTQEY-794** and **69-ITYQGLFPY-77** also interacted with two alleles as shown in **Table 2**. These three epitopes and their positions in the structural level of spike glycoprotein were shown in **Figure 5**.

Table 2: List of Top Epitopes that had Binding Affinity with MHC-I alleles. The position of peptides is according to the position of amino acids in spike glycoprotein of the MERS -**Coronavirus.**

Allele	Start	End	Peptide
HLA-A*02:06, HLA-A*29:02, HLA-A*30:02, HLA-B*15:01, HLA-B*18:01, HLA-B*15:02, HLA-B*35:01, HLA-C*12:03	*279*	*287*	***FQFATLPVY***
HLA-A*29:02, HLA-A*26:01, HLA-A*30:02, HLA-A*68:01, HLA-B*15:01, HLA-B*35:01, HLA-B*46:01, HLA-B*58:01, HLA-C*12:03	**786**	**794**	**FSFGVTQEY**
HLA-A*11:01, HLA-A*29:02, HLA-A*30:02, HLA-A*32:01, HLA-B*15:01, HLA-B*35:01, HLA-B*58:01, HLA-C*12:0	**69**	**77**	**ITYQGLFPY**

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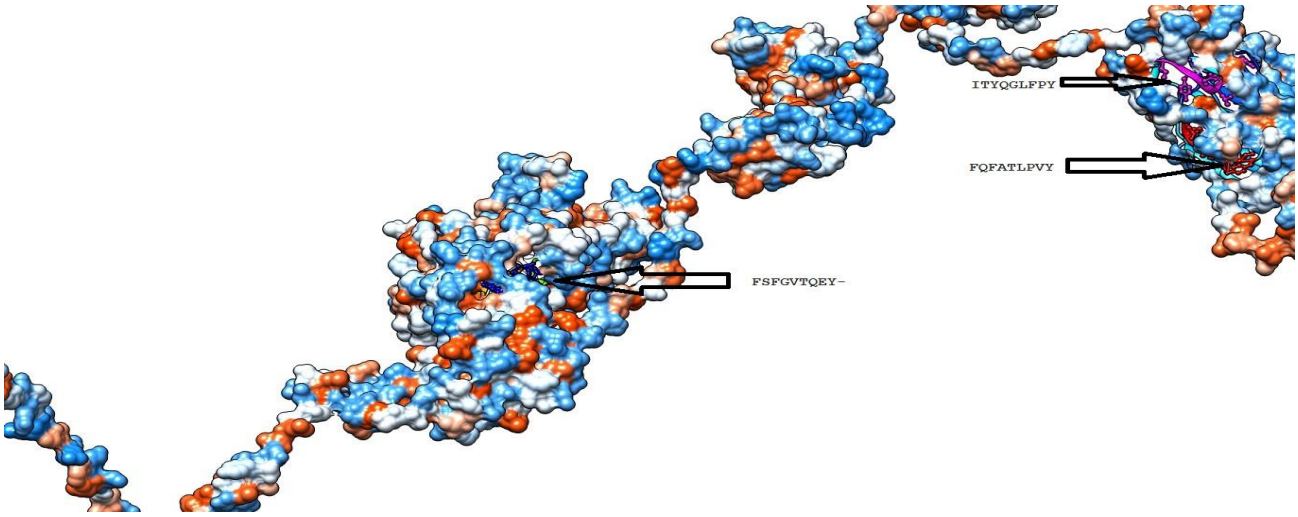

Figure 5: Position of Proposed Conserved T Cell Epitopes that Interact with MHC-I in Structural level of spike glycoprotein of MERS -Corona Virus

Table 3: List of Top Epitopes that had Binding Affinity with MHC-II alleles.

Allele	Start	End	Peptide
HLA-DQA1*05:01/DQB1*03:01	**923**	**937**	**AQYVAGYKV**
HLA-DRB1*01:01	**924**	**938**	
HLA-DRB1*07:01	**922**	**936**	
	926	**940**	
	925	**939**	
	920	**934**	
	921	**936**	
HLA-DPA1*03:01/DPB1*04:02	**1271**	**1285**	**ALNESYIDL**
HLA-DRB1*01:01	**1269**	**1283**	
HLA-DRB4*01:01	**1270**	**1284**	
	1272	**1286**	
	1268	**1282**	
HLA-DQA1*01:02/DQB1*06:02	**1299**	**1313**	**AGLVALALC**
HLA-DQA1*03:01/DQB1*03:02 HLA-DQA1*04:01/DQB1*04:02	**1300**	**1314**	
	1301	**1315**	
	1298	**1312**	
	1302	**1316**	
	1303	**1317**	
	1304	**1318**	

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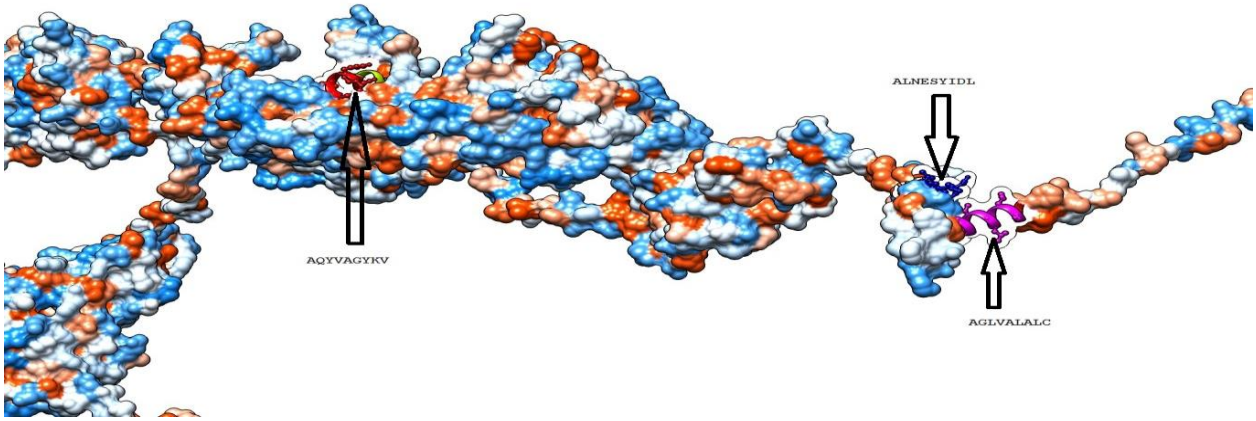

Figure 6: Position of proposed conserved T cell epitopes that interact with MHC-II

Population Coverage

Population coverage was performed for predicted T-cell epitopes and their respective MHC-I and MHC-II alleles preliminarily. We selected only three epitopes that interacted with most frequent MHC-I alleles **279-FQFATLPVY-287 and 786-FSFGVTQEY-794** and **69-ITYQGLFPY-77**. They demonstrated population coverage against the whole world **60.81%** Three epitopes, **924-AQYVAGYKV-938**, **1271-ALNESYIDL-1285** and**1300-AGLVALALC-1314**, demonstrated population coverage against the whole world 65.76% against MHC-II. Interestingly the epitope **924-AQYVAGYKV -938** was shown to interact with both MHC-I and MHC-II alleles. The overall epitope sets for the predicted epitopes against MHC-I and MHC-II alleles were **91.18%** as shown in Figure 6. We selected only nine countries of the MENA region while predicting population coverage, which are North Africa, Iran Israel, Jordan, Lebanon, Oman, Saudi Arabia, Turkey, and the United Arab of Emirate. In North Africa, population coverage was 74.33%, Iran 78.30%, Israel 69.89%, Jordan 66.94%, Lebanon 68.38%, Oman 55.47%, Saudi Arabia 84.68%, Turkey 79.73% and United Arab of Emirate 2.19%. The highest population coverage 84.68%, was seen for Saudi Arabia and the lowest at 2.19%.was seen for the United Arab of Emirate.

