# SUDAN MEDICAL LABORATORY JOURNAL

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# SUDAN MEDICAL LABORATORY JOURNAL

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FACULTY OF MEDICAL LABORATORY SCIENCES


SMLJ

وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا

وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي

فَضَّلَنَا عَلَى كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ

النمل15

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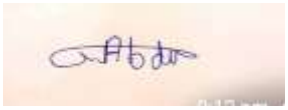
# Preface from the Editor-in-Chief

Dear readers and Colleagues,

It gives me a great pleasure to bring before you the first issue, volume 10 of the SMLJ assembled under our joint and articulate editor ship.
Sudan Medical Laboratory Journal (SMLJ) is issued by Omdurman Islamic University, College of Medical Laboratory Sciences. It is a refereed quarterly scientific journal (from 2011 - 2022). Issued it publishes scientific papers in the medical laboratory fields.
The journal can also consider a special issue for the purpose of avoiding delay or to publish a special theme for the conference.
We are continually receiving diverse and novel submissions. We hope that our readers continue to use SMLJ as their primary source for the most up to date knowledge in the field of laboratory sciences and medical researches comprising research papers, short communications, cases studies or reviews.

**Aims and scopes**

Sudan Medical Laboratory Journal (SMLJ) is issued by Omdurman Islamic University, College of Medical Laboratory Sciences. It publishes scientific papers in the medical laboratory Sciences.
The main objective of this journal is to publish the research papers well in time but with peer review by subject experts. We are confident that our editorial board in different specialties nationally and internationally reputed. The ultimate objective of dissemination of knowledge is to improve patient management and enhance health care delivery. In the process, the scientific work of the authors is viewed by a larger audience and is peer reviewed by global experts. The authors are, in turn, rewarded with appreciation, promotion and acknowledgement by peers.
This is a peer-reviewed journal published yearly. It is aims to reflect medical laboratory scientific research in various aspects of medicine as well as regional and international relevant research. Basic scientific research clinical practice, experiences that help in patient management are also welcome. Review articles, original articles, case reports are welcome. Local research in sciences education and history of laboratory and medicine will be considered for publication.
Thanks to the authors for their contributions to this issue and the editorial staff for their dedication. Thanks to our readers for their continued support and interest in our publications.



Dr. Abdelsamee Elobied Mohamed Elamin

Editor- in-Chief

# Editorial

*"We are judged by hope"*

*Arabian poem*

Dear SMLJ readers;

"In these difficult times that our country is going through, and after experiencing war in our streets, villages, cities, and inside our homes, we are now, after absorbing the shock, rising again in all fields to work on building our nation and looking far ahead to be better than we were before. We are governed by hope from our faith and upbringing.

We are publishing this issue after a pause due to the shock of the war, and the second issue will be released immediately after to continue the journey of scientific publishing through your leading journal. Scientific research must continue to build a nation worthy of it. We move forward together in all fields, and our country rises with knowledge."

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.

We would like to inform you that your journal available on-line and our URL is http://www.journal.oiu.edu.sd/ojs/index.php/mlj so, just and retrieve your article without the bother to request.
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And till we hear from you, we remain,

Yours

Dr. Abdulazeem Abdulsalam Ibrahim Alkhidir
*Executive Editor*

## Important Directives

# 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.

Articles for publication always should be prepared in a 1.5cm-spaced typewritten on size A4 paper and with 3.81cm margin on left side and 3 cm on all other sides. *Not to forget*, the alignment (***left to right***) through all the text, is required.

Names of authors should be followed by their official address indicated by the numbered superscript as follows:

Last name + initials i.e. Cornel, C. E[1]. Ford, E. J. H[2]. Evans, J[3].

Articles reviewed by a peer reviewer, are subject to acceptance, rejection or to acceptance after complying with amendments in the enclosed report.

Each author will receive a copy of his/her published submitted article sent via email address. Order for extra issues or articles of other authors will be charged for.

Article template consists of an **abstract** (less than 300 words). It is of high importance to give, *here*, the name and address (*namely email*) of the *Correspondence Address*, followed by an introduction (*a short concise overview of previous relevant research*), **materials and methods** (*a description of the methodology used*), **patient selection** (*inclusion and exclusion criteria*), **results** (*comprising text, tables and figures, avoiding repetition of data*), **discussion** (*of the results obtained*) and **references** (*in Vancouver Style*).

*Original Article* **Sudan Medical**

# Association of expression C/C .G/G, C/G Genotypes with Glycated hemoglobin (HbA1c) levels in Sudanese children with type 1 Diabetes

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**ABSTRACT**

**Background:** Type 1 diabetes (T1D) is a common complex metabolic disease characterized by the autoimmune destruction of insulin-producing B cells of the pancreatic islets. Due to a drastic loss of B cells, resulting in no to lowinsulin production,. Genetic factors play a significant role in the aetiology of T1D, However, the main causes of morbidity and mortality today are the complications that arise from even mild hyperglycaemia in T1D cases. Glycemic control is often measured using an assay called haemoglobin A1C (HbA1c), that provides an estimate of average blood glucose in individuals over a 3month period. Aim of the study to correlate between the frequencies of C/C,G/G and C/G genotypes and HBA1C levels in T1D patients.

**Objective:** To evaluate the significant correlation between frequencies of genotypes and HBA1C levels.

**Methods:** A total of 100 Sudanese subjects with T1D were enrolled in this study, on the average age between 5 to 18 years. The study was conducted in diabetes central hospitals in Khartoum state. In order to determine the HLA gene polymorphism, the allele-specific-refractory mutation system-polymerase chain reaction (ARMS-PCR) method was utilized.

**Results:** There were no significant correlation (r = 0.074, P-value = 0.497) between frequencies of genotypes and HBA1C levels.

**Conclusion:** This study identified that there's no significant correlation between frequencies of genotypes and HBA1C levels.

**Keywords: Genotypes, Glycated hemoglobin (HbA1c), Sudanese children, type 1 Diabetes**

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## Introduction

Type 1 diabetes (T1D) is an autoimmune disease in which insulin is functionally absent because of the destruction of the B cells in the pancreas by the immune system [1].The triggers for the autoimmune attack are not fully understood, but it is now widely accepted that both environmental and genetic factors contribute to it [2]. T1D was originally described as "juvenile diabetes" and, later, "insulin-dependent diabetes mellitus" (IDDM). T1D occurs most commonly in juveniles but can occur in adults, especially those in their late 30s and early 40s [1]. The incidence of T1D has risen over the past 20 years [3].T1D is one of

the most frequently studied complex genetic disorders [4]. The strong genetic contribution to T1D is illustrated by the fact that by age 60, 65% of identical twins of T1D probands will develop T1D themselves [5]. Furthermore, children born to a family with an affected family member have a 5% risk of T1D by age 20, compared with a 0.3% risk for children without affected family members [6], yet the great majority of T1D cases do not have a first-degree relative with diabetes [1] . The major histocompatibility complex (MHC) is reported to account for approximately 40% to 50% of the familial aggregation of T1D [7, 8]. The gene products from this region were originally discovered on the surface of white blood cells, so they became known as leukocyte antigens; thus, the human MHC is also referred to as the HLA complex. HLA molecules were originally studied for their ability to confer tolerance (histocompatibility) following tissue grafts [9]. The HLA genotype, which is the combination of HLA alleles inherited from both parents, is key for the development of T1D [10]. Glycated hemoglobin (HbA1c) reflects the level of blood glycemia and is commonly used to monito glycemic control in diabetes and to diagnose type 2 diabetes. Genome-wide association studies (GWAS) have revealed .60 genetic variants to be associated with HbA1c in individuals without diabetes (11–12). Arecent GWAS of multiple ethnic groups confirmed 18 variants previously associated with HbA1c and added 42 new loci (12). Some of the variants affect biological pathways maintaining glucose homeostasis. For example, single nucleotide polymorphisms (SNPs) in or near glucokinase (GCK) and melatonin receptor 1B (MTNR1B) are known to be associated with HbA1c, fasting plasma glucose, and b-cell function (13). HbA1c is further influenced by genes and pathways that affect erythrocytes. In particular, SNPs in hemochromatosis (HFE) and hexokinase 1 (HK1) (13) have an effect on HbA1c through nonglycemic pathways that affect erythrocyte physiology and survival HbA1c reflects the glycemic load over ;3 months (14), and traits such as hemoglobinopathies, alterations in intracellular glucose metabolism, or defects of glucose transport into erythrocytes affect HbA1c In addition, diet,drugs, and insulin administration affect HbA1c in individuals with type 1 diabetes (T1D) who have little to no endogenous insulin production and, thus, rely on an external source of insulin to ensure glucose uptake in cells. Despite a strong environmental stimulus, HbA1c levels are correlated between both dizygotic twins and monozygotic twins concordant or discordant for T1D (15).

**Materials and Methods:** This study was performed on 100 Sudanese subjects on the average age between 5 to 18 years. 100 cases with type1diabetes mellitus according to the diagnostic criteria established by National Diabetes Data Group (NDDG). And 100 (non – diabetic) with no clinical evidence or T1D history in their family, as a control groupThe DNA was extracted from peripheral blood samples of the patients and controls by utilizing a commercially available kit

(G-DEXTM11b Genomic DNA Extraction Kit (Blood)\200T catalog numbers 17241).

**Genotyping:** We designed an amplification refractory mutation system polymerase chain reaction (ARMS-PCR) for detection of rs3104413 (C/G) (Table1) [10]. Polymerase chain reaction (PCR) was performed by using commercially available PCR premix (AccuPower PCR Premix; BIONEER, Daejeon, Korea) according to the manufacturer's instructions. Briefly, 1 µl template DNA (~100 ng/µL), 1 µl of each primer (10 pmol/µl), and 15 µL DNase-free water were added to AccuPower PCR Premix. It was done in 20 µl reaction volume containing 100 ng of genomic DNA. The following thermal profiles were run; 3 min. at 95ºC for initial denaturation, followed by 30 cycles of 95ºC for 20s, 60ºC for 30s and 72ºC for 40s and final extension at 72°C for 5 min. for position rs3104413 (C/G). The amplified PCR products were analyzed by 2% agarose gel electrophoresis and ultraviolet visualization. The length of the expected PCR products were 372 bp for rs3104413 (C/G) polymorphisms. (Table1), Glycated protein is formed post-translationally through the slow, non enzymatic reaction between glucose and amino groups on protein..HbA1 is considered as a more reliable parameter in monitoring glycemia over the glycemic reading with the conventional glucometer (15). Test Procedure: 100 µl of hemolysis buffer was Drew and transfer it into detection buffer tube, 5µl of fingertip blood or tube blood was Drew using 5µl capillary tube, the capillary tube was putted into the detection buffer tube, the lid of the detection buffer tube was closed and the sample was mixed thoroughly by shaking it about 15 times, the cartridge half was take out from I-Chamber slot, 75µl of the sample was pipetted out, mixtured and loaded it into a sample well in the teats cartridge, waited till the sample mixture flow appears in the windows, the cartridge was -inserted into I-Chamber slot (30C◦), the cartridge was leaved in I-Chamber for 12 minutes before removing, to scan the sample – loaded

**(Table1) The sequences of primers used in the study for rs3104413 single-nucleotide Polymorphism**

| Gene polymorphism HLA rs1304413 | Primers Sequence (5′ to 3′) | Tm (˚C) | Product size |
|---|---|---|---|
| Reverse (C allele) | GGAGAAGCACGACAATAGGAC | 59 | C and G allele: 327 b p |
| Reverse (G allele) | GGAGAAGCAAGCCAATAGGAG | 59 | |
| Forward (common) | CTGCTTTTCACACCAACCTCT | 60 | |

**HbA1c:** cartridge was inserted it in to the cartridge holder of the instrument for I- chroma tests, the select button was Pressed on the instrument for I-chroma testes to start the scanning process, instrument for i-chroma tests was start scanning the sample loaded cartridge immediately and test result was read on the display screen of the instrument for I-chroma teast. Statistical Analysis:Data was examined using the statistical package of social science (IBMSPSS version 26.0)

for windows software package. A P. value of ≤ 0.05 was interpreted as statistically significant. Categorical variables, alleles and genotypes frequency were analyzed using a Pearson's Chi square test. Comparison of groups was done by Kruskal Wallis test. The strength of significant was done by calculating the contribution to chi square of each cell using adjusted residuals P values (adjusted P value =0.05/number of new adjusted residuals or cells).

**Results**

A total of 200 Sudanese subjects were enrolled in this study, on the average age between 5 to 18 years .100 cases with type1diabetes mellitus and 100 (non – diabetic) as a Control group. The study was conducted in diabetes central hospitals in Khartoum state. The patients' mean age were (12.00±3.735) and controls were (12.25±3.686) as shown in (Table1), in the control group, male frequency was (49%) and female was (51%) while the patients group, male (48.3%) and female was (51.7%) as shown in (Table2).

**Table 1: The Correlation between the frequencies of Genotypes and HBA1C levels in patients:**

| **GROUPS** | | | **FREQUENCY** |
|---|---|---|---|
| **CONTROL** | Gender | Females | 51.0(%) |
| | | Males | 49.0(%) |
| | Age | (mean ±Std.) | 12.25±3.686 |
| **PATIENTS** | Gender | Females | 51.7(%) |
| | | Males | 48.3(%) |
| | Age | (mean ±Std.) | 12.00±3.735 |

There were no significant correlation (Spearman's rho, r= 0.074, P value = 0.497) between frequencies of genotypes and HBA1C levels as shown Fig 1.