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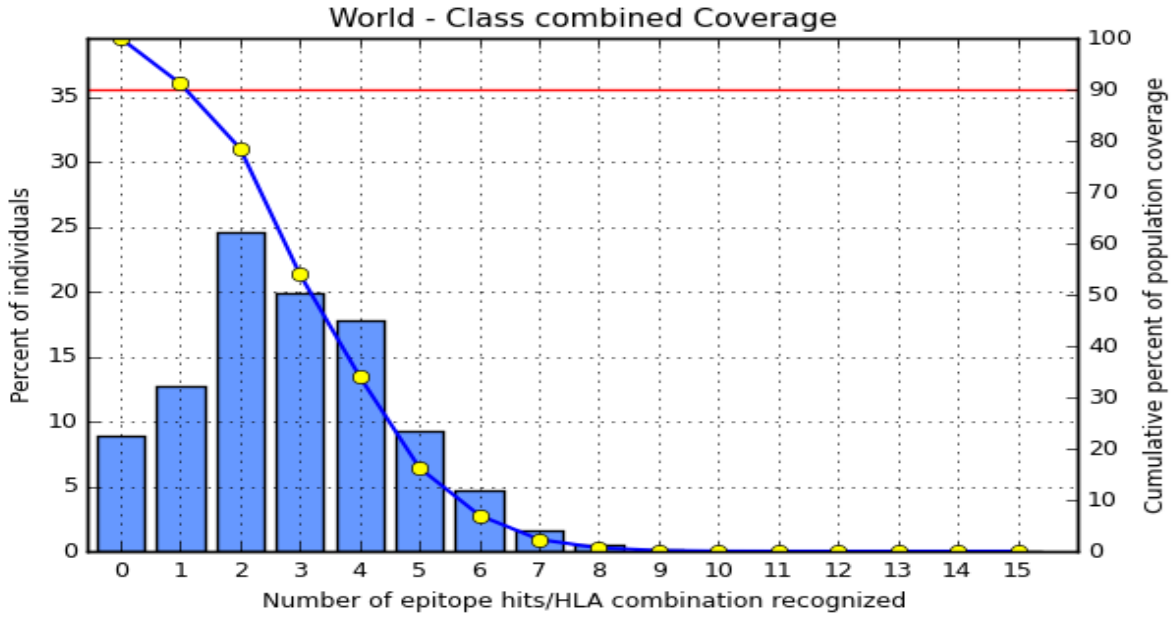

Figure 6 Combined population coverage of MHC class I and class II for proposed peptides from Selected Spike glycoprotein of MERS -Coronaviruses.

Discussion

Researchers to predict possible antigenic epitopes from MERS -Corona-virus proteins (especially Spike glycoprotein) for peptide-based vaccine development have instantly utilized advancements of immuno-informatics tools. *In-silico* adducts aided over *in vitro* experimental techniques and tools in vaccine design individually in terms of cost as well as time. Moreover, MERS -COV is being a positive-strand RNA virus is more vulnerable to mutation due to a lack of proofreading activity of RNA polymerase. However, for reliable vaccine candidates, a highly conserved epitope will ensure effective and long-lasting immunity. Therefore, this study aims to identify potential epitopes that can induce cellular and humoral immune reactions and act as a candidate for vaccine development. Therefore, we used immune- informatics tools to identify epitopes for multiple peptide vaccine for Spike glycoprotein. One epitope from the only conserved region was found to interact with Spike glycoprotein, It was found that the most satisfactory peptide is 6 amino acid **106 – SQDVKQ-111** B cell epitope from 106 to 111 with antigenicity score of 1.037 and 2.651 Score for Emini surface accessibility and chosen as a proposed peptide that can activate B cell to produce antibodies against the virus or it had a domain that can neutralize antibodies [28]. While three Epitopes from structural Spike glycoprotein interacted with MHC class I HLA alleles. T cell immune response is essential for longer-lastingresponses [28]. The proposed T cell peptide **279-FQFATLPVY-287 and 786-FSFGVTQEY-794** and **69-ITYQGLFPY-77** with potential population coverage of **60.81%** this peptide considered as a candidate for vaccine production. Three epitopes, **924- AQYVAGYKV-938**, **1271-ALNESYIDL-1285**, and **1300-AGLVALALC-1314**, demonstrated population coverage against

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the whole world 65.76% against MHC-II. Interestingly the epitope **924-AQYVAGYKV -938** was shown to interact with both MHC-I and MHC-II alleles. The following proposed peptides are recommended for multiple peptides vaccine designs against Coronavirus **106–SQDVKQ-111**, **279-FQFATLPVY-287, and 786-FSFGVTQEY -794** and **69-ITYQGLFPY-77, and 924-AQYVAGYKV-938**, **1271-ALNESYIDL-1285**, and **1300-AGLVALALC-1314**. This vaccine will ensure good population coverage **91.18%** and fewer side effects that can be seen with the live-attenuated vaccine with T cell response against the vaccine. The peptides found in the present study may prove more immunogenic as compared to the earlier reported peptides [29]. Predicted peptides might show the physicochemical instability, to overcome this limitation, several structural as well as physical modification strategies are available to enhance the poor physicochemical stability of peptides. These strategies are including peptidomimetic approach, prodrug approach, analog formations, hydrophobic ion pairing, conjugation with fatty acids, and use of substitute methods of drug administration. Researchers have been working to gather data linked to Corona-virus to understand its biology, transmission, and pathophysiology to eliminate the disease. Shortly, we anticipate that predicted epitopes have therapeutic potential with an outstanding scope. Our immune-informatics examinations have proposed a strong T cell epitope along with a B cell epitope that will efficiently support the development of potent peptide-based vaccines to deal with the MERS -Corona-virus challenge. In recent studies, novel antigenic epitopes of some essential and vital proteins revealed that could victoriously elicit the response of the immune system, therefore, becoming great peptide vaccines targets and protecting the host from virus attack. Therefore, the current research was conducted to predict antigenic epitopes of spike glycoprotein of MERS -Corona-virus. We carried out sequence, structure, and conservation analysis as well as homology modeling of spike glycoprotein of Corona-virus. These epitopes were capable to induce a particular immunologic response. Hopefully, that few of the antigenic epitopes suggested and screened in this work might present a preliminary set of peptides for future vaccine development against MERS -Corona-virus for control and prevention of this devastating epidemic. Limitation occurs where there is no alleles of Sudanese have not been screened. There are only 29 sequences of Spike glycoproteins available in the database; more sequences are needed to increase the significance of the result. This vaccine will ensure a good population. Coverage and fewer side effects that can be seen with life attenuated vaccine.

Conclusion and Recommendations

The efficacy and safety of predicted epitopes by this computational analysis are needed to evaluate animal model studies, to confirm whether they can induce a protective immune response or not. More sequences are needed to increase the significance of the result. The following proposed peptides are recommended for multiple peptides vaccine

designs against MERS **106–SQDVKQ-111**, **279-FQFATLPVY-287, and**, **1300-AGLVALALC-1314**. This vaccine will ensure a good population. Coverage and fewer side effects that can be seen with life attenuated vaccine.

Recommendations: Using Animal model studies, to confirm whether they can induce a protective immune response or not.

Declaration: The views expressed in the submitted article are the author's own and not an official position of the institution or funder.

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Original Article

Sudan Medical Laboratory Journal

Anthropometric measurements and indicators of body fat distribution in patients with bronchial asthma

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Abstract

Background: Previous studies repeatedly demonstrate a higher prevalence of bronchial asthma (BA) in subjects with high as well as low body mass index (BMI). This U-shaped associations between BMI and BA raise a question whether indicators of body fat distribution are helpful to predict BA prevalence and symptom control. The aim of this study was to evaluate anthropometric measurements and indicators of body fat distribution in asthmatic patients.

Materials and Methods: One hundred and twenty asthmatic patients were recruited from chest refer clinics - Military Hospital, Khartoum, Sudan and served as the test group. Another 59 non–asthmatic subjects were recruited from co-patients, University students/employees and served as the control group. Following clinical and spirometric evaluation of the studied subjects, the following were measured: body weight, height, waist circumference (WC), hip circumference (HC), triceps (TSF), biceps (BSF), subscapular (SSSF) and suprailiac (SISF) skinfolds thicknesses and the ratio between waist and hip circumferences (WHR). Body fat percent (BF %) and BMI were calculated. Based on BMI, studied subjects were categorized into four classes: underweight, normal weight, overweight, and obese.

Results: Although BMI, HC, TSF, BSF, SSSF, SISF and BF% were higher in asthmatic patients compared to non-asthmatic subjects, the difference of each of these parameters did not reach statistical significance. WC and WHR were significantly higher in asthmatic patients (88.50 (78.00- 101.75), 83.00 (78.47- 90.17)) compared with non-asthmatic subjects (81.00 (72.00- 92.00), 80.00 (75.67- 85.10), P = 0.004, 0.003). Presence of BA in underweight subject was comparable to normal BMI subjects (OR=1.05). However, presence of BA increases steadily in overweight (OR=1.46) and obese subjects (OR=2.67) compared with normal BMI subjects. Presence of symptoms at the time of the study increases in underweight (OR=3.55), overweight (OR=2.13) as well as obese (OR=3.43) compared to normal BMI subjects.

Conclusion: The results of the present study provide further evidence for the association between BA and obesity. Although all indicators of body fat distribution were higher in asthmatic patients compared to non-asthmatic subjects, only WC and WHR reached statistical significance, which points to the importance of abdominal obesity in the pathophysiology of BA.