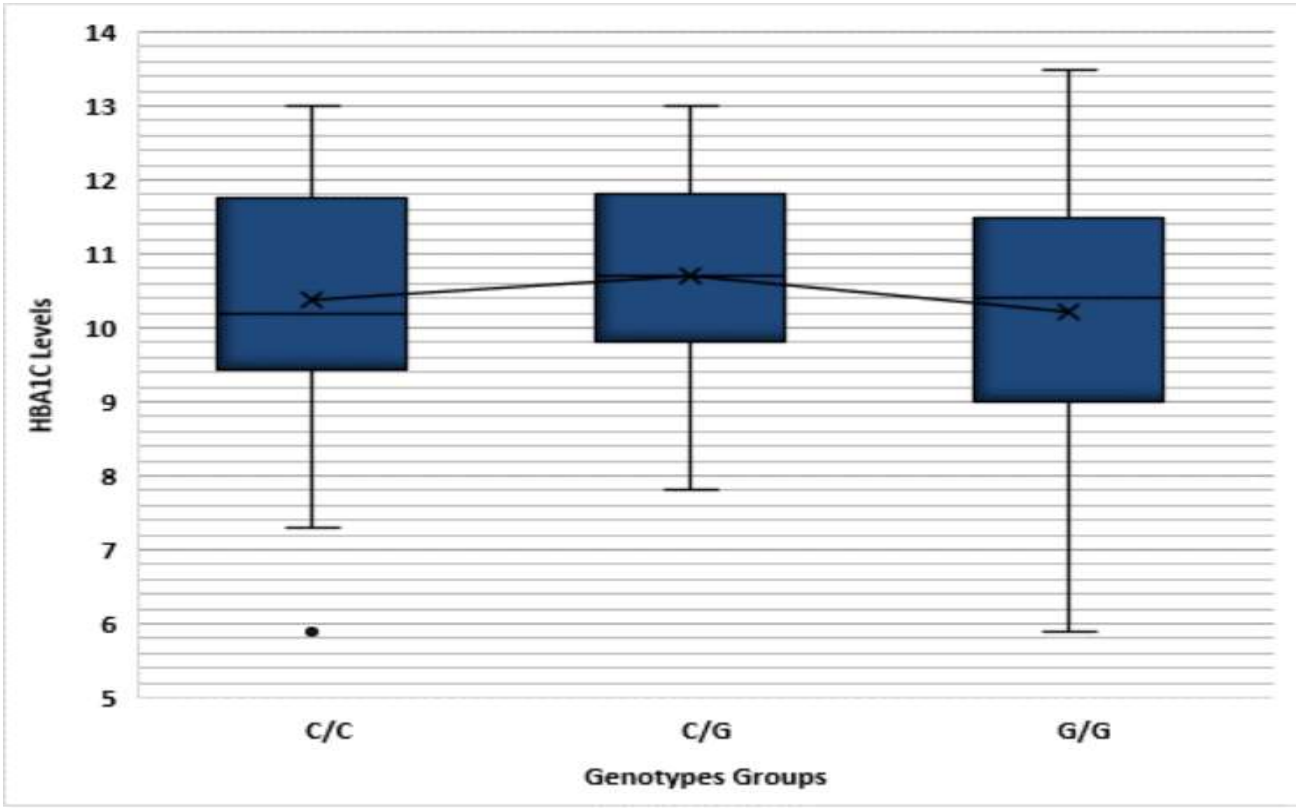


**Fig1: The Correlation between the frequencies of Genotypes and HBA1C levels in patient**

**Discussion**

Once T1D is diagnosed, the average time to develop micro and macro vascular complications of the disease is 15-20 years (19). The epidemiological risk factors associated with the development of complications include: duration of diabetes, glycemic control (typically as measured from hemoglobin A1c (HbA1c)), age, weight and waist-hip ratio, smoking status, cholesterol, triglycerides, and blood pressure. Of these. (16,18 ,17 ) Glycemic control of T1D can be measured by frequent monitoring of glucose in the blood or urine or by the percent of glycated haemoglobin in the blood, so called- haemoglobin A1C (HbA1c). Normal levels of HbA1c are lower than 6%, while a HbA1c value of 6.5% or more can be diagnostic of hyperglycemia (19). HbA1c levels have been shown to be heritable, with heritability estimates ranging from 47% to 59% (20). A genome wide association study identified 11 single nucleotide polymorphisms (SNPs) in 10 different genetic loci associated with HbA1c levels (21). Collectively, they explained approximately 2.4% of the variance in HbA1c levels and 5% of heritability of HbA1c. However, environmental factors also influence variation in HbA1c; age and levels of C-reactive protein (a measure of stress) were found to be the strongest determinants of variation in HbA1c levels among Finnish men without diabetes (22). Nevertheless, HbA1c is a marker of long-term glycemia. Prolonged hyperglycemia causes a number of micro and macro-vascular complications tissues, and plays a causative role in the micro-vascular complications in T1D,

including retinopathy, nephropathy and neuropathy (23). High HbA1c can also cause an increase in the number of reactive oxygen species inside RBCs, which can alter the cell membrane properties and lead to blood cell aggregation and impaired blood flow, or lead to inflammation that can result in atherosclerotic plaques, both of which become risk factors for CVD (24). This study to Correlation between the frequencies of Genotypes and HBA1C levels in a total of 100 Sudanese subjects with type1 diabetes mellitus , on the average age etween 5 to 18 years, our study found that there were no significant correlation (Spearman's rho, r= 0.074, P value = 0.497) between frequencies of genotypes and HBA1C levels. Another study Anna Syreeni, Niina Sandholm etal identified that: a locus on chromosome 13 near RXFP2 that is associated with HbA1c in individuals with T1D (25) Sara Victoria Good found that weakly suggestive associations between imputed gene expression and mean HbA1c for nine genes expression with Glycated haemoglobin (HbA1c) levels in people with type 1diabetes. (26) Conclusion This study identified that there's no significant correlation between frequencies of genotypes and HBA1C levels. Data Availability When all data is publicly available, this manual is a review of pub-lished research. Conflicts of Interest The authors declare no conflicts of interest. Authors' Contributions Hiba Omer- designed the project. Data collection. xperimental design. Lab working . .Data analysis. Manuscript writing. Sababil Salih- Co manuscript writing. . Data collection Sakeena NourEldine - Data collection Abdulaze Abdulsalam – Manuscript reviewing data analysis. Mohamed Abdelgadir – Assisting in study design, lab working, data analysis

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*Original Article* **Sudan Medical Laboratory Journal**

# Assessment of Serum Triglyceride and High Density Lipoprotein Cholesterol among Sudanese Patients with Deep Venous Thrombosis

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**Abstract**

**Background:** Deep Vein Thrombosis (DVT) and Vein Thrombo Embolism (VTE) are a major health problem with high mortality worldwide. Dyslipidemia are used as a marker of increased DVT risk.

**Objectives:** This study aimed to assess serum triglyceride (TG), High Density Lipoprotein cholesterol (HDL-C) levels among Sudanese DVT patients.

**Methods:** This was case control study conducted in Omdurman Teaching Hospital in Khartoum State during period between February to May 2019,total of 100 Sudanese patients with DVT were enrolled in the study, including 50 DVT patients and 50 apparently healthy individuals as controls matching in age and gender.

Serum TG and HDL-C were measured by ROCHE DIAGNOSTIC – COBAS INTEGRA400 PLUS. Data were analyzed using Statistical Package of Social Science (SPSS version 20).

**Results:** The result showed, thrombosis most common among age between (20-40) years (70%), (24%) between (41-60) years and (6%) between (61-80) years. (80%) of patients were females, while (20%) were males. TG level increased (164 ± 31 mg/dl versus 88 ± 18.8 mg/dl, *P. value* = 0.00) while HDL-C decreased (16.2 ± 6.5 mg/dl versus 50 ± 7.7 mg/dl, *P. value* = 0.00) in cases compared to control group. There were insignificant differences in the mean of TG and HDL-C according to gender: TG (176 ± 31.8 mg/dl versus 161 ± 30.4 mg/dl, *P .value* 0.15), HDL.C (14 ± 6.6 mg/dl versus 17 ± 6.5 mg/dl, *P. value* 0.26). TG level increased in patients who take warfarin treatment compared to patients who take heparin (171 ± 31.7 mg/dl versus 161 ± 30.9 mg/dl, *P.value* (0.00) and there was no difference in level HDL- C (12 ± 3.8 mg/dl versus 18 ± 6.5 mg/dl, *P.value* 0.34). Results also showed that there were no correlation between TG, HDL-C and ages of patients (r = 0.09, *p .value* = 0.5) (r = -0.25, *p. value* = 0.08) respectively.

**Conclusion:** This study concluded that the level of TG is increased, while HDL- C is decreased in DVT patients; this indicates that these parameters are a promising novel biomarker for predicting treatment outcome in patients with thrombosis.

**Key words: DVT, TG, HDL-C.**

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**Introduction:** Thrombosis is the formation of blood clot inside a blood vessel, obstructing the flow of the blood through the circulatory system, blood clots may form in the body under certain conditions, clot or apiece of clot, that breaks free and begin to travel around the body is known as an embolus [(1)].

Annual incidence rates ofDeep vein thrombosis (DVT) ranged from ~45 to 117 in 100,000 adults per year [(2)].Triglyceride (TG) is one of most important lipids in the body, containing three fatty acid molecules attached to one molecule of

glycerol linked by ester bond in one of three stereochemically distinct bonding position referred as sn-1, sn-2 and sn-3, measurement of TG useful in evaluation of cardiovascular (CV) risk has long been associated with multiple issues [(3)]. High Density Lipoprotein cholesterol (HDL-C) is one of the five major groups Lipoproteins. HDL-C correlates with cardiovascular risk. Low HDL-C should prompt examination of additional metabolic and inflammatory pathologies. An increase in HDL.C through lifestyle change has positive effects and recommended [(2)]. Relationship between lipid levels and risk of venous thrombosis is not well established; several studies in the past decade have shown that patient with venous thrombosis (Deep vein thrombosis) have an increased of subsequent arterial disease [(4)]. Lipid levels can be modulated by life style intervention and drug therapy.

Serum HDL-C concentration is reduced in patients with thrombosis while serum TG concentration is increased [(3)]. Higher levels of serum TG and low level of HDL-C were associated with postoperative DVT after total Heparin arthroplasty in patients with non-traumatic osteonecrosis of the femoral head. Serum HDL-C concentration is reduced in patient with thrombosis while serum TG concentration is increased [(5)]. High serum HDL-C level was associated with decreased risk of venous thrombosis after adjustment in hospitalization, malignancy, height and weight. In contrast elevated TG level were associated with an increase risk [(5)].

**Material and Methods:** This was comparative case control hospital base study, carried out in Sudanese patients with DVT in Omdurman Educational Hospital in Khartoum state during February to May 2019. Total of 100 Sudanese patients with DVT were enrolled in the study; 50 patients with DVT, aged range between 20 -80 years as case group and50 apparently healthy individuals as controls matching in age and gender.

**Ethical consideration:** This study was approved by scientific committee of college of medical laboratory science, Sudan University of Science and Technology, ethical committee No (SCI -01 – 19), Verbal informed consent was obtained from each participant.

**Data and sample collection:** Clinical data were obtained and recorded on questionnaire sheet. Four ml blood was collected by standard venipuncture method, directly in plain container and the serum was separated after centrifugation for 5 minutes at 5000 rpm at room temperature. Estimation of triglyceride and HDL-C concentrations using full automated ROCHE DIAGNOSTIC – COBAS INTEGRA400 PLUS. Pathological and normal control sera were used to assure the accuracy of the results.

**Data analysis:** Collected data were analyzed by a computer system using statistical package for social sciences (SPSS version 20), T test and person correlate were used for comparison and correlation.

**Results:** 70% of patients aged between 20-40 years, 24% between 41-60 years and 6% between 61-80 years. 20% of patients were males while females represent 80% as in table (1). The level of TG increased and HDL.C decreased in DVT patients compared to control group: TG (164 ± 31 mg/dl versus 88 ± 18.8 mg/dl, P .value =0.00), HDL.C (16.2 ± 6.5 mg/dl versus 50 ± 7.7 mg/dl, P.

value =0.00) as shown in table (2). There were insignificant differences in the mean of TG, HDL - C levels according to gender: TG (176 ± 31.8 mg/dl versus 161 ± 30.4 mg/dl, P. value 0.15), HDL.C (14 ± 6.6 mg/dl versus 17 ± 6.5 mg/dl, P. value= 0.26) as in table (3). Level of TG increased in patients who take warfarin treatment compared to patients who take heparin, there was no significant difference in the level of HDL - C; (171 ± 31.7 mg/dl versus 161 ± 30.9 mg/dl, P. value =0.00) for TG (12 ± 3.8 mg/dl versus 18 ± 6.5 mg/dl, P. value = 0.34) for HDL- C as in table (4). The results showed that, there were no correlations between TG, HDL-C and ages of patients (r = 0.09, p .value =0.51) (r = -0.25, p. value =0.08) as in figure (1 and 2).

**Table 1:** Gender and age distributions among case group.

| Variable | Number | Percentage |
|---|---|---|
| Age 20-40 | 35 | 70% |
| Age 41-60 | 12 | 24% |
| Age 61-80 | 03 | 6% |
| Sex male | 10 | 20% |
| Sex female | 40 | 80% |

**Table (2):** Comparison of mean levels of TG and HDL-C in case and control group.

| Variable | Case: N=50<br>Mean ±SD | Control: N=50<br>Mean ±SD | *p. value* |
|---|---|---|---|
| TG mg/dl | 164±31 | 88±18.8 | 0.000 |
| HDL – C mg/dl | 16.2±6.5 | 50±7.7 | 0.000 |

Result given in mean ± SD, P. value ≤0.05 consider significant.
Independent sample T test was used for comparison.