Keywords: Anthropometric measurements, bronchial asthma, obesity.

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Introduction

Previous studies demonstrate a close relationship between bronchial asthma (BA) and obesity (1-7). From physiological standpoint, adipose tissue releases a group of cytokines which synergistically enhance airways mucosal inflammation associated with BA (8). In addition, obesity induces restrictive ventilatory defect, increases elastic work of breathing and consequently oxygen demands (9). This may explain the augmented perception of dyspnea in patients with already compromised lung like those suffering from BA (6, 10). Several studies were able to demonstrate higher prevalence of BA in patients with high as well as low body mass index (BMI) (2, 11, 12). The predominance of BA in both extremes of BMI raises a question whether indicators of body fat distribution e.g. waist circumference (WC), hip circumference (HC), the ratio between waist and hip circumferences (WHR), triceps (TSF), biceps (BSF), subscapular (SSSF) and suprailiac (SISF) skinfolds thicknesses would be helpful to predict BA prevalence and symptom control. The aim of this study was to evaluate anthropometric measurements and indicators of body fat distribution in patients suffering from BA.

Materials and Methods

This study received ethical approval from the Ethical Review Committee - Al-Neelain University Board, Khartoum, Sudan. All studied subjects signed informed consents before being enrolled in the study.

The study was conducted in the Military Hospital - Sudan during the period June 2016 –January 2017. One hundred and twenty asthmatic patients were recruited from chest refer clinics - Military Hospital, Khartoum, Sudan and served as the test group. Another 59 non–asthmatic subjects were recruited from co-patients, University students/employees and served as the control group. Asthmatic patients were defined as self-reported, physician diagnosed BA cases for at least two years. Cigarette smoking, pregnancy, age below 20 years or above 40 years and chronic diseases like diabetes mellitus and hypertension were excluded in all studied groups.

Medical history, clinical examination, results of lung function tests and anthropometric measurements were collected using a prearranged data collection sheet. The examined anthropometric measurements were body weight, height, waist circumference (WC), hip circumference (HC), triceps (TSF), biceps (BSF), subscapular (SSSF) and suprailiac (SISF) skinfolds thicknesses. The ratio between waist and hip circumferences (WHR) was calculated for each subject. BMI was calculated using the formula: BMI = body weight (kg)/height^2 (m^2) (13). Based on BMI, studied subjects were categorized into four classes: underweight (< 18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese (> 30 kg/m^2) (14). The body fat was calculated from the four measured skinfold thicknesses using Durnin-Womersley formula (15). Body fat percent (BF %) was calculated using the formula: BF% = (fat mass/body weight) X100. Statistical evaluation was performed using SPSS (version 20, Chicago, SPSS Inc. USA). Normal

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distribution of variables was examined using Shapiro-Wilk test. The normally distributed variables were described with mean and standard deviation (SD). Studied variables with abnormal distribution were described with median and 25th–75th interquartile (Q1–Q3). Unpaired T-test was used to assess statistical difference of the mean for normally distributed variables. Alternatively, significant statistical differences of abnormally distributed variables were assessed by comparing median (25th–75th interquartile) and giving the P value of Mann-Whitney U test. P < 0.05 was considered significant for all statistical tests.

Results

Distribution of age and gender were comparable in the studied groups, table 1. In contrast, FEV1% and PEFR were significantly lower in asthmatic patients compared to the control group, table 1.

Table 1: Characteristic of the studied groups

	Non-asthmatic N = 59 Median (25th – 75th Quartile) N (%)	Asthmatic N = 120 Median (25th – 75th Quartile) N (%)	P
Age (Years)	28.00 (25.00- 33.00)	28.00 (24.00- 36.00)	0.674
Male N (%)	30 (50.85%)	58 (48.33%)	0.752
FEV1%	82.99 (74.26- 88.07)	75.60 (67.63- 82.46)	< 0.001
PEFR (L/min)	410.0 (350.0- 510.0)	300 (242.5- 360.0)	< 0.001

The indicators of body fat distribution in the studied groups is shown in table 2. Although BMI, HC, TSF, BSF, SSSF, SISF and BF% were higher in asthmatic patients compared to non-asthmatic subjects, the difference of each of these parameters did not reach statistical significance in the studied groups, P > 0.05.WC and WHR were higher in asthmatic patients (88.50 (78.00- 101.75), 83.00 (78.47- 90.17)) compared with non-asthmatic subjects (81.00 (72.00- 92.00), 80.00 (75.67- 85.10), P = 0.004, 0.003), table 2.

Table 2: Indicators of body fat distribution in the studied groups

	Non-asthmatic N = 59 Mean (SD)Median (25th – 75th Quartile	Asthmatic N=120 Mean (SD)Median (25th – 75th Quartile)	P
Weight (Kg)	67.60 (58.70- 79.00)	73.72 (17.31)	0.106
Height (m)	1.67 (0.09)	1.67 (0.09)	0.503
BMI	24.13 (20.48- 27.77)	26.22 (21.73- 30.75)	0.060
WC (mm)	81.00 (72.00- 92.00)	88.50 (78.00- 101.75)	0.004
HC (cm)	101.00 (95.00- 110.00)	106.00 (95.00- 116)	0.217
WHR	80.00 (75.67- 85.10)	83.00 (78.47- 90.17)	0.003
TSF (mm)	18.00(10.00- 25.00)	17.00 (9.25- 24.00)	0.719
BSF (mm)	9.00(5.00- 14.00)	10.00 (6.00- 20.75)	0.078
SSSF (mm)	18.00 (12.00- 22.00)	18.00 (12.25- 26.00)	0.594
SISF (mm)	19.00 (12.00- 22.00)	19.00 (12.0- 24.75)	0.622
BF %	25.40 (20.40- 33.70)	27.10 (19.40- 35.55)	0.905

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As shown in figure 1, presence of BA in underweight is comparable to normal BMI subjects (OR=1.05, 95% CI: 0.35−3.03). However, presence of BA increases steadily in overweight (OR=1.46, 95% CI: 0.68−3.12) and obese subjects (OR=2.67, 95% CI: 1.07−6.63) compared with normal BMI subjects. Presence of symptoms at the time of the study increases in underweight (OR=3.55, 95% CI: 0.41−31.00), overweight (OR=2.13, 95% CI: 0.66−6.86) as well as obese (OR=3.43, 95% CI: 0.87−13.54) compared to normal BMI subjects, figure 2.

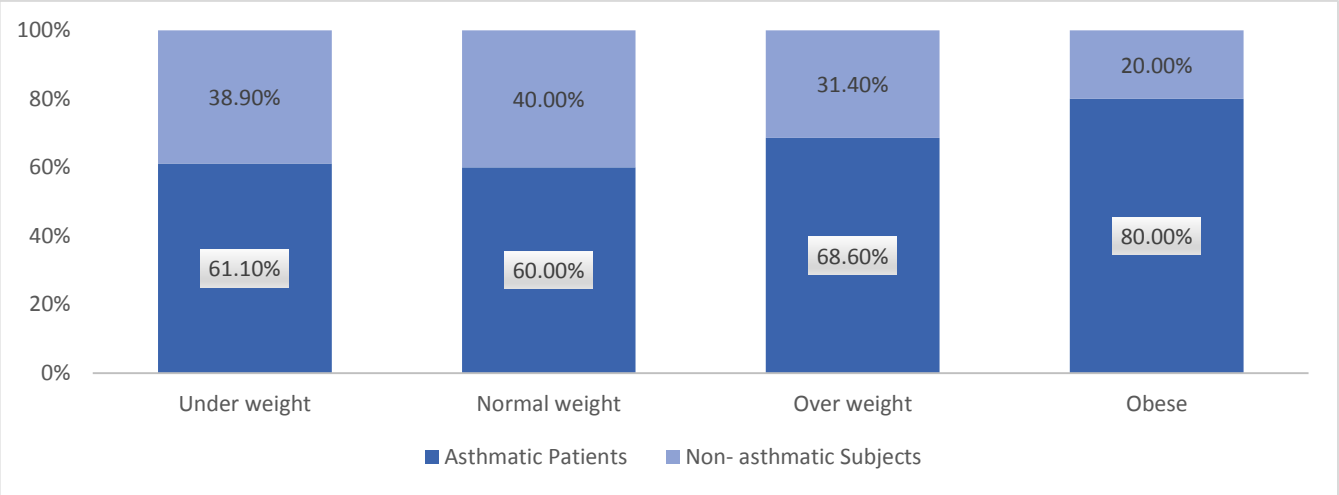

Figure 1: Percentage of BA among different BMI categories.