**Table 3:** Comparison of TG and HDL – C levels in patients according to gender.

| Variable | Male: N=10<br>(Mean ± SD) | Female: N=40<br>(Mean ± SD) | *p.value* |
|---|---|---|---|
| TG | 176±31.8 | 161±30.4 | 0.15 |
| HDL - C | 14±6.6 | 17±6.5 | 0.26 |

Result given in mean ± SD, P.value>0.05 consider insignificant.
Independent sample T test was used for comparison.

**Table 4:** Comparison between Heparin and Warfarin in case group.

| Variable | Warfarin: N=12<br>(Mean ± SD) | Heparin: N=38<br>(Mean ± SD) | *p.value* |
|---|---|---|---|
| TG | 171±31.7 | 161±30.9 | 0.00 |
| HDL.C | 12±3.8 | 18±6.5 | 0.34 |

Result given in mean ± SD, P. value ≤0.05 consider significant
Independent sample T test was used for comparison.

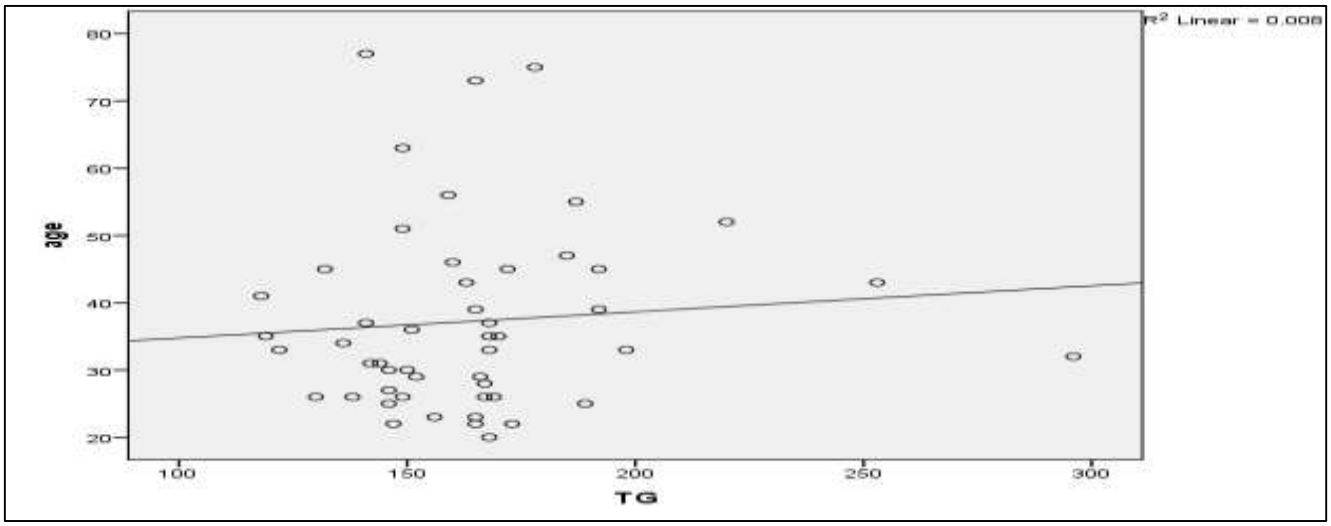


**Figure1:** Correlation between TG level and age in case group (r = 0.09, p. value =0.5).

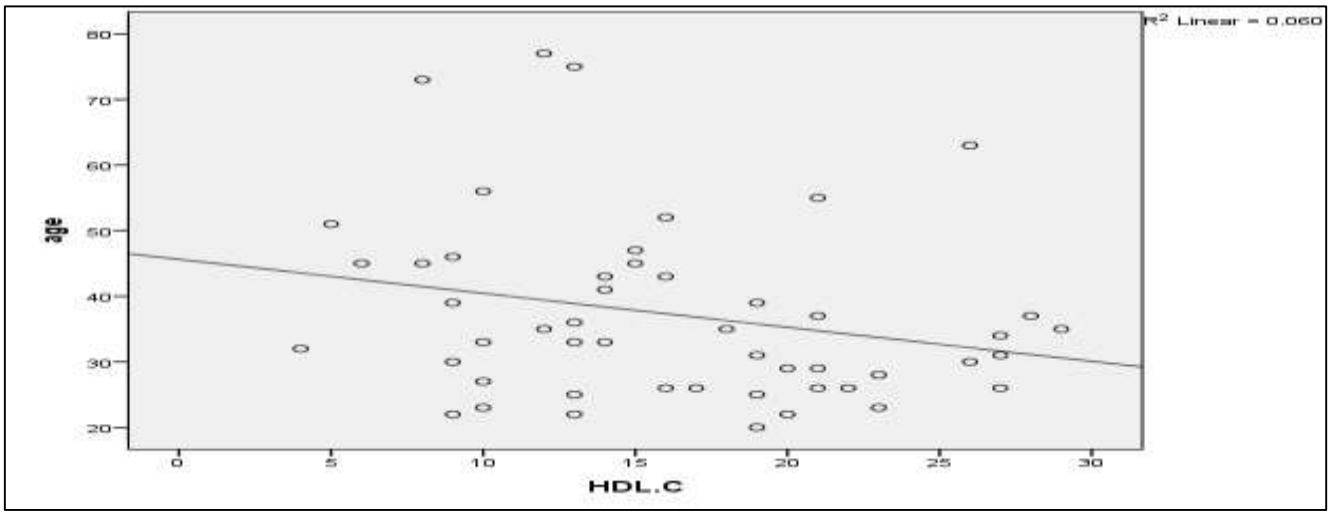


**Figure 2:** Correlation between HDL - C level and age in case group (r = - 0.25, p. value =0.08).

**Discussion:** Thrombosis is the formation of blood clot inside a blood vessel, lead to obstructing the flow of the blood through the circulatory system [(2)]. In current study DVT most common among age between 20-40 years; this is agreed with study carried by Morelli et *al.*, [(9)]. Also this study found 80% of patients were females, while 20% were males. Similar frequency reported in previous study conducted by Silverstein *et al.*, [(10)] and also agree with previous studies found that females had a higher risk of DVT than males [(13,14)]. Also similar finding reported in meta-analysis in 2018 found that women were in risk to develop distal DVT than men.The result of this study showed, there was significant increase in triglyceride level and decreased HDL- C level in thrombosis patients compared to control group (*P .value* = 0.000), this agreed with study showed that serum TG level increased and HDL-C decreased, the occurrence of this case in thrombosis patients due to the role of TG-rich lipoproteins in signaling mechanisms via

apolipoprotein C-111 and free fatty acids leading to activation of NFKB , VCAM-1 and other inflammatory mediators which lead to fatty streak formation and advanced atherosclerosis and hypertriglyceridaemia can lead to change in gene expression in endothelium producing to a prothrombotic state[6].Also this study showed insignificance difference in the mean of TG and HDL- C in males compared to females (*P. value* = 0.15) and (*P. value* = 0.26) respectively; this finding similar to another result which reported there were no significant difference in the mean of HDL-C and TG in males compared to females[10] and disagree with previous studies which suggest HDL-C distribution differed by ethnicity and gender[15,16,17]; and the study done Davis et al. report that sex difference in HDL-C level of six countries and assessed that female had higher HDL-C level than male[16]. Another study carried out by Akem*et al* suggested that prevalence of low HDL-C of was 22.4 %, distributed into 32.8% in males and 12.5% in females[18].

This study also showed that TG level increased in patients who take warfarin treatment compared to patients who take heparin treatment (P. value =0.00).warfarin act as enzyme inhibitor called vitamin K epoxide reductase which lead to lack of fibrin and so on improve the functioning of blood circulating so best lipid metabolism [11].Also previous study Noted that treatment of warfarin has an effect on the adjustment of cholesterol levels compared to normal rates and levels of cholesterol ,LDL are decreasing well compared to rates of TG where rates decrease after treatment, but stay slightly higher than normal rates[19]. Also in this current study there were no correlation between HDL.C, TG and age of thrombosis patients (r = -0.25, p. value =0.08) (r= 0.09, p. value =0.5) respectively. This result disagreed with another result, which showed that, there were significant positive correlation between TG, HDL, C and age of thrombosis patients [12].

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*Original Article* **Sudan Medical Laboratory Journal**

# Blood Cupping (AL-hijamah) Potentially Affects Biomarkers Associated with Hypertension and Cardiovascular Diseases

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**Abstract**

**Background:** Cupping therapy (CP) local name (Al-hijamah) is one of the oldest documented medical techniques. Al-hijamah used in Middle Eastern cultural practice for centuries. Despite blood cupping is oldest medical procedures therapy, the secrets of its mechanisms still mysterious.

**Objectives:** To investigate the impact of blood cupping (Al-hijamah) on biochemical parameters. Furthermore, to demonstrate their relation to hypertension and cardiovascular diseases (CVDs).

**Methods:** In analytical, quasi-experimental study, 40 subjects who underwent blood cupping at Al-Almi and Al-Rawabih centers, were included. Their demographic data were obtained. Blood pressure (BP), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), creatinine, uric acid, Alanine transaminase (ALT), Aspartate transaminase AST, Albumin, C- reactive protein (CRP) and creatine kinase (CK) levels were measured pre- and post-Al-hijamah.

**Results:** Compared to pre-samples BP, TC, LDL-C, Uric acid, and CRP levels were significantly decreased. While HDL-C, albumin and creatinine levels were significantly increased. No significant changes were observed in AST and CK, while ALT revealed significant increase. SBP negatively correlated with age (r= -0.353, *P*= 0.026), BMI (r= -0.380, *P*= 0.042) and positively correlated with uric acid (r= 0.388, *P*= 0.013). Albumin negatively correlated with TC (r= -0.502, *P*= 0.001) and LDL-C (r= -0.311, *P*= 0.044).

Conclusion: Blood cupping has a potential effect on the biomarkers associated with hypertension and CVDs.

**Key words:** Blood cupping, Al-hijamah, CRP, Blood pressure, lipid profile, Sudan

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**Introduction:** Cupping therapy (CP) local name (Al-hijamah) is one of the oldest documented medical techniques. Al-hijamah used in middle eastern cultural practice for centuries.1,2 Despite blood cupping is oldest medical procedures therapy, the secrets of its mechanisms still mysterious. 3,4

Recently in Sudan modern centers have been stablished for cupping traditional medicine. Therefore, the present study carried out to investigate the effect of Al-hijamah on some biochemical parameters and their relation to blood pressure. Mahmoud et al (2013) has reported that, Al-hijamah considered as a prophetic medicine and among the best remedies according to the hadith (The best among what you use in therapy is Al-hijamah).[3] Moreover, believed that seventy percent of diseases, pains and ailments are due to

the blood being unable to reach certain parts of the body.[2] Furthermore, many benefits have been linking to cupping blood, first encourages blood flow to that region which have poor blood circulation, thus cause relief of pain and diseases. Second dry cupping and wet cupping enhanced remove of the toxins onto the surface of the skin. Moreover, blood diverted replaced by healthy blood. Third it stimulates local lymphatic circulation, therefore enhancing their primary effect, namely, the mobilization and removal of toxic waste material.[5,6]

**Materials and methods:** In analytical, quasi-experimental study, 40 patients from 2 centers (Al-Almi for alternative medicine and Al-Rawabih Charity Organization for Al-Hijamah) in Khartoum State were enrolled. The patients were randomly selected according to their medical record (BP, age, gender, BMI and health status). Patients those receiving hypertensive, statin and/or steroids drugs were excluded. After taking verbal informed consent, demographic characteristics and medical history were gathered using structured questionnaires. Pre- and 3 days post-blood cupping samples were collected. Serum and heparinized plasma were obtained after centrifugation at 3000 rpm for 10 min.

**Ethical considerations:** This study was approved from local ethical committee of Sudan University of Science and Technology in accordance of Helsinki 2000. Moreover, each participant was informed by the aims of the study.

**Estimations of biochemical parameters:** Brief according to manufacturer total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), uric acid, creatinine, alanine transaminase (ALT), aspartate transaminase (AST) and creatine kinase (CK) were measured using full-automated chemistry analyzer BS-380.

**Estimation of C-reactive protein:** Latex agglutination immunoassay was used to determine C-reactive protein (CRP) concentration (BioSystem Spain). According to manufacturer's protocol, briefly monoclonal IgGs onto latex reagent binding with CRP. The intensity of developed turbidity was proportional to concentration of CRP, which measured by using spectrophotometer (BTS-310).

**Statistical analysis:** Descriptive data were assessed using paired *t*-test. Person's correlation was employed to assess the association between study parameters and variables. Results expressed as percentage, Mean (SD), regression coefficient. Statistical significance was set at *P value* $\leq .05$. All data analyses were performed using SPSS version 21.0 software.

**Results:** The characteristics of study groups are presented in figure1: A, B, C, and D. Of the 40 patients 26 (65%) were females and 14 (35%) were males. Whereas, 16(40%) of our population were normal weight followed by 13 (32.5%) obese and 11(27.5%) overweight. Among patients 23 (57.5%) were (20 to 39 years old) followed by 12 (30%) (40 to 59 years old) and 5 (12.5%) (> 60 years old). Moreover, 12 (30%) were hypertensive patients followed by 18 (25%) with other diseases, 9 (22.5%) with headache and 9 (22.5%) had back pain.