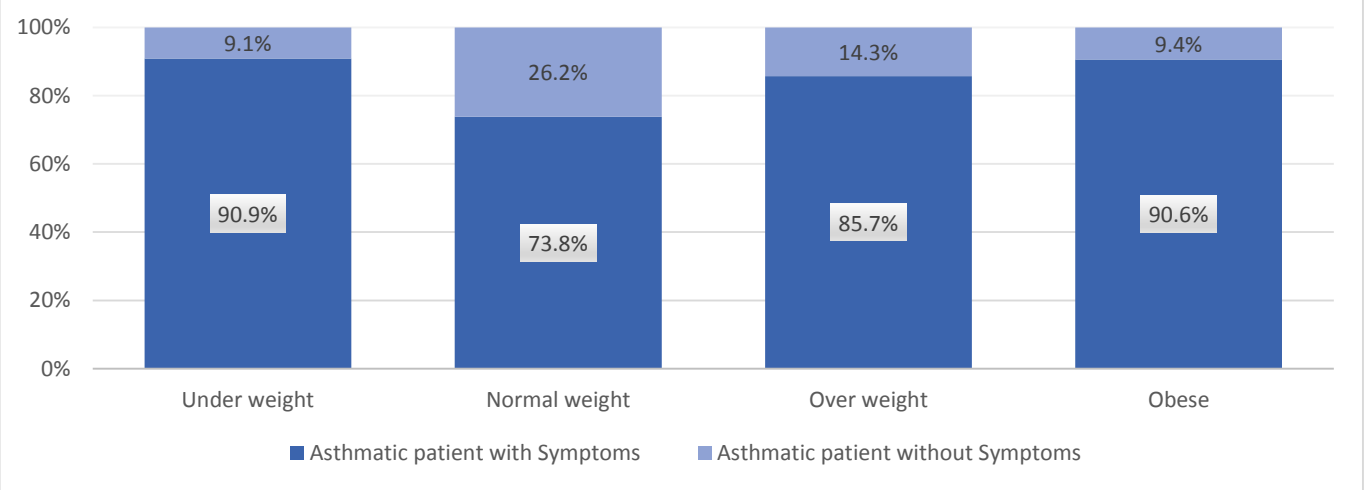

Figure 2: Distribution of symptoms among BA patients with different BMI

Discussion

The results of the present study revealed three main findings: firstly, although all indicators of body fat distribution were higher in asthmatic patients compared to non-asthmatic subjects, only WC and WHR reached statistical significance. Secondly, presence of BA in underweight patients is comparable to normal BMI subjects, however the presence of BA increases steadily in overweight and obese subjects compared with normal weight subjects. Thirdly, presence of BA symptoms at the time of the study increases in underweight, overweight as well as obese subjects compared to normal BMI subjects.

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Previous investigations repeatedly demonstrate an intimate relationship between BA and obesity in women (5, 16-18), except for a few reports (4). The first report on the association between BA and obesity was probably in the mid-eighties of the last century. In 1986, data correlating BMI to health service consumption for over 20 years old Dutch adults demonstrated significant association between overweight and BA in women, but not for men (16). Several studies thereafter were able to confirm significant association between high BMI to BA in females (5, 17, 18). Camargo et al demonstrated that women who gained weight after age 18 were at higher risk of developing BA during the following 4 years (19). The association between BA and obesity was also demonstrated in the results of a study exploring the relationship between BMI and prevalence of common chronic diseases in Italian National Health Survey (20). According to a longitudinal cohort of the Tucson Epidemiologic Study of Airways Obstructive Diseases, BMI equal to or more than 28 Kg/m^2 increased the odds of BA diagnosis 2.1 times, irrespective of gender (5). Increased prevalence of BA among high BMI adults was also established in people from 1970 British Cohort Study (BCS70) when they were surveyed at the age of 26 years about past history of BA (18). After controlling for possible confounders, the odds ratio comparing uppermost with lowermost BMI quintile in BCS70 was 1.72. In addition, the association between fatness and BA was more evident in females (18).

Luder et al explored the association between BMI and BA in over 18 years old adults of diverse ethnic and socioeconomic background (2). The results showed that BA prevalence was significantly higher in women with a BMI 25 kg/m^2 or more. According to the same study, the prevalence of BA in men was increased in those with BMI less than 22 kg/m^2 as well as BMI equal to or more than 30 kg/m^2. This U-shaped association was more evident in the males with age range 18-49 years. In a separate large scaled cross-sectional study in Chinese adults, both extremes of BMI distribution were also associated with airway hyperresponsiveness (AHR) in both men and women (11). The same U-shaped associations between BMI and AHR were demonstrated in both gender when data from European Community Respiratory Health Survey were analyzed (12). In the present study, although presence of BA in underweight is comparable to normal BMI subjects, it increases steadily in overweight and obese subjects. In contrast, presence of BA symptoms at the time of the study is equally high in underweight and overweight asthmatic subjects. The predominance of BA in both extremes of BMI may explain why most indicators of body fat distribution failed to reach statistical difference when asthmatic patients were compared to non-asthmatic subjects in the present study.

Interestingly, WC and WHR were statistically higher in the studied asthmatic patients compared to non-asthmatic subjects. This finding points to the probable importance of abdominal obesity in pathophysiology of BA. In a large cohort of 88,304 female teachers, Von Behren et al examined the association between BA prevalence and WC as a measure of abdominal obesity (3). The study confirmed that large WC was associated with

increased BA prevalence even among women with a normal BMI. According to the same report, the OR for BA was also higher among abdominally obese women compared to those with less WC. In a separate prospective study involving 23,245 subjects living in Nord-Trøndelag, Norway in 1995-2008 (HUNT study), abdominal obesity remained a risk factor for BA development after adjustment for BMI (1). The implication of HUNT study was reproduced by the North West Adelaide Health Study, which used WC and WHR as measures of central obesity (7).

Physiologically, obesity reduces respiratory system compliance and consequently lung volumes and capacities (9, 21) . Another consequence of restrictive ventilatory defect associated with obesity is increased elastic work of breathing and consequently higher oxygen cost of pulmonary ventilation (9). Increased oxygen demands give further burden on the already compromised lung in obese subjects as described above. This may explain augmented perception of dyspnea in obese asthmatic patients (6, 10). Recently, adipose cells were proved to express powerful endocrine function that recruit immune cells and enhance inflammatory responses (8), especially among those with central obesity (22). The endocrine role of adipocytes gives an explanation for the higher prevalence of BA among obese subjects as described in the present study and many other previous reports (5, 16-18).

Conclusion

The results of the present study provided further evidence for the association between BA and obesity. Although all indicators of body fat distribution were higher in asthmatic patients compared to non-asthmatic subjects, only WC and WHR reached statistical significance. This finding points to the importance of abdominal obesity in the pathophysiology of BA, as described in the literature. The present results also showed that presence of BA and intensity of symptoms increases steadily with BMI, possibly because of the mechanical and endocrine effect of obesity on the respiratory system.

Abbreviations

AHR airway hyper-responsiveness; BA bronchial asthma; BF% body fat percent; BMI body mass index; BSF biceps skinfold thickness; FEV1% forced expiratory volume in the first minute percent; HC hip circumference; OR odds ratio; PEFR peak expiratory flow rate; Q1–Q3 25th–75th interquartile; SD standard deviation; SISF suprailiac skinfold thickness; SPSS Statistical package for the social sciences; SSSF subscapular skinfold thickness; TSF triceps skinfold thickness; WC waist circumference; WHR the ratio between waist and hip circumferences.

Ethics approval and consent to participate

The study received ethical approval from the Ethical Review Committee - Al-Neelain University Board, Khartoum, Sudan. All studied subjects signed informed consents before being enrolled in the study.

Consent for publication: Not applicable

Availability of data and materials: The data supporting the present findings are contained within the manuscript.

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Original Article

Sudan Medical Laboratory Journal

Association between Inflammatory Cytokines and Liver Functions in Rheumatoid Arthritis Patients

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Abstract

Background: Rheumatoid Arthritis (RA) associated with abnormal liver tests, and medications used for RA are often hepatotoxic. Therefore, this study aims to investigate an association between proinflammatory, antiinflammatory cytokines and liver function tests in rheumatoid arthritis patients.

Materials and Methods: In a descriptive cross-sectional study 88 RA patients were included, 84 were females and 4 were males, age ranged from 21 to 81 years old. Serum interleukin 10 (IL-10), interleukin 17 (IL-17), Osteopontin OPN and liver function tests were measured. **Results:** The frequency of RA is higher among adults >41 Years 72(81.8%) than young adults ≤41 Years 16(18.2%), RA is common in females 84(95.5%) than males 4(4.5%) approximately 21:1 fold. Young adults had higher abnormal IL-10 than adults RA patients (OR = 3.72, *p*-value 0.044). Abnormal IL-17 (OR= 5.67, *p*-value 0.034) were found to be increased in young adults RA patients. No association observed between age and OPN. No association between duration of disease and IL-10, IL-17 and OPN. No association found between types of treatment and IL-10, IL-17 and OPN. No association observed between IL-10, IL-17, OPN and liver parameters (AST, ALT, ALP, ALB, TP, and GGT).**Conclusion:** in conclusion proinflammatory and antiinflammatory cytokines are not associated with liver functions as has been demonstrated in RA patients.