To investigate whether the blood cupping has an impact on biochemical parameters, paired *t*-test

analysis was performed. Our observations show that, systolic blood pressure and diastolic blood pressure (SBP and DBP) were deceased significantly to (118 ± 21.6 and 73 ± 15.0) in pre-cupping than post-cupping (122 ± 27.7 and 76.0 ±16.6) with ($p$=0.040 and 0.037) respectively.

Post-cupping findings were significantly lower of TC (157 ± 44.8 mg/dl) and LDL-C (89.6 ± 43.8 mg/dl) in comparison with pre-cupping (172± 52.0 mg/dl) and (109 ± 46.9 mg/dl) with ($p$=0.002) and ($p$=0.001) respectively. While HDL-C was significantly increased to (51.2 ± 24.2 mg/dl) post-cupping than (45.4±19.2mg/dl) pre cupping ($p$=0.047).

To determine whether cupping treated cardiovascular diseases (CVD), immunoassay was performed on CRP levels, which is the early predictor marker of CVD. It was observed that, the mean CRP of the post-cupping was significantly lower (16.5 ± 7.51 mg/dl) than pre-cupping (27.3 ± 8.90 mg/dl) with ($p$=0.013). Furthermore, blood cupping increased albumin significantly to (4.32±0.32 g/dl) than (4.11± 0.57 g/dl) ($p$=0.043).

Our results revealed that, uric acid levels were significantly decreased to (3.50 ± 1.50 mg/dl) post-cupping compared to (4.40 ± 1.70 mg/dl) pre-copping ($p$=0.000). In contrast creatinine levels were significantly increased to (1.40 ± 0.29 mg/dl) than (1.20 ± 0.23 mg/dl) with ($p$=0.000).

Enzymes activity were measured pre- and post-cupping. The two groups were matched and there were no significant changes in the post-cupping of AST and CK activities in comparison with the pre-cupping ($p$=0.732) and ($p$=0.461) respectively, while ALT activity was significantly increased to (5.95 ± 4.02 Ul) than (4.31 ± 4.30 U/l) with ($p=0$.003).

To assess whether the levels of the study parameters were associated with study variables, a person's correlation was performed. Thus, correlation dot plot indicated that, SBP were negatively correlated with age (r= -0.353, $P$=0.026) and BMI (r= -0.380, $P$=0.042), moreover was positively corelated with uric acids (r= 0.388, $P$=0.013). In addition, albumin levels were correlated negatively with TC (r= -0.502, $P$=0.001) and LDL-C (r= -0.311, $P$=0.044). Also, TC was correlated negatively with AST (r= -0.357, $P$= 0.024).

**Table 1:** Comparison analyses for biochemical parameters of post-cupping versus pre-cupping.

| Characteristics | Post-Cupping Mean (SD) | Pre-Cupping Mean (SD) | *P value* |
|---|---|---|---|
| **SBP** mmHg | 118 (21.6) | 122 (27.7) | 0.040 |
| **DBP** mmHg | 73.0 (15.0) | 76.1 (16.6) | 0.037 |
| **Cholesterol** mg/dL | 157 (44.8) | 172 (52.0) | 0.002 |
| **LDL-C** mg/dL | 89.6 (43.8) | 109 (46.9) | 0.001 |
| **HDL-C** mg/dL | 51.2 (24.2) | 45.4 (19.2) | 0.047 |
| **Creatinine** mg/dL | 1.40 (0.29) | 1.20 (0.23) | 0.000 |
| **Uric acids** mg/dL | 3.50 (1.50) | 4.40 (1.70) | 0.000 |
| **ALT** U/L | 5.95 (4.02) | 4.31(4.30) | 0.003 |
| **AST** U/L | 15.8 (12.9) | 15.3 (13.0) | 0.732 |
| **Albumin** g/dL | 4.32 (0.32) | 4.11 (0.57) | 0.043 |
| **CK** U/L | 80.9 (29.4) | 74.3 (28.2) | 0.461 |

| | | | |
|---|---|---|---|
| **CRP** mg/dL | 16.5 (7.51) | 27.3 (8.90) | 0.013 |

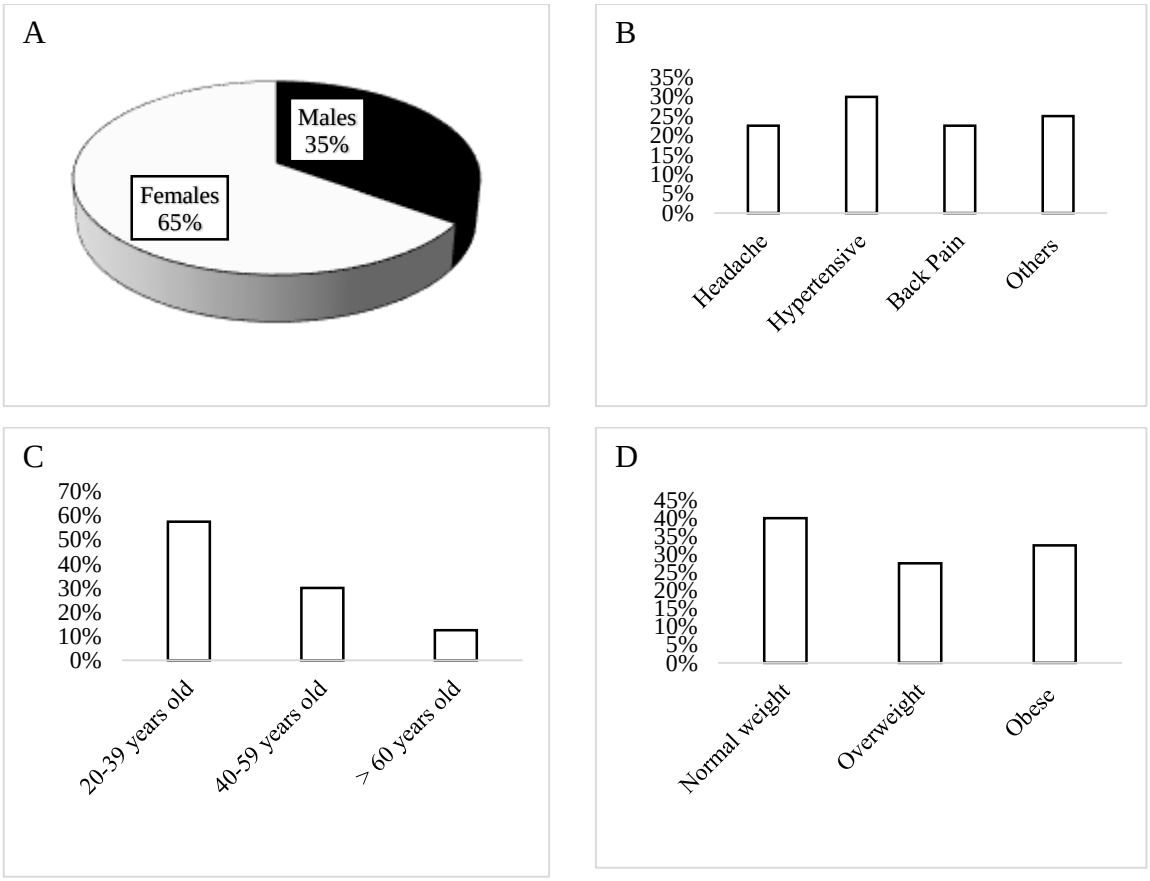


**Figure 1:** Characteristics of study population (A: gender, B: Health status, C: Age groups and D: BMI).

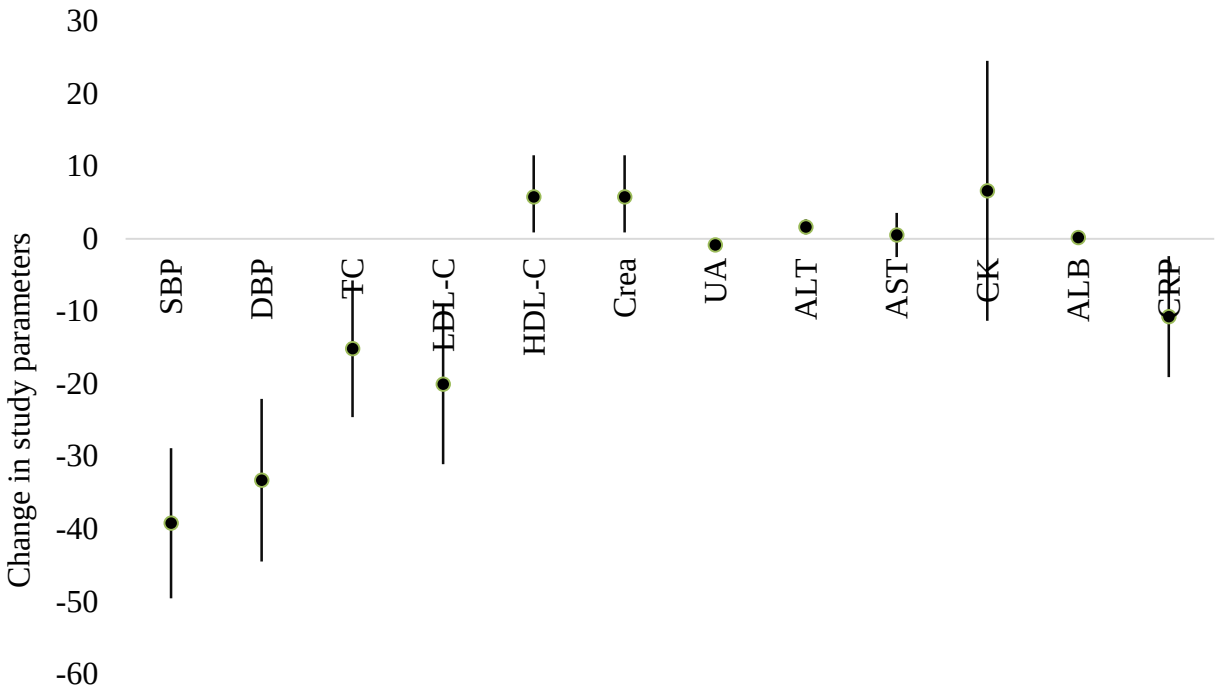


**Figure 2:** Change in study parameters obtained by deduced pre- from post-cupping results.

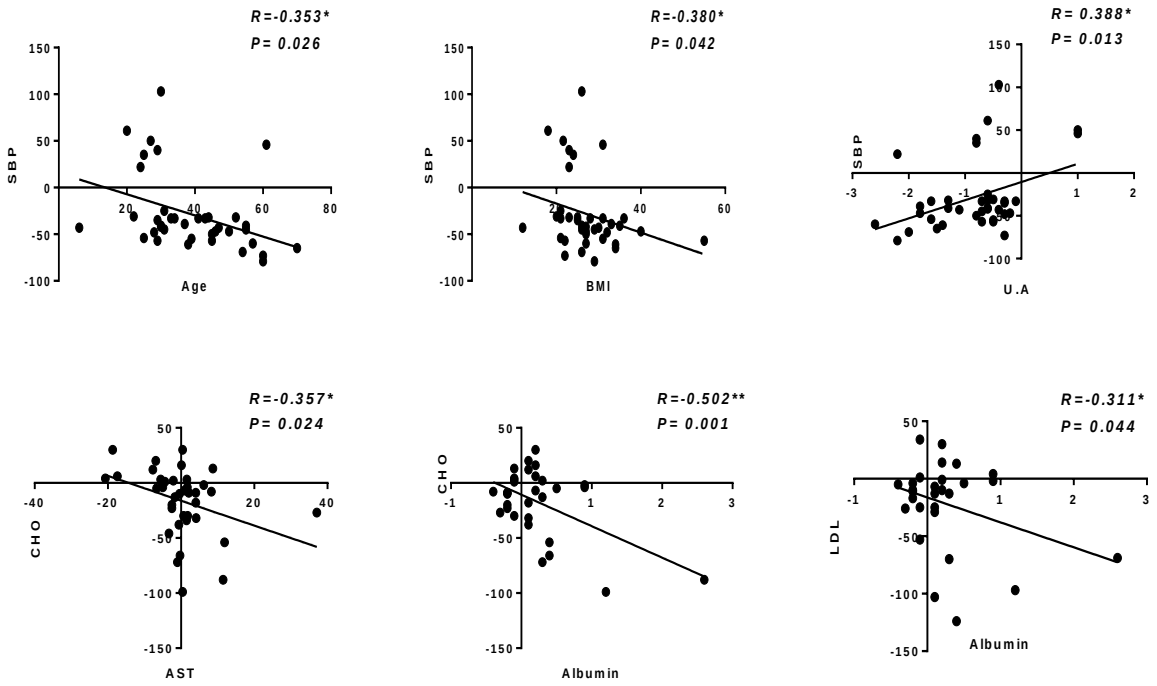


**Figure 3: Dot plot regression analyses of change in study parameters and variables**

## Discussion

This study shows a significant reduction of TC and LDL-C levels, with elevation of HDL-C in post-cupping than pre-cupping. Moreover, SBP positively associated with TC and LDL-C. These findings reinforced our hypothesis that, hypolipidemia results from blood cupping, raise on the benefits of Al-hijamah. Hence oxidized LDL and decreases HDL level, activates macrophage leading to foamy phagocytic cells, consequently atherosclerosis and CVD. Indeed, HDL-C levels associated with coronary events, HDL-C inversely related.[7]

Previous studies have shown that, there were associations between atherosclerosis, CVD and hypertension.[8] Based on past reports, the present study hypnotized that, blood cupping prevents atherosclerosis formation, hence relieve sequence of event that could lead to CVD and hypertension. Therefore, BP was investigated pre and post-cupping, where it was demonstrated that, both SBP and DBP were decreased after treatment with Al-hijamah. Further supported by previous finding that, 10 days after blood cupping significantly decreased both SBP and DBP compared with the baseline.[9,10]

In addition, it is also speculated that, the reduction of CRP by blood cupping could improve pathogenesis of CVD. Since CRP may induce atherogenesis by activating the inflammatory cascade CVD.[11] Concurrent with the previous finding, the present study demonstrated that, blood cupping significantly decreased CRP.[12,13] Therefore, suggesting that, Al-hijamah might possess a therapeutic effect against CVD. Soedamah *et al* found that, LDL-cholesterol and CRP below target levels achieved by statins trial, supported the non-lipid benefits of statins.[14] To our knowledge this is the first study documented the

potential benefit of blood cupping in relation to hypertension and CVD in Sudanese population.

Interestingly, this study revealed that, blood cupping significantly lower serum uric acids. Moreover, the negative association between uric acids and SBP was demonstrated. Therefore, these findings indicate that, blood cupping might treat hypertensive patient. The present study was compatible with previous report that, elevated uric acids mediate renal vasoconstriction, causes a reduction in endothelial levels of nitric oxide, lead to activation of renin-angiotensin system, probably because uric acids independently causes microvascular renal disease, which inducing the development of hypertension.[15] On the other hand, blood cupping is known to be enhanced remove of toxins and blood supply,[5,6] therefore resulting in lower uric acids level after blood cupping.

On the contrary, blood cupping significantly increased serum albumin. Furthermore, albumin level inversely correlated with TC and LDL-C, which means that, the more higher serum albumin levels the lower TC and LDL-C, probably benefit from blood cupping. Likewise, previous studies reported that, Albumin inhibits lipids peroxidation and LDL-C oxidation thus prevent CVD.[16] In addition, serum albumin level prevent deterioration of circadian BP rhythm in essential hypertensive patients, moreover might predict nocturnal systolic BP.[17]

Although controversial previous results that, after blood cupping serum creatinine increased.[9] The present study found that, Al-hijamah was demonstrated to be increased creatinine level. Which further attributed to higher CK activity post-blood cupping shows in this study. This finding was similar with previous result reported by Mohammad *et al*.[18] Rafiq *et al* stated that, CK correlates positively with serum creatinine level.[19] Recently Zervou *et al* demonstrated that, CK activity and their isoforms improved cells survival induced by hypoxia, thus were protected against cells damage.[20] Based on this finding, we demonstrated that, increase CK activity caused by blood cupping could protect against cells damage, resulting from hypoxia. Moreover, no significant differences were observed in mean ALT and AST activity. Which were concurrent with previously reported results.[1,9]

**Conclusion**

In conclusion, the data of present study suggests that, blood cupping had potentially deceased the BP, TC, LDL-C, uric acids and increased HDL-C levels. Moreover, uric acids are positively correlated with SBP. Meanwhile, Al-hijamah has a potential effect on the biomarkers associated with hypertension and CVDs. Therefore, mechanism of therapeutic interactions needs further study.

**Limitations**

In this present study, in addition to the small sample size, the clinical history and health status were obtained from patients using structured questionnaire, which was not considered to determine accurately the disease onset. Since the hypertension and CVD are asymptomatic at early stage. Therefore, further study is recommended to investigate the exact mechanisms and therapeutic action of blood cupping.

**Conflict of interest:**

The authors have no potential conflict of interest to declare.

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*Original Article* **Sudan Medical Laboratory Journal**

# Detection of Theileria and Babesia infection and their co-infection in ruminants in Wad Madani town, Gezira State, Central Sudan

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**Abstract**

**Introduction**: The Poor animal health is often a consequence of parasitic diseases; ticks are transmitting a number of diseases to indigenous and exotic ruminants in Sudan and cause large economic losses in productivity.

This study was designed to investigate Theileria and Babesia infection in ruminants (cows, sheep and goats) from ruminant's farms and ruminants market (Al mwashi market) in Wad Madani town, central Sudan..

**Method:** A total of 130 blood samples were collected from December 2020 to March 2021. Samples were collected from ruminants from 13 farms in Wad Madani town and Al mwashi market. Ruminants were chosen according to the breed, sex and age. For hematological examination for identification of the parasites in the prepared blood smears which were tested using Giemsa stain. Then Complete Blood Count (CBC) were done for each ruminants, measuring RBC, WBC, LYM, MID, GRAN, MCH, MCHC, MCV, HGB, HCT and RDW.

**Result:** revealed that 30 (23.1%) samples were positive for co-infection of Babesia and Theilera. (22.5%) in cows, (15.4%) in goats and (29.4%) in sheep. Results showed that sheep are the most one which have the co-infection with both Theileria, Babesia, infection, ruminants, Sudan

**Kayword: Theileria and Babesia.**

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**Introduction:** Tick-borne diseases (TBDs) are present throughout the world especially in the tropical and subtropical regions and affect approximately 80% of the world's ruminants population. They are considered a significant threat to the global food security (Ibrahim *et al.*, 2020). Losses directly attributed to haemoparasitic diseases include morbidity, mortality, production losses together with the costs of veterinary diagnosis/treatment and vector control (Ganguly *et al.,* 2020). The economically most important tick-borne diseases are theileriosis, babesiosis, ehrlichiosis (heart water) and anaplasmosis (ElGhali and Hassan, 2012). Tropical theileriosis is a parasitic disease caused by the hemoprotozoan *Theileria annulata* that is transmitted to ruminants by ixodid ticks of genus Hyalomma (Uilenberg *et al.,* 2013). *H. anatolicum* is the most important tick species in ruminants and buffalo (Hayati *et al.,* 2020). *Theileria annulata* is widely distributed throughout Europe, the Middle East, Russia, China, and Africa,

whereas *Theileria parva*, termed East Coast fever, is primarily distributed in Africa (Jia, *et al.,* 2020). It is a destructive disease that affects ruminants of all ages, breeds and sex and leads to severe losses in production and reproduction. Tropical theileriosis is highly endemic in Al Gezira State, Central Sudan due to the high prevalence of *H. anatolicum*, which is the main vector of this disease (Ibrahim *et al*, 2020).

Clinical signs shown by the affected animal include high fever ($107^{O}$F), anorexia, weight loss, debility, long lasting anaemia, jaundice, tachypnea, cough, salivation, lacrimation, petechiae on conjunctiva, enlarged lymph nodes and abortions or stillbirths (Reddy and Lakshman, 2020). Nasal discharge, enlargement of superficial lymph nodes, anorexia, reduced appetite (Niaz *et al.*, 2021). Lethargy, increased cardiac and respiratory rates, respiratory failure, decreased milk production (Eamens, *et al.*, 2013; Khatoon *et al.,* 2015). Ruminants that are left untreated usually die from the disease (Anupama *et al.,* 2015). Beside to that Bovine babesiosis is a tick-borne (Tb) parasitic disease of ruminants caused by *Babesia* species, a haemoprotozoan parasite including two predominant species i.e. *B. bigemina* and *B. bovis*. These species mostly affect ruminants and buffalos and are prevalent worldwide. With significant distribution in the tropical and subtropical areas of Africa, Asia, Australia, central and South America (Siddique *et al.*, 2020). The transmission of the disease by ticks and the principal of vectors of *B. bovis and B. bigemina are Rhipicephlaus* formally *Boophilus* species (Embiyale *et al.*, 2018). *Babesia divergens*, transmitted by the tick *Ixodes ricinus* (Springer *et al.,* 2020). Affected animals suffered from marked rise in body temperature, loss of appetite, cessation of rumination, labored breathing, emaciation, progressive hemolytic anemia, various degrees of jaundice (Icterus) (Yusuf, 2017). From paleness in mild case to sever yellow discoloration of conjunctival and vaginal mucous membranes in more progressive cases; haemoglobinuria, accelerated heart and respiratory rates, ocular problems and drop in milk production. In ruminants, fever during infections in some cases cause abortion to pregnant ruminants (El Moghazy *et al.,* 2014). Coffee colored urine is the characteristics clinical feature of babesiosis (Wodaje *et al.,* 2019). The detection methods of *Babesia* and *Theileria* spp. are morphological examination (Habibi et *al.,* 2020). Smears are preparations made using liquid substances. The function of making smears preparations is to observe cells in body fluids, for example in the blood. Diagnostic techniques were tested using Giemsa's staining technique. The blood parasites were then identified and their prevalence was determined (Ritonga *et al.,* 2020).

**Aim of the study:**The purpose of current study is to investigate the presence of *Theileria* and *Babesia* infections and their co- infection in ruminants in Wad Madani twon, Gezera state, central Sudan.

**Materials and methods: Study design area and population:** Cross sectional study for ruminants, conducted during the period of December 2020 – June 2021 was take place at Wad Madani town, Gezira State, Central Sudan. Which it is located 5,223.49 mi (8,406.40 km) south of the North Pole and has a hot desert climate. Two sites

were chosen according to the availability of animals: 13 private ruminant's farms and the main ruminants market in the town (Al mwashi market). Ruminants were chosen according to the breed, sex and age. The age ranged between >1 - <3 years, mostly sheep and goats were examined, followed by cows. The number of males (25, 13, 15) and the females were (26, 26, 25) animals respectively.

Three species of ruminants were chosen to investigate Theileria and Babesia infection (cows, sheep and goats). A total of 130 blood samples were collected: 40 from cows, 39 from sheep and 51 from goats. Sample size was determined according to the availability of the animals and willing of the owners to include their animals in the study.

**Blood collection:** Three ml of blood collected using disposable sterile syringes from each animal in heparized tubes for Complete Blood Count (CBC), thin and thick blood smears stained with Giemsa stain were done.

**Complete Blood Cell Count (CBC):** Complete Blood Count (CBC) were done from the whole blood of each ruminant for analysis of: RBC, WBC, LYM, MID, GRAN, MCH, MCHC, MCV, HGB, HCT and RDW.

**Blood Smear Technique (BST):** Thin blood smears were prepared from each sample. The blood smears were made in newly labeled glass slides according to McCosker(1975) by putting ne drop of blood on a microscope slide and using another slid to spread the blood at an acute angle that makes a thin film. Then slides were air died and fixed by absolute methanol for 2-3 minutes before staining using 10% solution of Giema stain for 30 minutes. The slides were washed with distilled water; air dried. Stained thin blood films examined by microscope using the X100 magnification using oil immersion lens for presence of *Theileria* and *Babesia spp*. piroplasms.

**Results: Infection rate according to breeds:**

Results showed Babesia infection rate was highest in sheep (51.0%), and then in cows (30.0%) and the lowest infection rate was in goats (20.5%). The Theileria infection rate was highest in sheep (64.7%), and then in goats (56.4%) and the lowest infection rate was in cows (55.0%). While the percentage of the co-infection with Babesiosis and Theileriosis was highest in sheep (29.4%), and then in cows (22.5%) and the lowest infection rate was in goats (15.4%) Table (1).

**Table (1):** Infection rate according to breeds:

| Breed | Babesiosis (%) | Theileriosis (%) | Co-infection (%) | Total |
|---|---|---|---|---|
| cows | 12 (30.0) | 22 (55.0) | 9 (22.5) | 40 |
| goats | 8 (20.5) | 22 (56.4) | 6 (15.4) | 39 |
| sheep | 26 (51.0) | 33 (64.7) | 15 (29.4) | 51 |
| **Total** | **46 (35.4)** | **77 (59.2)** | **30 (23.1)** | **130** |

**Infection rate according to age:**

**Babesia** infection rate according to age group was highest in cows at the age group 1-3 years (35.3%), and then in cows less than 1 year (33.3%) and the lowest infection rate was in cows more than 3 years (18.2%).

**Babesia** infection rate according to age group was highest in goats less than 1 year (36.8%) and then in goats more than 3 years (14.3%), while the lowest infection rate was in goats between 1-3 years (0%).

**Babesia** infection rate according to age group was highest in sheep more than 3 years (66.7%) and than in sheep between 1-3 years (52.6%), while the lowest infection rate was in sheep less than 1 year (40%).

**Theileriosis** infection rate according to age group was highest in cows at age group 1-3 years (70.6%) and then cows less than 1 year (50%) while the lowest infection rate was in cows more than 3 years (36.4%).

**Theileriosis** infection rate according to age group was highest in goats at age group less than 1 year (68.4%) and then in goats between 1- 3 years (53.8%), while the lowest infection rate was in goats more than 3 years (28.6%).

**Theileriosis** infection rate according to age group was highest in sheep at age group more than 3 years (66.7%) and then in sheep less than one year (65%), while the lowest infection rate was in sheep between 1-3 years (63.2%).

**Co-infection** infection rate according to **age** in cows was highest at age group less than one year (33.3%) and then in cows more than 3 year (18.2%), while the lowest infection rate was in cows between 1- 3 years (17.6%).

**Co-infection** infection rate according to **age** in goats was highest at age group less than 1 year (13.6%) and then in the other categories (0%).

**Co-infection** infection rate according to **age** in sheep was highest at age group more than 3 years (41.7%) and then in sheep between 1-3 years (31.6%) while the lowest infection rate was in sheep less than 1 year (20%), Table (2).