Keywords: Cytokines, Liver Functions, and Rheumatoid Arthritis

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Introduction:

Rheumatoid arthritis (RA) is common autoimmune inflammatory disease. Although the prevalence of RA is lower globally 0.5-1% it is still associated with socioeconomics burden, and higher risk of mortality rate (Kwan *et al*., 2019). Recent studies have demonstrated that, the treatments used for RA improved outcome, and also account as risk for hepatic complications (Sundbaum *et al*., 2019). The adverse effects of RA treatments includes asymptomatic elevations of liver enzymes, fibrosis and may be fatal hepatic necrosis (Conway and Carey, 2017). On the other hand liver disorders were noted in untreated RA patients (Rakuomi *et al*., 2017).

increasing amounts of IL-10can be detected in the synovium of RA patients, a potent anti-inflammatory cytokine (Shikhpour *et al*., 2018),. Another fact is that the activity of RA cannot be attenuated by IL-10 administration (Holdsworth and Yi, 2015), Many researchers suggest that IL-10 plays an important role in chronic liver diseases (Zhang and wang, 2006). IL-17 is a pro-inflammatory cytokine, which contributes and upregulates in many autoimmune diseases such as RA. A high level of IL-17 is produced in different samples of RA (Elvira *et al*., 2018; Mengesha and Conti, 2017). Some investigators suggest that IL-17 plays a key role in many liver diseases, and also associated with the progress of disease (Du *et al*., 2013; Tan *et al*., 2013; Zheng *et al*., 2013), Osteopontin (OPN) is a pro inflammatory cytokine that induces RA (Shi *et al*., 2018; Athanasiadous *et al*., 2018; Luukkonen *et al*., 2017), That OPN is included in many liver diseases, beyond its roles, is still controversial (Iida *et al*., 2018). Therefore this study carried out to find out the association between pro inflammatory, anti-inflammatory cytokines and liver function tests among RA patients.

Materials and Methods

A descriptive cross sectional hospital based study on 88 RA clinically diagnosed according to the criteria of the American College of Rheumatology1987 (ACR), were examined at the common rheumatoid arthritis clinics in Khartoum State (military, alamal hospital and Zain clinic). Ethical permits for the studies were obtained from ethical review committees at the sites where patients were recruited, and all patients gave informed consent for their participation in the studies. All patients received treatment, the demographic data, type of treatment and duration of disease for each patient were recorded, 4 male and 84 female, age range 28 -90 years, Non Sudanese patients with RA and the doubtful diagnosed patients were excluded. Serum from each subject were centrifuged at 3000*g* for 10 minutes after clotting for 30 minutes at room temperature and were stored at -40°C until analysis, All samples were investigated for OPN , IL 17 and IL10 by sandwich enzyme linked immune sorbent assay (ELISA) (ELISA Development; Thermo Fisher scientific Systems-USA) according to the manufacturer's instructions, also liver functions test TP, Albumin, AST, ALT, GGT, and ALP were analyzed using full automated Mindary Chemistry Analyzer (BS 200), Data was statistically analyzed by Statistical Software Packages SPSS (version 16), Results were

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expressed as number and percent. *Chi square* was used to determine the level of significance (*P*-value of > 0.05 was considered to be statistically significant)

Results:

RA is more common in adults 72(81.8%) than young adults 16(18.2%), the frequency of the RA was found to be higher in female 84 (95.5%) than male 4 (4.5%). The patients receiving steroids was 52(59.1%) and the rest 36(40.9%) was on non steroids treatment. Moreover, 62(70.5%) was a duration of disease ≤6 and others 26 (29.5%) was >6 Years old. Abnormal IL-10 was found in 63(71.6%), was 25(28.4%) have normal percentage. The results of characteristic data shows, 80(91%) of RA patients had normal IL-17 whereas 8(9%) had abnormal. Normal OPN was observed in 76(86.4%) RA patients while 12(13.6%) was abnormal, the results presented in table-1. Chi-square analysis revealed that, young adults group had higher abnormal IL-10 than adults RA patients (OR = 3.72, *p*-value 0.044). Also abnormal IL-17 (OR= 5.67, *p*-value 0.034) were found to be increased in young adults RA patients whereas no association observed between age group and OPN (OR= 2.67, *p* value 0.144), shows table-2. Furthermore no association reported between duration of disease and IL-10, IL-17 and OPN with *p*-value (0.410, 0.176, 0.502) and OR (0.77, 0.37, 1.30) respectively, the results noted in table-3. No association found between types of treatment and IL-10, IL-17 and OPN with *p*-value (0.246, 0.286, 0.351) and OR (1.53, 2.21, 0.65) respectively, shows table-4. Person correlation analysis revealed that there were no association observed between IL-10, IL-17, OPN and liver parameters (AST, ALT, ALP, ALB, TP, and GGT), results reported in table-5.

Table (1)

	Variables	**Frequency (%)**
Age	≤41 Years	16 (18.2%)
	>41 Years	72 (81.8%)
Sex	Male	4 (4.5%)
	Female	84 (95.5%)
Treatment	Steroid	52 (59.1%)
	Non-steroid	36 (40.9%)
Duration	≤6 Years	62 (70.5%)
	>6 Years	26 (29.5%)
Cut off IL10	Abnormal	63(71.6%)
	Normal	25(28.4%)
Cut off IL17	Abnormal	8(9%)
	Normal	80(91%)
Cut off OPN	Abnormal	12(13.6%)
	Normal	76(86.4%)
	Total	**88 (100%)**

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Table (2)

Variables	Age		OR	CI-Lower CI-Upper	*p*-value
	≤41 Years	>41 Years			
IL 10					
Abnormal	14 (23.0%)	47 (77.0%)	3.72	(0.78-17.7)	0.04
Normal	2 (7.4%)	25 (92.6%)			
IL 17					
Abnormal	2 (40.0%)	6 (60.0%)	5.67	(1.24-25.7)	0.03
Normal	12 (15.0%)	68 (85.0%)			
OPN					
Abnormal	4 (33.3%)	8 (66.7%)	2.67	(0.69-10.2)	0.14
Normal	12 (15.8%)	64 (84.2%)			

Table (3)

Variables	Duration		OR	CI-Lower CI-Upper	*p*-value
	≤6 Years	>6 Years			
IL 10					
Abnormal	42 (68.9%)	19 (31.1%)	0.77	(0.28-2.13)	0.41
Normal	20 (74.1%)	7 (25.9%)			
IL 17					
Abnormal	4 (50.0%)	4 (50.0%)	0.37	(0.08-1.65)	0.17
Normal	58 (72.5%)	22 (27.5%)			
OPN					
Abnormal	9 (75.0%)	3 (25.0%)	1.30	(0.32-5.25)	0.50
Normal	53 (69.7%)	23 (30.3%)			

Table (4)

Variables	Treatment		OR	CI-Lower CI-Upper	*p*-value
	Steroid	Non-steroid			
IL 10					
Abnormal	38 (62.3%)	23 (37.7%)	1.53	(0.61-3.83)	0.24
Normal	14 (51.9%)	13 (48.1%)			
IL 17					
Abnormal	6 (75.0%)	2 (25.0%)	2.21	(0.42-11.6)	0.28
Normal	46 (57.5%)	34 (42.5%)			
OPN					
Abnormal	6 (50.0%)	6 (50.0%)	0.65	(0.19-2.21)	0.35
Normal	46 (60.5%)	30 (39.5%)			

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Table (5)

Parameters		*p*- value	R^2
IL10	AST	0.62	0.12
	ALT	0.20	0.66
	ALP	0.80	0.05
	ALB	0.16	-0.13
	TP	0.56	0.02
	GGT	0.25	0.15
IL17	AST	0.18	-0.15
	ALT	0.82	0.02
	ALP	0.82	-0.02
	ALB	0.23	0.12
	TP	0.59	0.05
	GGT	0.17	-0.14
OPN	AST	0.50	0.07
	ALT	0.25	0.12
	ALP	0.89	0.01
	ALB	0.29	-0.11
	TP	0.49	0.07
	GGT	0.98	-0.02

Discussion:

The abnormal liver functions were observed in RA patients. The researchers further attributed the abnormality to immune aggregations and others justified that by drugs toxicity. Accordingly, this study carried out to assess whether the pro inflammatory, anti-inflammatory cytokines associated with liver functions in rheumatoid arthritis patients.