**Table (2):** Infection rate according to age

| Breed | Age group | Babesiosis (%) | Theileriosis (%) | Co-infection (%) | Total |
|---|---|---|---|---|---|
| Cows | <1 year | 4 (33.3) | 6 (50) | 4 (33.3) | **12** |
| | 1-3 years | 6 (35.3) | 12 (70.6) | 3 (17.6) | **17** |
| | > 3 years | 2 (18.2) | 4 (36.4) | 2 (18.2) | **11** |
| Goats | <1 year | 7 (36.8) | 13 (68.4) | 6 (13.6) | **19** |
| | 1-3 years | 0 (0) | 7 (53.8) | 0 (0) | **13** |
| | > 3 years | 1 (14.3) | 2 (28.6) | 0 (0) | **7** |
| Sheep | <1 year | 8 (40) | 13 (65) | 4 (20) | **20** |
| | 1-3 years | 10 (52.6) | 12 (63.2) | 6 (31.6) | **19** |
| | > 3 years | 8 (66.7) | 8 (66.7) | 5 (41.7) | **12** |

**Infection rate according to sex:** Babesia infection rate according to sex in cows was higher in males (40%) than in females (24%).

Babesia infection rate according to sex in goats was higher in males (30.8%) than females (15.4%).

Babesia infection rate according to sex in sheep was higher in males (52%) than females (50%), Table (3).

Theileria infection rate according to sex in cows was higher in females (60%) than males (46.7%).

Theileria infection rate according to sex in goats was higher in males (61.5%) than females (53.8%).

Theileria infection rate according to sex in sheep was higher in females (69.2%) than males (60%).

Co-infection infection rate according to sex in cows was higher in females (24%) than males (20%).

Co-infection infection rate according to sex in goats was higher in males (23.1%) than females (11.5%).

Co-infection infection rate according to sex in sheep was higher in females (34.6%) than males (24%) Table (3).

**Table (3):** Infection rate according to sex

| Breed | Sex: | Babesiosis (%) | Theileriosis (%) | Co-infection (%) | Total |
|---|---|---|---|---|---|
| Cows | Male | 6 (40) | 7 (46.7) | 3 (20) | 15 |
| | Female | 6 (24) | 15 (60) | 6 (24) | 25 |
| Goats | Male | 4 (30.8) | 8 (61.5) | 3 (23.1) | 13 |
| | Female | 4 (15.4) | 14 (53.8) | 3 (11.5) | 26 |
| Sheep | Male | 13 (52) | 15 (60) | 6 (24) | 25 |
| | Female | 13 (50) | 18 (69.2) | 9 (34.6) | 26 |

**Association between infection rate and CBC parameters:**

**Small ruminants (goats and sheep):**Results of infections (babesia, theileria and their co-infection) and CBC parameters indicated that there were significant association only between babesia infections and some CBC parameters (LYM% (0.022), GRAN% (0.018), MCH (0.006) and MCHC (0.008)). Table (4).

**Table (4):** Association between infection rate and CBC parameters in small ruminants (goats and sheep)

| CBC | Babesiosis -ve (N56) | Babesiosis +ve (N34) | Theileriosis -ve (N35) | Theileriosis +ve (N55) | Co-infection -ve (N69) | Co-infection +ve (N21) |
|---|---|---|---|---|---|---|
| WBC $10^3$/ul | 34.51 | 38.56 | 34.61 | 36.19 | 36.47 | 34.62 |
| ***p*-value** | 0.299 | | 0.686 | | 0.679 | |
| LYM% | 50.74 | 60.29 | 53.65 | 54.79 | 53.31 | 57.77 |
| ***p*-value** | **0.022*** | | 0.787 | | 0.358 | |
| MID% | 10.65 | 8.83 | 9.59 | 10.19 | 10.05 | 9.66 |
| ***p*-value** | 0.148 | | 0.645 | | 0.788 | |
| GRAN% | 38.61 | 30.91 | 36.75 | 35.03 | 36.64 | 32.61 |
| ***p*-value** | **0.018*** | | 0.606 | | 0.220 | |
| RBC$10^6$/ul | 2.43 | 3.06 | 2.48 | 2.70 | 2.58 | 2.96 |
| ***p*-value** | 0.097 | | 0.552 | | 0.367 | |
| HGB g/dl | 8.65 | 8.68 | 8.58 | 8.57 | 8.78 | 8.24 |
| ***p*-value** | 0.950 | | 0.977 | | 0.341 | |
| HCT% | 10.87 | 13.73 | 11.05 | 12.14 | 11.53 | 13.32 |
| ***p*-value** | 0.108 | | 0.529 | | 0.377 | |
| MCV fl | 43.56 | 44.16 | 43.20 | 43.38 | 43.70 | 44.05 |
| ***p*-value** | 0.200 | | 0.673 | | 0.531 | |
| MCH pg | 62.20 | 39.69 | 55.34 | 51.93 | 57.60 | 40.85 |
| ***p*-value** | **0.006*** | | 0.683 | | 0.050 | |
| MCHC g/dl | 150.04 | 93.49 | 132.85 | 125.82 | 138.35 | 96.89 |
| ***p*-value** | **0.008*** | | 0.742 | | 0.059 | |
| RDW-SD fl | 17.24 | 17.20 | 17.29 | 17.18 | 17.22 | 17.23 |
| ***p*-value** | 0.890 | | 0.698 | | 0.973 | |
| RDW-CV% | 12.37 | 12.17 | 12.38 | 12.25 | 12.32 | 12.22 |
| ***p*-value** | 0.266 | | 0.469 | | 0.611 | |

*significant at ($P < 0.050$)

**Cows:** Results of infections (babesia, theileria and their co-infection) and CBC parameters indicated that there were significant association only between babesia infections and some CBC parameters (MCH (0.003) and MCHC (0.003)). Table (5).

**Table (5):** Association between infection rate and CBC parameters in cows

| CBC | Babesiosis -ve (N28) | Babesiosis +ve (N12) | Theileriosis -ve (N18) | Theileriosis +ve (N22) | Co-infection -ve (N31) | Co-infection +ve (N9) |
|---|---|---|---|---|---|---|
| WBC $10^3$/ul | 27.04 | 25.92 | 27.62 | 26.70 | 27.57 | 25.54 |
| ***p*-value** | 0.727 | | 0.759 | | 0.447 | |
| LYM% | 56.89 | 59.05 | 59.78 | 55.70 | 57.01 | 59.37 |
| ***p*-value** | 0.574 | | 0.316 | | 0.554 | |
| MID% | 7.45 | 6.13 | 6.10 | 7.84 | 7.14 | 6.76 |
| ***p*-value** | 0.197 | | 0.069 | | 0.734 | |
| GRAN% | 35.66 | 34.82 | 34.12 | 36.46 | 35.85 | 33.88 |
| ***p*-value** | 0.794 | | 0.506 | | 0.535 | |
| RBC $10^6$/ul | 5.07 | 5.25 | 5.24 | 5.12 | 5.12 | 5.35 |
| ***p*-value** | 0.648 | | 0.754 | | 0.600 | |
| HGB g/dl | 8.51 | 9.58 | 9.28 | 8.78 | 8.83 | 9.60 |
| ***p*-value** | 0.208 | | 0.529 | | 0.503 | |
| HCT% | 26.79 | 26.67 | 27.67 | 26.81 | 27.01 | 27.82 |
| ***p*-value** | 0.956 | | 0.686 | | 0.716 | |
| MCV fl | 52.43 | 50.83 | 51.94 | 52.79 | 52.42 | 52.36 |
| ***p*-value** | 0.247 | | 0.463 | | 0.963 | |
| MCH pg | 16.21 | 17.92 | 17.42 | 16.89 | 17.00 | 17.57 |
| ***p*-value** | **0.003*** | | 0.377 | | 0.460 | |
| MCHC g/dl | 31.56 | 36.38 | 33.88 | 32.30 | 32.73 | 33.97 |
| ***p*-value** | **0.003*** | | 0.336 | | 0.561 | |
| RDW-SD fl | 26.92 | 25.22 | 25.99 | 26.75 | 26.35 | 26.61 |
| ***p*-value** | 0.189 | | 0.534 | | 0.883 | |
| RDW-CV% | 16.24 | 15.55 | 15.89 | 16.15 | 16.00 | 16.14 |
| ***p*-value** | 0.196 | | 0.604 | | 0.848 | |

*significant at ($P < 0.050$)

**Discussion**

In the present study the infection of babesia, theileria and their co-infection were detected. The overall Babesia infection rate was (35.4%) from the study population, (59.2%) for theileria and (23.1%) for their co-infection. The risk factors for these diseases studied here include: breeds, age and sex which may explain this differences.

In current study the overall infection of babesiosis was relatively highly significant (0.007). Where the highest infection rate was in sheep (51.0%) then in cows (30.0%), and goats (20.5%). However, this study disagree with (Mohammed *et al*., 2018) who found Babesiosis infection rate higher in goats (60.77%) than sheep (46.70%) in Sennar state, Sudan.

In this study the infection rate of Theileriosis was high (59.2%). Infection was found to be higher in sheep (64.7%) than goats (56.4%) and lowest in cows (55.0%). According to sex in cows, the female's rate was higher than the rate in males. This study agrees with (Abakar *et al.,* 2018) who found the prevalence rate of *T. annulata* antibodies in female (80%) was higher than in males (20%). In addition in this study, the infection rate according to age group was highest in cows between 1-3 years (70.6%), while he found the infection rate was highest in cows between 1-3 years (84.2%), and disagree with him that the infection rate was followed by cows less than 1 year (50%) and the lowest was in cows more than 3 years (36.4%), while his study showed that the infection rate was followed by cows more than three years old (75.5%) and the lowest rate was in cows less than one years (60.8%) in Elhoush district in south Gezira State.

In this study the prevalence of Theileriosis in sheep was higher in more than three years (66.7%) than the other categorical, this study agree with (Magzoub *et al.,* 2020) who found the highest prevalence rate was recorded in more than three years (21.6%) than the other. And disagree with him that the infection rate according to sex was higher in females (69.2%) than males (60%) while his study showed that the infection rate was higher in male (17.9%) than female (12.2%) in El Huda station in Al Gezira State and El Nuhud in West Kordofan State. However this study agree with (Ahmed *et al.,* 2018) who found infection rate was higher in female (83.3%) than male (52.4%) in Alhuda National Sheep Research Station, in Alhuda town.

In this study the co-infection between Theileria and Babesia infection rate was higher in sheep (29.4%) than goats (15.4%), this study agree with (Mohammed *et al*., 2018) who found the co-infection between Theileria, Babesia and Anaplasma infection rate was higher in sheep (31.66%) than goats (18.62%) in Sennar state.

In this study, Hematological analysis revealed haemato- Parameters values in Theileriosis in ruminants, which include decreases in the RBC count, WBC count, lymphocytes (LYM), packed cell volume (PCV), haemoglobin (Hgb) concentration, mean corpuscolar haemoglobin concentration (MCHC) with increased mean corpuscular volume (MCV), this result agree with ( Agina *et al.,* 2020).who found the same result above in Holstein ruminants in Malaysia.

In this study, Haematological analysis revealed haemato- Parameters values in Babesiosis in ruminants, expect of increasing in the RBC count, and haemoglobin (Hgb) concentration, this result disagree with (Abdalsalam, N.A. and Hazawy, S., 2017) who found Haematological analysis revealed haemato- Parameters values, expect of increasing in lymphocyte absolute count and mid-range absolute count (WBC), within the normal range in farm in ElWisata location in El-Gabal El-Akhdar area, El-Beida, Libya.

**Conclusion:**

Current study concluded that co-infection between Babesiosis and Theileriosis are detected in all types of ruminants tested.

CBC test is useful in detecting infection by Babesiosis and Theileriosis, and co-infection especially in late stages of infection.

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*Original Article* Sudan Medical Laboratory Journal

# Estimation of Plasma Tsriglyceride Level among Diabetic Mellitus Patients, Wad Medani, Gezira State, Sudan, 2022

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**Abstract**

**Background**: In Sudan and around the world, Diabetes Mellitus is a growing health concern. It is a serious, ongoing condition .The Middle East and North Africa (MENA) area, which includes Sudan, has the world's highest prevalence of diabetes. It was predicted to be about 25%. More concerning is the prediction that by 2030.

**Methods:** This is a case control study conducted in Aldaraga Health Center for Diabetic Care and Wad Medani Pediatric Teaching Hospital , Wad Medani, Gezira State, Sudan to estimate the plasma triglyceride level among diabetic patients. A total of 160 participants were included in the study, ninety patients were case group, their mean age was (40.90±24.00) years and seventy were control group their mean age was (45.78±22.38) years. Among case, 37 (41%) were males while 53 (59%) were females. Data were collected by using questionnaire and analyzed by computer program SPSS (Version 20). After fasting (8-12) hours, 2 ml of heparinized plasma and 1.5 ml EDTA whole blood was collected for estimation of triglyceride and HbA1c respectively and then plasma was stored at -20$^0$C till assay was done by Cobas C 311.

**Results:** It showed high rate of poor glycemic control in Sudanese diabetic patients (81%). There was significance differences in plasma triglyceride between case and control (P value =0.002), age groups (P value =0.000 ), and gender (P value =0.019). However, there were no statistically significant difference when compared plasma triglyceride levels with glycosylated hemoglobin and duration of the disease (P values = 0.514 and 0.096 respectively).

**Conclusion:** Diabetes, age and gender, but not glycosylated hemoglobin or disease duration, were found to have an impact on triglyceride levels in this study.

**Key words: Triglyceride, Glycosylated Hemoglobin, Diabetes mellitus, Sudan.**
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**Introduction:** Diabetes mellitus is a chronic, lifelong disease resulting from a defect in insulin secretion, insulin resistance, or both (1). In Sudan, diabetes mellitus is a serious health issue. It is estimated about 19% in urban areas and 2.6% in rural areas (2). The Middle East and North Africa (MENA) area, which includes Sudan, has the world's highest prevalence of diabetes. It was predicted to be about 25%. More concerning is the prediction that by 2030, there will be 28 million people living in Africa who are affected by DM, rising from 14 million. In north Sudan's regions, diabetes affects 19% of the population. and 8.3% among members of the Danagla tribe who reside in Northern Sudan. In rural areas in north Sudan, the incidence of undiagnosed diabetes was 2.6% (3). Type 1 Diabetes, which affects the production of insulin (a hormone that controls blood sugar), and

Type 2 Diabetes, which affects the body's ability to utilize the insulin that is produced, are both serious, chronic diseases. Nearly 90% of the disease's burden is caused by type 2 diabetes mellitus, and the remaining 10% is caused by type 1 diabetes or gestational diabetes(4).

The glycosylated hemoglobin (HbA1c) test is freq uently used to determine glycemic status. Intensiv e glycemic control is achieved through frequent ex ercise, diet, medication use, and insulin injections to keep blood glucose levels within the normal ran ge or very close to it (4). The American Diabetes Association defined glycemic control status for HbA1c level. When an adult's HbA1c level of 7% or less indicates adequate glycemic control and when an adult's HbA1c level of more than 7% indicates poor glycemic control (5).

Poor glycemic control has been linked to diabetes problems, which can be prevented by maintaining adequate diabetic control. In different settings, it has been documented that varying rates and different factors (age, gender, obesity, education, and exercise) are linked to poor glycemic control. While there are a lot of published material on glycemic control and its related variables in the various African nations. Few published studies on glycemic control in Sudan exist. According to the American Diabetes Association (ADA), each 1% increase in HbA1c increases the risk of diabetes-related death by 25%. Additionally, it has been calculated that each percentage point increase in HbA1c is associated with a 35% increase in the risk of micro vascular problems and an 18% increase in the risk of major vascular complications (6).

A variety of metabolic dysfunctions are brought on by uncontrolled diabetes mellitus as a result of impaired insulin secretion or insulin resistance. Poor glycemic control, which predisposes to micro- and macrovascular complications, is the main concern faced by this disease. Neuropathy, retinopathy, and nephropathy are examples of micro vascular consequences. Coronary artery disease and peripheral artery disease are macro vascular problems. Each extra unit of Hcy concentration above 14 mol/L in diabetic patients was discovered to be associated with an increased risk of diabetic retinopathy and renal failure (7). According to recent studies, Sudan had a serious health problem with diabetes and its associated problems (5). Low levels of very low density lipoprotein cholesterol (VLDL-C), excess levels of low density lipoprotein cholesterol (LDL-C), and elevated triglyceride levels are all symptoms of dyslipidemia (8).

Because key enzymes and metabolic pathways involved in lipid metabolism are impacted by insulin resistance or deficiency, lipid abnormalities are common in diabetes mellitus. The production of apo proteins, control of lipoprotein lipase, action of cholesterol ester, transfer proteins, and hepatic and peripheral actions of insulin are specifically impacted. Furthermore, it has been suggested that diabetic dyslipidemia's lipid particle makeup is more atherogenic than that of other forms of dyslipidemia (8). There is a relationship between hyperglycemia and hypertriglyceridemia and an increased risk of cardiovascular disease (9). The two basic categories of hypertriglyceridemia (HTG) will be covered: "mild to moderate," defined as triglyceride levels between 150 and 499

mg/dL, and "severe HTG," defined as triglyceride levels of 500 mg/dL. other HTG patients, it is crucial to consider additional risk factors such as lipodystrophy, obesity, alcoholism, renal disease, drugs (such as steroids, blockers, retinoid, oral estrogens, tamoxsifen, protease inhibitors, and bile acid sequestrants), and pregnancy (10).

**Methods:** This is a case control study conducted in Gezira State among Known diabetic patients Attending Aldaraga Health Center for Diabetic Care and Wad Medani Pediatric Teaching Hospital. One hundred and sixty participants were enrolled in the study, 90 were diabetic as case group, their average age was (40.90±24.00) years and 70 healthy individual as control group their average age was (45.78±22.38) years. 73(71%) uncontrolled and 17(29%) controlled they were recruited by trained field workers and gave their written consent voluntarily.

Fasting diabetic Sudanese nationality patients who gave agreement for participation were included in the study. Newly diagnosed diabetic patient, smokers, obese, pregnant and lactate women, patient with other illness (Renal failure, hypertension, liver disease, cancer, thyroid disorder), and patients use these drugs (e.g. steroids, β blockers, retinoid, oral estrogens, tamoxsifen protease inhibitors, and bile acid sequestrants), all these were excluded from the study.

Ethical approval was obtained from the Ministry of Health, Gezira State. Ethical permission was obtained from Manager of *Aldaraga* Health Center for Diabetic Care and Manager of Wad Medani Pediatric Teaching Hospital. The specimens and information was collected from the individuals under privacy and confidentiality and was not used for any purposes rather than this study.

The data was collected by using questionnaire that designed to include personal, clinical information, and laboratory results.

Venous blood sample collection was done by clean venipuncture technique under a septic condition and 3.5 ml of venous blood was obtained from patient with overnight fasting at least (8-12 hours). A non-allergenic adhesive spot placed over the venipuncture site. The blood divided in to two blood containers tube, one containing $K_3$EDTA for HbA1c estimation by colormetric method by Cobas C311. The other tube contain lithium heparin for TG estimation the tubes were labeled and centrifuged at 5000 rpm for 5 minutes to separate the plasma. After centrifugation laboratory analysis was done immediately or the plasma separated and stored in labeled Eppendorf tube at -20C until it analyzed by enzymatic colormetric test by used Cobas C311.

The diagnostic criteria used for the parameters were follows: glycosylated hemoglobin in Non-diabetic = ≤6mg/dl, Control diabetic = ≤7mg/dl, Uncontrolled Diabetic = more than 7mg/dl (5) Triglyceride normal rang was (<1.70mmol/L = <150mg/dL). Hypertriglyceridemia (HTG) was discussed under two broad categories: "mild to moderate, "in which the triglycerides are between 150 to 499 mg/dL, and "severe HTG"when the triglyceride levels are ≥500 mg/dL (10).

Data were analyzing by using Statistical Package for Social Sciences (SPSS) computer program (Version 20). Descriptive statistics were calculated

for the data in the form of mean and standard deviation (SD±) using student T test for quantitative data and frequency and distribution for qualitative data.

**Results:** This study was carried out on 160 participants included 90 diabetic patients and 70 healthy individuals at the mean age (40.90±24.00) years, (45.78±22.38) years respectively. 41% of the participants were men, while 59% were women. Regarding the duration of diabetes, 44% of patients had a range of less than 5 years, 47% had a range of 6 – 10 years, and 9% had a range of more than 10 years. 56% of patients were from rural areas, while 44% were from urban areas. 84% of patients have a history of diabetes in their families(Table 1). The mean of triglyceride was significant between case and control (Table 2). The distribution of participants by HbA1c level revealed that the majority of diabetic patients are uncontrolled 81%, (Table 3).

In terms of triglyceride level with glycemic control and disease duration, the results did not indicate any statistically significant changes (Table 4 and Table 7)**,** respectively. However, differences in triglyceride levels associated with age and gender (Table 5 and Table 6), respectively were statistically significant.

**Table 1:** Frequency and percentage of Socio-demographic data

| **Demographic/clinical variables** | | **Frequency** | **Percent (%)** |
|---|---|---|---|
| **Category** | **Sub-category** | | |
| **Gender** | **Male** | 40 | 41 |
| | **Female** | 50 | 59 |
| **Age group** | **Less than 17 years** | 38 | 42 |
| | **17 – 50 years** | 23 | 26 |
| | **More than 50 years** | 29 | 32 |
| **Disease duration/ year** | **Less than 6 years** | 40 | 44 |
| | **6 – 10 years** | 42 | 47 |
| | **More than 10 years** | 8 | 9 |
| **Residence** | **Rural** | 50 | 56 |
| | **Urban** | 40 | 44 |
| **Family history** | **Yes** | 76 | 84 |
| | **No** | 14 | 16 |

**Table 2:** The mean of Triglyceride between case and control.

| | **N** | **Mean** | **STD** | **P value** |
|---|---|---|---|---|
| **Case** | 90 | 109.32 | 65.92 | |
| **Control** | 70 | 135.00 | 79.00 | **0.020** |

**Table 3:** Frequency and percentage of Triglyceride in Controlled and uncontrolled diabetic groups.

| | Frequency | Percent |
|---|---|---|
| Triglyceride level | | |
| Normal | 72 | 80 |
| Moderate | 18 | 20 |
| HbA1c level | | |
| Controlled | 17 | 19 |
| Uncontrolled | 73 | 81 |

**Table 4: The mean of triglyceride level at different glycemic control.**

| | HbA1c | N | Mean | STD | P value |
|---|---|---|---|---|---|
| Triglyceride | Controlled | 17 | 118.59 | 85.52 | 0.514 |
| | Uncontrolled | 73 | 106.89 | 61.14 | |

**Table 5:** The mean of triglyceride level according to Age groups.

| **Age** | **N** | **Mean** | **STD** | **P value** |
|---|---|---|---|---|
| **Less than 17 Years** | 38 | 71.84 | 29.24 | **0.000** |
| **17 – 50 Years** | 23 | 117.83 | 69.03 | |
| **More than 50 Years** | 29 | 151.00 | 72.24 | |
| **Total** | 90 | 109.1 | 66.03 | |

**Table 6:** The mean of triglyceride level according to gender:

| | **Gender** | **N** | **Mean** | **STD** | **P value** |
|---|---|---|---|---|---|
| **Triglyceride** | **Male** | 37 | 90.86 | 50.476 | **0.019** |
| | **Female** | 53 | 121.83 | 72.773 | |

**Table 7:** The mean of triglyceride level according to disease duration.

| **Triglyceride** | **N** | **Mean** | **STD** | **P value** |
|---|---|---|---|---|
| **Less than 6 Years** | 40 | 92.65 | 62.02 | 0.096 |
| **6 – 10 Years** | 42 | 120.48 | 67.06 | |
| **More than 10 Years** | 8 | 131.63 | 68.95 | |
| **Total** | 90 | 109.10 | 66.03 | |

**Discussion:** In this study, the distribution of patients by HbA1c level revealed that 81% of diabetic patients are uncontrolled. This proportion was nearly identical to or equivalent to those of numerous African nations, including Uganda (79.2%), Botswana (82%), Nigeria (62%) and Congo (68). Oman, Saudi Arabia, the United Arab Emirates, and Bahrain all had higher than average rates of controlled diabetes in the Gulf region: 35%, 32.3%, 21.2%, 41%, and 21.8%, respectively. So, like other Gulf region nations, Sudan has a similar proportion of uncontrolled diabetes. However, it was projected that 52.5% of Americans and 49.1% of Koreans had controlled diabetes. These findings show that glycemic control represents a major challenge not just in African nations but also in

Western and Asian nations (11).

In a prior study, Rania and et al. found that levels of CHOL, TRI, HDL, and LDL varied amongst three groups (normal weight, overweight, and obese) but that the differences were not statistically significant. High amounts of COHL and LDL were found in the overweight group, but high levels of TRI and HDL were seen in the obese group. Increased quantities of fat in the cells block the effects of insulin, resulting in insulin resistance and the eventual emergence of type II DM. The rising prevalence of obesity has been largely related to dietary practices, such as consuming a lot of fatty and sugary foods and dates and getting little exercise. Very low density lipoprotein (VLDL) secretion from the liver may be enhanced and delayed as a potential cause of hypertriglyceridemia (12).

According to the study's findings, there was no statistically significant difference in the triglyceride levels in individuals with various levels of glycemic control and disease duration (P value = 0.514 and 0.096). This finding is consistent with research conducted by Omer and colleagues (2019) in Gadarif State, Eastern Sudan, to evaluate glycemic control among adult patients with type 2 diabetes. They discovered that triglycerides and the duration of the disease were not related to poor glycemic control (P value = 0.170 and 0.085 respectively). This however, conflicts with several studies that examined the relationship between triglycerides and glycosylated hemoglobin and were conducted in different countries around the world. It differs with studies carried out by Azad and colleagues in Saudi Arabia in 2017 (P value = 0.036) (13), as well as studies carried out by Mullugeta and colleagues in India in 2012 (P value = 0.050) (14), Vinod Mahato in Nepal in 2011 (P value = 0.030 ) (15) , and Ismail in Iraq in 2017 (P value = 0.042) (6).

The results of this study showed that there was statistical significance difference in triglyceride in case and control (P value = 0.020) also with patients age and gender (P value = 0.007) (P value = 0.019) respectively. This agree with the all studies mentioned above in and out of Sudan. There may be differences in ethnicity, lifestyle, patient fasting length, and sample size between this study and studies conducted in other countries.