The current study revealed that there is no association between interleukins and liver function tests. In fact that, abnormal liver tests were noted in patients with RA (Dinic *et al*., 2018). Concurrent with many previous studies indicated that, the frequency of RA is higher in elderly subjects (Mursal *et al*., 2016; Elsedig *et al*., 2014). Possible explanation might be that, in elder the protective mechanisms are decreased, resulting in decreased immunotolerance, decreased cytokines synthesis and T cells proliferation (Kobak and Bes, 2018). The demographic data indicated that, the prevalence of RA was found to be 21 fold higher in females than males. In contrast with previous study in Sudan that, the ratio is 9:1 females to males (Abdelsalam *et al*., 2011). Since the change in sex hormones after puberty associated with high prevalence of RA in females, moreover the female's immune system is potentially more reactive than males. Present study reported that, young adults more likely to have abnormal IL-10 and IL-17. These results disagreed with previous studies (Abd Elazeem *et al*., 2018; Akdeniz *et al*., 2018). Whereas no association found between age groups and OPN level. Concurrent with this finding a relationship between age and OPN has

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previously reported (Iwadate *et al*., 2013). Similar to other results, no associations between IL10, IL17, OPN level and duration of disease have been demonstrated (Abd Elazeem *et al*., 2018; Akdeniz *et al*., 2018; Al Zifzaf *et al*., 2015). In spite of IL-17 has been decreased after used of steroids therapy, whereas IL-10 was increased (Negera *et al*., 2018). The present study revealed no associations between IL-10, IL-17, OPN levels and types of treatment. It have become clear that, steroids directly modulate the pro inflammatory cytokine, or through suppression of cytokines producing cells (Negera *et al*., 2018; Noack *et al*., 2016).

Conclusion:

The data of present study concludes that, female at higher risk to have RA. Moreover, young adult RA patients are more likely had abnormal IL-10 and IL-17. Furthermore proinflammatory and antiinflammatory cytokines are not associated with liver functions as has been demonstrated in RA patients.

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OriginalArticle Sudan Medical Laboratory Journal

Risk Factors and Prevalence of Taeniasis among Displaced peoples in Ombadda Locality- Omdurman -Sudan

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Abstract

Background. Taeniasis is infection of human with intestinal cestode tape worm parasites belonged to the genus Taenia, species saginata and solium.

Objective: The study aimed to determine the risk factors and prevalence of taeniasis among displaced peoples district in Ombadda Omdurman state- Sudan.

Material and Methods: This is a cross sectional study was conducted in Ombadda and Basheer hospitals in Omdurman-Sudan from the period September to November 2021. A total of 150 stools samples were collected and examined using direct microscopy, formal ether concentration technique and Ziehl-Neelsen staining method for detecting Taenia Spp.

Results: A total of 150 stools samples collected and examined, 10 (6.3%) out of 150 faecal samples were positive and 140 (93.7%) were negative sample. Out of 10 positive samples 6 (60%) were males and 4(40%) were females their age groups range from 20 – 60 Years old, The Eggs stage of Taenia saginata and Taenia solium was identified by using direct microscopy and formal ether concentration t techniques and differentiated by the Ziehl-Neelson staining method.

Key words: Taeniasis, risk factors of transmission, displaced peoples.

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Introduction:

Taeniasis is infection of human with intestinal tapeworms cestode parasites belonged to the genus Taenia and It is a significant zoonotic disease (6) Because it may cause cysticercosis in the tapeworm carriers, family members, and other close contacts (17). The most important human pathogen species in the genus Taenia are *Taenia solium* the pork tapeworm and Taenia saginata the beef tapeworm. The other species *Taenia asiatica* is found only in East Asia (9). Taenia saginata and Taenia soliun are worldwide distributed, taenia saginata found in the countries where cattle are raised a while Teania solium is most common found in latin America, Southeast Asia and Africa practically prevalent in rural area where domestic pigs are found (3 ,19). Human infected with Teania saginata and Teania solium when they consumption infected beef meats or pigs' liver which were poorly cooked and eaten (8). These parasites have been complate indirect life cycle in two hosts, the definitive host was human which harbors the adult worm a while Porks

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and Cattles are intermediate host which was harbors the larval infective stage (15). Eggs or gravid proglottids are passed with feces of human the eggs are highly infectious and can survive for days to months in different environmental sources (7). Cattles and pigs become infected by ingesting vegetation contaminated with eggs or gravid proglottids. Taenia saginata and Teania solium consider to be like other several parasitic diseases cause mortality, T. solium cysticercosis is one of the most lethal parasitic diseases and is the most important foodborne Parasite (16). In terms of health and economic burden, T. solium has been reported as the first, and Taenia saginata as the nineteenth foodborne parasite at the global level [4]. Taenia saginata has a global distribution in Sudan than T.solium because it is considered one of the public health problem it is increasing mostly in the area where catles were arizing in community and if untreated causes economic burden and sever complication in human.

Material and Methods:

A cross sectional study conducted in Ombadda and Basheer hospitals in Omdurman State-Sudan from September to December 2021. A total of 150 faecal samples were collected in containers contain 10% formal saline as preservative reagents. were examined by microscopy and forma ether concentration as diagnostic methods, preserved faeses in formal saline were emulsified and homogenized, one drop of emulsified faecse suspensions placed on a slide microscope, covered with cover class, examined microscopy by wet preparation technique, using the 10 x objective and 40x objective of microscope lens for detecting segments and eggs of Taenia SPP in faeces. Using formal ether concentration method, 1 g of faeces emulsified in 4 ml of 10% forma water in test tubes, 3-4 ml of formal water added further in test tubes mixed well, emulsified faeces sieved in beaker, 2-3 ml of diethyl ether added to the suspension and mixed in the test tubes, homogenate suspension was centrifuged at 750-1000 g for 1 minute and the sediment was examined microscopically by using the 10 X objective and 40 x to identify eggs and segment of Taenia Spp. The Eggs stage of Taenia saginata and Taenia solium was detected, identified and differentiated by the Ziehl-Neelson staining method.

Results

A total of 150 faecal samples collected from displaced peoples in Ombada hospitals Omdurman - state examined. 10 (6.3%) out of 150 faecal samples were positive and 140(93.7%) were negative samples for Taenia Spp (Table1). The 10 positive samples that were seen in faecal samples Taenia saginata was9 (90%) and Taenia solium 1(10%) the eggs stag of Taenia Spp was identified using direct microscopy and formal ether concentration as diagnostic methods and differentiated by the Ziehl-Neelson staining method (Table3). Among these10 positive samples 6(60 %) were found to occur in males' and 4 (40 %) were found in female's patients the difference was found to be statistically in significant (*P*. Value=0.454) as shown in (Table2) .All patients came from displaced area their age groups range from 20 – 60 Years old and The age groups 31-40 to51-60 Years old were most commonly infected

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by Taenia SPP this differences was found to be statistically in significant (*P*. Value=0.425) (Table2) The Infection rate decreased in Patients their jobs 1(10%) were students and 2(20%) were non workers this differences was found to be statistically in significant (P.Value=0.566)in (Table2) . Patients jobs 5(50%) Slaughters and 2(20%) farmers were more susceptible to Taenia Spp infection in both sexes than other occupied else where the differences was found to be statistically significant (*P*. Value0.001) (Table 2). High infection rate occur in Patients had past history of eating under cooked meat of beef 9(90%) and eating under cooked meats of pork 1(10%) there was correlation association between Taenia SPP infection and peoples has cultural practices eating under cooked meat of beef and meat of pork the differences was found to be statistically significant (*P*. Value0.001) (Table 4.). infection rate increased in patients not using toilet for defecation and decreased in patients using toilet for stool disposable (Table 4).

Table (1): Prevalence of Taeniasis among displaced peoples in Ombadda and Basheer hospitals Omdurman state.

No Examined	Taenia *SPP*		Total Number
	Positive%	Negative%	
150	10(6.7%)	140(93.3%)	150

Table (2): Demographic characteristics of 10 patients with Taeniasis in Omdurman state.

	Variables	Taeniasis SPP		Total No	P-Value
Gender		T.saginat %	T.solium %		
	Males	5(60%)	1(10%)	10 (100%)	0.454
	Females	4 (40%)	0 (0%)		
Ages	20-30	2(20%)	0(0%)	2(20%(	
	31-40	2(20%)	1 (10 %)	3(30%)	0.425
	51-60	5 (50%)	0(0%(	5(50%)	
	Total	9(90%)	1(10%)	10(100%)	
Occupations	Student	1(10%)	0 (0%)	1(10%)	0.566
	Non workers	1(10%)	1(10%)	1(10%)	
	Farmers	2(20%)	0 (0%)	2(20%)	0.001
	Slaughters	5 (50%)	1(10%)	1(10%)	
	Total	9(90%)	1(10%)	10(100%)	

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Tables (3): Morphological differences between T. saginata and T. solium eggs using the Ziehl-Neelson staining method in 10 patients in Ombada hospital.

Total of feacal examined	Taeniasis positive	Ziehl-Neelson		Total No
		T.saginat	T.solium	
150	10	9(90%)	1 (10%)	10(100%)

Table (4) prevalence of Taeniasis and associated risk factors in displaced peoples

History of eating raw meats	T.saginata	T.solium	Total No	P.Value
Pork raw consumption +ve (1)	0 (0 %)	1(10%)	9(90%)	
Beef raw consumption +ve	9(90%)	0(0%)	1(10%)	0.001
Total	9(90%(	1 (10%)	10(100%	
Method of stool disposable				
Open defecation yes (8)	8 (80%)	0(0%)		
Using Toilet yes (2)	1(10%)	1(10%)		0.001
Total	9(90%)	1(10%)	10(100%)	

Discussions:

The results obtained in the present study indicated that the overall infection rate of Taeniasis among the displace people was 6.4% this percentage results considered to be higher rate of infection when compared by previous study obtained by(Ghada etal., 2016) (10) found that(1.6%) in Sudan and higher than percentage results by(McCleery et al..,2015) (13) reported a low prevalence rate(2.9%) of taeniasis among refugees living on the Thai–Myanmar border in Thailand and were extremely lower when compared by previous results reported by(wongsaroi etal., 2014)(20) in Korea reported that 18.2% of Taeniasis among Korean people probably due to consumption raw meats or under cooked meats of beefs and porks which was containing infective stage of Taeniais .

Our results in the present study showed that Taeina saginata infection rate was 9(90%) and Taenia solium infection rate was 1(10%) this indicate that Taenia saginata was more prevalent in displace people than Teania solium due to Muslim religion in the area and population raises cattle's houses, for food or for breeding. which was risk factor for taeniasis this results obtained when compared by previous study by (Anantaphruti etal., 2007) (2) in Thailand showed significant differences. When we analyzed the taeniasis infection in the gender, the prevalence rate was highest in males 6(60%) and lower in females 4(40%) the middle-aged groups ranging from 20–60 years old to 31–40 years old and51-60 Years old were most commonly infected by *Taenia saginata* this significant differences of a higher infection in males agreement with previous

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study by(Ng-Nguyen et al., 2018) (14) reported in highlands of Vietnam where it was noted that males has history of consumption raw meats and undercooked meats of beefs and pork's as cultural practices in dis place area .The results obtained showed that there are no Taeniasis infection reported in children age group(< 20 Years old)this may be due to fact that the samples size of stools was collected in sufficient to declared that children are not in risk of infection by Taeniasis when compare this results with previous studies showed that highly significant differences has been reported by (Lit etal.,2019) (11) in China ,(Madinga etal.,2012)(12) in Chan go and (Xud-M etal., 2010)(21) in Philippines reported that the highly Taeniasis infection found in children age group(< 20 Years old) .In the present study the Infection rate of Taeniasis carriers decreased in Patients their jobs 1(10%) were students and 2(20%) were non workers this results agree with the previous results reported by **(**Openshaw etal., 2018) (1) in western Sichuan, People's Republic of China and were predominantly occur in Patients their jobs 5(50%) Slaughters and 2(20%) farmers infection in both sexes than other occupied elsewhere this differences declared that the displace peoples a history of consuming raw or undercooked raw pork. , beef raw meats , working in corn fields and by cooking at their work places which was the most predominant risks factor for prevalence of Taeniasis similar results reported by (Swastikaetal.,2017) (18) in Karangasem Villages Indonesia .The present study showed that infection rate increased in patients has history of passing stools outside their houses not using toilet for defecation and decreased in patients using toilet for stool disposable which pre-oral infection by Taenia eggs These are important risk factors of taeniasis when compared by previous results reported by (Ito etal., 2020) (23) showed similarity.

Conclusion: Taeniasis is the major public health problem mostly associated with those have poor hygiene habit, lack of meat inspection, bush defecation and poor waste disposable which has been considered risk factors for Taeniasis, slaughtering practices have contributed to the establishment and transmission of disease, Taenia saginata is more common and prevalent indisplaced people than Taenia solium, consumption under cooked Beef meats and pork meats has been hazardous and risk factors facilitated transmission of Taeniasis.The results obtained showed that the Ziehl-Neelson staining method used more effective in parasites differencation than other techniques used

Conflict of interest: there are no conflict of interest.

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Original Article

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Measurement of Alkaline Phosphatase Activity and Calcium Levels in Sudanese Patients with Breast Cancer

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Abstract

Breast cancer is the most common cancer affecting women in Sudan, it is characterized by metastatic pattern in liver and bone. Changes in serum levels of biochemical parameters especially alkaline phosphatase (ALP) may be valuable in diagnosis of breast cancer. This study was conducted to detect alkaline phosphatase activity and calcium level in Sudanese patients with breast cancer,who attended the Radiation and Isotope Center of Khartoum hospital.during the period from March -May 2015.

In this retrospective, case-controlled, hospital- based study; 40 female patients with breast cancer (aged 25-85 years), and 40 healthy female control (aged 25-85 years) were enrolled in the study. A questionnaire containing demographic and clinical data was completed for every patient. Venous blood (2.5ml) was taken in a plane container from every volunteer. Samples for alkaline phosphatase and calcium were processed and analyzed using spectrophotometric method.

The present study showed that there was a highly significant increase in alkaline phosphatase activity in patients compared to control ($P<0.01$), whereas insignificant increase in calcium level in patients compared to healthy control ($P>0.05$).Thus, it was concluded that analysis of serum alkaline phosphatase and calcium in patients with breast cancer may help in monitoring metastasis, and to follow up of treatment response.

Key words: Breast cancer, alkaline phosphatase, calcium

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Introduction

Among females, breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death (Freddie *et al*., 2018; Jemal. *et al*. 2011).There are several types of breast cancer, include noninvasive breast cancer which may be ductal carcinoma *in situ* (DCIS), in which a malignancy involving the ductal system of the breast that is wholly confined within basement membrane (Malone *et al*., 1993). Another type is invasive breast cancer (secondary or metastatic breast cancer), in which the cancer cell spreads from the ducts or lobules into surrounding breast tissue, lymph node in the armpit or to other areas of the body such as bones, liver or lung. No single agent has been found to cause the disease, but a

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number of predisposing factors have been identified,such as geriatric factors (Allen *et al*., 2009), genetic and family history (Bishop *et al*., 2010),which includes menstrual cycle, pregnancy history, breastfeeding, overweight, history of breast cancer,oral contraceptive use and ionized radiation, alcohol, smoking, lack of exercise, race, chemicals used in cosmetic, food , water and plastic(Singh *et al*., 2013) .

Breast cancer is characterized by a distinct metastatic pattern mostly in liver and bones and leads to elevation of some biochemical metabolites such as alkaline phosphatase(ALP) and calcium. Alkaline phosphatase belongs to a group of enzymes that catalyzes the hydrolyzesvarious phosphor-monoester at an alkaline pH to liberate inorganic phosphate from the organic phosphate ester with the concomitant production of an alcohol. It is present in high concentration in intestine, liver,bones,spleen, placenta and kidneys (Singh*et al*., 2013).

Elevation of alkaline phosphatase level is frequently associated with a variety of diseases such as extra hepatic bile obstruction, intrahepatic cholestasis, hepatitis, cirrhosis, cancer, and bones disorder. Some studies found that women with breast cancer have alkaline phosphatase activity higher than normal health women and the progressive increase in serum alkaline phosphatase activity with breast cancer is an indication of metastasis (Kamal Eldin, 2007).

Calcium is one of abundant mineral in the human body. Ninety nine percent of the total calcium in the body is found in bones and 1% is found extracellular in soft tissue and plasma(Ferro and Roberto, 2012).The calcium ion is an essential structure component of skeleton and play a key role in muscle contraction, blood coagulation, enzyme activity, neural excitability, secondary messenger hormone release and membrane permeability (Ferro and Roberto, 2012).

Methods

This study was conducted to detect alkaline phosphatase activity and calcium level in Sudanese patients with breast cancer during the period from March 2015 toMay 2016 who attended the Radiation and Isotope Center of Khartoum hospital. Forty female patients with breast cancer (aged 25-85 years), and 40 healthy female control (aged 25-85 years) were enrolled in this study.Patients with myocardial infarction, liver disease, pancreatic disease, diabetes mellitus were excluded from the study.

Informed consent was obtained from each participant and protocol was approved by Omdurman Islamic University, Radiation and Isotopes Center Khartoum and Ministry of health, and conducted in accordance with international ethical guidelines.

Venous blood samples, (2.5 ml) each,were obtained from participants using standard vein puncture technique, blood specimens were collected in plain blood containers, allowed to clot at room

temperature for 30 minute and centrifuged at 500 x g for 5minutes to obtain the serum. Then serum was separated and preserved at- 20 ^{0}C until analysis of sample.

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To monitor the reliability of result, control sera with known alkaline phosphatase and calcium values were run with samples. Alkaline phosphatase activity was measured by enzymatic method using photometer 4040. The kit supplied by Biosystems (Spanish, Cat. NO 11805).Calcium was measured by chemical method using Med Lab Plus; the kit supplied by Biosystem (Spanish, Cat. NO 11583). Data were analyzed using statistical package for social sciences (SPSS) software (version 11.5).A p value <0.05 was considered statistically significant.

Results:

General characteristics of the participants were shown in table (1)

Table (1): Characteristic of study subjects

Variables		Control (n= 40)	Patients (n= 40)	Percentage (%)
Age	(years)	34. 3± 11. 4	46. 1± 13.1	-
Stages	Stage1 Stage 2 Stage 3	- - -	5 25 10	12.5% 62.5% 25%
Menopausal	Premenopausal Postmenopausal	- -	19 21	47.5% 52.5%
Cancer site	Left Right	- -	18 22	45% 55%
Metastasis	With Without	- -	3 37	7.5% 92.5%
Treatment	Untreated Hormonal Surgical Chemotherapy	- - - -	4 30 5 1	10% 75% 12.5% 2.5%

As shown in table (2), ALP was found to be significantly higher in patients compared to control (P=0.01), whereas calcium showed insignificant difference between patients and control (P=0.05), although women with breast.

Table (2) Comparison between controls and patients regarding ALP and calcium level

**: significant at 0.01 level of probability, ns: no significant difference

Variables	Control	Patient	P. value
ALP(U/L)	48.15	99.39	0.01^{**}
Calcium (mmo/l)	2.12	2.26	0.05^{ns}

Regarding age factor, it is obvious that ALP activity decreases significantly in patients with the increase of age (P=0.03); whereas calcium level in not (P=0.246)

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Table (3): Age factor regarding ALP activity and calcium levels (using "Duncan's New Multiple Range Test"

Age (years)	ALP (U/L)		Calcium (mmol/l)	
	Patients	Control	Patients	Control
25- 40	107[a]	46.92[c]	2.18[a]	2.13[a]
41-**55**	98[ab]	52.02[c]	2.30[a]	2.14[a]
>55	88.0[b]	53.07[c]	2.30[a]	2. 01[a]
	0.03[**]		0.246[ns]	

Means within the Colum which having similar letters are not significantly difference between level of probability according to DNMRT,

**: significant at 0.01 level of probability,

Table (4): Showing ALP activity and calcium levels regarding the stage of breast cancer

Stage of disease	ALP(U/L)	Calcium (mmol/l)
Stage1 (n=5)	48.00	2.30
Stage 2 (n=25)	104.75	2.23
Stage 3 (n=10)	111.69	2.31
P-value	0.22[ns]	0.81[ns]

Comparing premenopausal and postmenopausal phases regarding the activity of ALP and calcium levels, showed no significant difference concerning both ALP (P= 0.25) and calcium level (P = 0.44), (table 5).

Table (5): Level of ALP and calcium as affected by menopausal of breast cancer patients

Variables	Premenopausal	Postmenopausal	P. value
ALP(U/L)	113.1	87.0	0.25[ns]
Calcium(mmo/l)	2.21	2.30	0.44[ns]

As shown in (table 6), metastasis has no role concerning ALP activity or calcium levels (P=0.07) and (P=0.88) respectively.

Table (6): Showing ALP activity and calcium levels in patients with metastasis

Variables	With metastasis	Without metastasis	P. value
ALP(U/L)	77.4	117.4	0.07
Calcium(mmo/l)	2.27	2.25	0.88

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As shown in (table7), both ALP activity and calcium levelinsignificantly affected by treatment of breast cancer, but untreated patients reported higher mean of ALP activity, followed by hormonal treatment, chemotherapy and finally surgical, while the highest level of calcium was obtained by surgical, followed by chemotherapy, untreatedwomen and hormonal treatment.

Table (7): Effectof treatment on ALP activity and the calcium levels using "Duncan's New Multiple Range Test".

Parameters	ALP (U/L)	Calcium (mmol/l)
No treatment	108.8a	2.25a
Chemotherapy	77.2a	2.27a
Hormonal	98.0a	2.20a
Surgical	57.3a	2.36a
P. value	0.503ns	0.954ns

As shown in (table 8), no correlation was found when comparing the three parameters (age, ALP activity, and calcium levels) with each other.

Table (8): Showing no correlation when comparing the three parameters (age, ALP activity, and calcium levels)

Variables	Coefficient	Age	ALP	Calcium
Age	r	1.000	- 0.096 ^{ns}	0.215 ^{ns}
	P	-	0.555	0.183
ALP (U/L)	r	-	1.000	0.187 ^{ns}
	P	-	-	0.248
Calcium (mmol/l)	r	-	-	1.000
	P	-	-	-

Discussion

Breast cancer mostly cancer affects women in Sudan. Generally, in order to improve breast cancer consequences and ensure survival, early detection is crucial. Early detection approaches are screening and early diagnosis.When breast cancer cells invade bones, they cause anosteolysis lesion which increases alkaline phosphatase and calcium in blood, hencealkaline phosphatase and calcium may help in monitoring the stage of breast cancer.

The present study was primarily designed to measurethe calcium level and ALPactivity in Sudanese patients suffering breast cancer compared to healthy individuals.In this study, 40 females with breast cancer (aged 25-85 years), in addition to 40 healthy females(aged 25-85 years) were enrolled as control.This study revealed that,

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mean alkaline phosphatase activitywas markedly elevated in patients with breast cancer compared to the control(P< 0.01).These findings agreed with the results obtained by Nathaniel*et al.* 2010 andSingh*et al.* 2013. Although some studies have reported high sensitivity of ALP for bone and overall metastasesdetection, but these studies use specific isoenzymes in addition to total ALP, which reported by Keshaviah *et al*., 2007.Also, our study revealed thatalkaline phosphatase activity was found to increase significantly with the decrease of patientsages, whilethere wasno significant difference in control, these findingsagreed with the results obtained byNathaniel*et al.* 2010. The increased activity of this enzyme observed in patients enrolled in our study may be due to osteolytic bone metastases in breast cancer leading to increased osteoclastic activity and bone resorption.

In addition to that the present study foundincreased ALP activityin the advanced stage of breast cancer patients.These findings agreed with the results obtained by Singh *et al*. 2013, Amritpal Kaur in 2015whofound that serum ALP activity was significantly increased with the progressof stage of cancer and the maximum increase being observed at stage IV, andChan drack*et al*. 2016 serum ALP levels increased significantly as the stage of the cancer progressed.

Regarding treatment of patients onALP activity: This study revealed that alkaline phosphatase showed insignificant decrease for all treatments typebut after surgeryshowed low one. These findings agreed with the results obtained by Chandrakanth *et al*. 2016. This may be due to removal of cancer tissue. But disagree with Keshaviah*et al.*2007 and Prabasheela *et al.* 2012; they reported an increased ALP activity in some of the cases after surgery, which might be due to recurrence.

The present study showed that calcium levels are insignificantly increased in patients compared with control (P=0.05). Some studies such as that carried out by Nathaniel and his colleagues (2010), reported a significant increase in serum levels of calcium. The hypercalcemia in breast cancer has beenattributed in part to osteolytic bone metastases andthis account for 20-30% of the hypercalcemia casesin oncology patients. The skeletal invasion anddestruction by tumor induced by tumor-productionof various cytokines such as transforming growthfactor-á (TGF-á), tumor necrosis factorá (TNF-á), TNF-â, interleukin-1 and interleukin -2, leads toincreasing bone osteolysisand modification of thereabsorption, excretion and resorption of calcium and phosphate ion(Francini*et al*.,1993).

Conclusion

From this study it could be concluded that alkaline phosphatase activity increases significantly in patients with breast cancer, but unlike calcium level which showed no significant increase. The increase in the serum ALP activities with breast cancer is an indication of metastasis. Therefore, measurement of ALP activities could be valuable in monitoring of metastasis andfollow up of treatment response. However, more studies (better if cohort study), are required including more

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sample size to determine the role of ALP and calcium in monitoring breast cancer.

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