**Conclusion:** In this study, it was discovered that triglyceride levels were influenced by diabetes, age, and gender but not by glycosylated hemoglobin or the duration of the disease. According to the distribution of patients by HbA1c level, 81% of diabetic patients have uncontrolled blood sugar. **Acknowledgements**:

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*Original Article* Sudan Medical Laboratory Journal

# Frequency of Methylenetetrahydrofolate Reductase (C677T) genetic polymorphism among Sudanese patients with myocardial infarction

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**Abstract**

**Background**: Myocardial infarction is a leading cause of mortality worldwide. Age, smoking, obesity, diabetes, hypertension, dyslipidemia, and low levels of physical activity are one of the known risk factors for the development of Ischemic Heart Diseases. However, the occurrence of Ischemic Heart Diseases in the absence of any of these factors raises the possibility of additional risk factors, such as genetic ones. Genetic risk factors for myocardial infarction have been established in the previous investigation and the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene polymorphism was one of the most often examined ones. MTHFR C677T polymorphism increases serum homocysteine level which was believed to lead to a hypercoagulable state. Accordingly, this study aimed to examone the association between the risk of developing myocardial infarction and MTHFR C677T polymorphism in Sudan.

**Methods:** A total of 50 patients with myocardial infarction and 50 healthy controls who were matched by age and gender were included in the study. MTHFR C677T polymorphism was studied in both cases and healthy controls, 2 ml of blood samples were collected from both patients and controls into EDTA anticoagulated tubes, the extraction of DNA was done using the salting out approach, The primers, were used to amplify the MTHFR C677T fragment, the amplified fragment was detected on an agarose gel electrophoresis. and count the blood cells.

**Results:** Ten percent (n = 5) of cases had the MTHFR C677T polymorphism while none of the healthy controls (0%) had it and there was a significant association between the polymorphism and having myocardial infraction in this study (P = 0.028).

**Conclusion:** this study revealed a statistically significant association between the risk of developing Myocardial infarction and having the MTHFR C677T gene polymorphism with none of the healthy controls reporting MTHFR C677T gene polymorphism.

**Key words: Methylenetetrahydrofolate Reductase, C677T, genetic polymorphism, myocardial infarction, Sudanese.**

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**Introduction:** Myocardial infarction (MI) is the death of cardiac cells due to insufficient blood supply resulting from coronary artery blockage. It is a leading cause of mortality worldwide and according to the World Health Organization, almost 50 million people die yearly due to Ischemic Heart Diseases (IHD), particularly myocardial infarction (1). Many modifiable and non-modifiable risk factors for the development of IHD such as age, smoking, Obesity, Diabetes, Hypertension, dyslipidemia, and low physical activity have been identified (2–4). However, the presentation of IHD without any known risk factor raises the question of the presence of other risk factors such as genetic ones (5). Previous studies have identified genetic risk factors for myocardial infarction and 5,10-methylenetetrahydrofolate reductase (MTHFR) gene polymorphism was

among the most commonly studied ones (6)(7).

The MTHFR gene is found at location 'Ip36.3' on chromosome 1 with its matching cDNA sequence that consists of 11 exons spanning 2.2 kb (8). The protein product of this gene contains 656 amino acids and functions as a catalyzing factor for the irreversible transformation of 5,10-methylenetetrahydrofolate (5,10-CH2-FH4) into 5-methyltetrahydrofolate (5-CH3-FH4), the main circulating form of folic acid that acts as a co-substrate for the re-methylation of homocysteine to methionine (9)(10). This 5-methyltetrahydrofolate is the form of folate that participate in the synthesis of nucleotides, the formation of S-adenosylmethionine, the re-methylation of homocysteine to methionine, and the methylation of DNA, proteins, and phospholipids (11).

The MTHFR gene's C677T polymorphism causes alanine to change to valine in the binding site of the flavin adenine dinucleotide, the cofactor of the MTHFR enzyme, by changing cytosine (C) to thymine (T) at position 677 (12). As a result, this MTHFR C677T allele makes it easier for the enzyme to become separated from its cofactor, which lowers its activity and thereby increases plasmatic homocysteine levels (13).

There is mounting evidence that patients with or without hypertension, diabetes mellitus, and other traditional risk factors are at a greater risk for cerebrovascular, peripheral vascular, and cardiovascular illness when their plasma Homocysteine level is elevated (14)(15). Several studies have examined the association between MTHFR C677T polymorphism and myocardial infarction before, however, different conclusions have been yielded. Accordingly, this study aimed to address this association in Sudan.

**Materials and method:** This was a case-control study that included 50 patients with myocardial infarction and 50 healthy controls who were matched by age and sex. MI patients were obtained from the Al-Shaab teaching hospital, in Khartoum, Sudan. Serum troponin I was tested in all MI patients, and all were positive. Before data collection and analysis, informed consent was taken from both cases and controls.

2 ml of blood samples were collected from both patients and controls into EDTA anticoagulated tubes and the laboratory work was done at the department of hematology, faculty of medical laboratory sciences, Alneelain University, Sudan. The extraction of DNA was done using the salting out approach. The forward primer, 5'-TGAAGGAGAAGGTGTCTGCGGGA-3', and the reverse primer, 5'-AGGACGGTGCGGTGAGTG- 3', were used to amplify the MTHFR C677T fragment. The thermocycler (Techne TC-412, UK) was used to do the amplification after a 5-minute denaturation stage at 94°C. After that, there were 40 cycles that each had three steps: a denaturation step at 94 °C for 30 seconds, an annealing step at 59 °C for 1 minute, an extension step at 72 °C for 1 minute, and the last extension step taking place at 72 °C for 7 minutes. A final 20 µl volume containing 4 µl of the pre-mixed, ready-to-use 5x FIREPolmaster mix (Solis BioDyne, Russian), 11 µl of DNAase-free DW, 3 µl of genomic DNA, and 1 µl from each primer was used for the PCR reaction. After being digested overnight with 10 U of

Hinf1endonuclease (New England Biolab, UK), the amplified fragment was detected on an agarose gel electrophoresis. An automatic cell counter was used to count the blood cells (Sysmex KX-21N).

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 25. Categorical data were presented as percentages and numbers (n (%)), and the frequency distribution between groups from different variables were compared using the Chi-square test. A P-value of less than 0.05 was considered statistically significant.

**Results:** A total of 50 patients with myocardial infarction were included in the study with their ages ranging from 35 to 65 years. Of them, 32 (64%) were males and 18 (36%) were females. Fifty healthy controls were matched to diseased subjects by age and gender. MTHFR C677T polymorphism was studied in both cases and healthy controls. Ten percent (n = 5) of cases had the MTHFR C677T polymorphism while none of the healthy controls (0%) had it and there was a significant association between the polymorphism and having myocardial infraction in this study (P = 0.028) (Table 1).

**Table 1:** Comparison of Methylenetetrahydrofolate Reductase (C677T) gene polymorphism frequency between patients and control

| Genotype | Patients n (%) | Controls n (%) | Sig. |
|---|---|---|---|
| **CC** | 45 (90%) | 50 (100%) | **0.028*** |
| **CT** | 5 (10%) | 0 (0%) | |

Associations between myocardial infarction and known risk factors such as diabetes, hypertension, smoking, and obesity were tested in this study, including patients and controls. We found a significant association between all these factors and the disease (P = 0.000). Hb levels were measured for both patients and controls and no significant difference was reported between the two groups (P = 0.8010)

**Discussion:** This study is the first of its kind in Sudan to examine the association between MTHFR C677T gene polymorphism and the risk of developing MI. in this study we reported a significant association between the two and none of the healthy controls reported this polymorphism in their genes. Other previous studies have examined this association and the effect of this polymorphism on developing hypercoagulability state and thrombosis such as deep vein thrombosis (DVT) due to the increase in serum homocysteine level. Studies conducted in Morocco (7) and China (16) revealed no significant association between the risk of MI and MTHFR C677T polymorphism, however, in a previous systematic review, a significant association was reported between the two, particularly among young patients (17). Furthermore, a study conducted in Georgia, revealed a significant association between MTHFR C677T polymorphism and the risk of developing arterial thrombosis (18), while a previous Sudanese

study reported an insignificant effect of this polymorphism on DVT occurrence (19). All these suggest that there is inconclusive evidence regarding the effect of MTHFR C677T polymorphism on the development of MI and other hypercoagulable diseases. This study is limited by the small sample size and inclusion of patients from only one hospital. Moreover, the lack of financial ability and resources to measure serum homocysteine levels among both patients and controls is another limitation of this study.

In conclusion, this study examined the association between the risk of developing Myocardial infarction and having the MTHFR C677T gene polymorphism and a statistically significant association was reported between the two. Moreover, none of the healthy controls in this study reported MTHFR C677T gene polymorphism.

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*Original Article* **Sudan Medical Laboratory Journal**

# Antimicrobial, Antioxidant activities and GC/MS analysis of clove oil (*Syzygium aromaticum*)

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**Abstract**

**Introduction:** Essential oils are volatile, complex, strong-smelling natural compounds formed by aromatic plants as secondary metabolites. Usually, it is obtained by steam or hydrodistillation.
In nature, essential oils play an important role in the protection of plants, Essential oils can be synthesized by all plant organs, i.e. buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretory cells, cavities, canals, epidemic cells or glandular trichomes.
**Method**: Extraction of Clove Oil Steam hydro-distillation is selected as a method to isolate clove oil, the antimicrobial and antioxidant activity was detected, and the chemical content of clove oil. Clove oil was tested against five standard bacteria strains (Bacillus subtilis, Staphylococcus aurous, Pseudomonas aeruginosa. salmonella typhi, Escherichia coli), and two fungi (Asperiglus nigur, Candida albicans) using the cup-plate agar diffusion method. Also tested was the antioxidant effect using DPPH. The chemical constituents of clove oil were also detected using GC/MS. Clove oil showed high activity against Salmonella typhi, Staphylococcus aurous, Pseudomonas aeruginosa, Escherichia coli, Asperiglus nigur and Bacillus subtilis
**Result:** Clove Oil extraction high activity against all types of bacteria under study (*Bacillus subtilis*, *Staphylococcus aurous*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*), and it had high activity against *Aspergillus nigur*, while it had low activity against *Candida albicans* at a concentration of 100 mg/ml.. interestingly, the antioxidant activity of clove oil was higher compared to Propyl gallate (Standard). Chemical detection by GC/MS showed the presence of 4 compounds. The main compounds in clove oil were Eugenol (58.38) and Caryophyllene (34.87).

**Kayword: Antimicrobial, Antioxidant, GC/MS, clove oil, *Syzygium aromaticum*.**
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**Introduction:** Essential oils are volatile, complex, strong-smelling natural compounds formed by aromatic plants as secondary metabolites. Usually, it is obtained by steam or hydro-distillation (1). In nature, essential oils play an important role in the protection of plants. They also may attract some insects to promote the dispersion of pollens and seeds or keep away other undesirable insects. Thus, EOs can play a role in mediating the interactions of plants with the environment (2). Essential oils can be synthesized by all plant organs, i.e. buds, flowers, leaves, stems, twigs, seeds, fruits, roots,transplacental transfer from the mother usually following acute maternal infection. If

congenital toxoplasmosis occurs early in pregnancy, it may lead to severe damage or abortion.(4) Though the infection is frequently asymptomatic or mild and self-limiting (fever, agitation, lymph- wood or bark, and are stored in secretory cells, cavities, canals, epidemic cells or glandular trichomes (1). Clove (*Syzygium aromaticum* L.) Merril. & Perry, is a member of the family Myrtaceae. The clove of commerce is its dried unopened flower buds. Whole and ground cloves are used to enhance the flavor of meat and rice dishes. They are highly valued in medicine as a carminative and stimulant and are said to be a natural anthelmintic (3). Clove oil has been listed as a ‘‘Generally Regarded As Safe’’ substance by the United States Food and Drug Administration when administered at levels not exceeding 1500 ppm in all food categories (4). Clove oil its antimicrobial properties, antioxidant, analgesic, anesthetic, anti-inflammatory, and insecticidal activity It is also used for preventing degenerative diseases because of its antioxidant effects. Clove bud oil is a common healing agent for wounds and burns in traditional medicine (5). The major constituents of the clove essential oils are eugenol, β-caryophyllene, α-humulene and humulene epoxide. These constituents are known to possess antibacterial antifungal and anti-carcinogenic properties. According to these various biological activities, clove oils find uses in toothpaste, mouth washes, soaps and other cosmetic items (6).

The purpose of this research is to investigate Antimicrobial, Antioxidant activities and GC/MS profiling of clove oil (*Syzygium aromaticum*).

**Material and Methods: Plant Collection:** The sample of clove was purchased from Omdurman local market. clove used was identified and authenticated at the herbarium of the botany department. Faculty of Science and Technology, Omdurman Islamic University.

**Oil Extraction:** Extraction of Clove Oil Steam hydro-distillation is selected as a method to isolate clove oil. A hundred grams of dried clove flowerswere put in a steam flask. The steam distillation was conducted several times such as 3, 4, 5, and 6h. The time started to be counted when the first drop of distillate comes out.

**Antimicrobial activity : 3.2.2.1.1Tested bacteria and fungi :** Five standard bacteria and two fungi species were obtained from Microbiology lab, Omdurman Islamic University. They were gram positive (G+ve): *Bacillus subtilis*, *Staphylococcus aurous* and gram negative (G-ve): *Pseudomonas aeruginosa*, *salmonella typhi*, *Escherichia coli*, and fungi. *Asperiglus nigur* and *Candida albicans*. The bacteria and fungi were activated and cloned three successive times in nutrient agar and stored on nutrient agar slants at 4°C. Subsequent cultivation and tests were done on nutrient agar medium.

**Preparation of bacterial suspensions :** One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108-109c.f.u/ml. The suspension was stored in a refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable

counting technique (7). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes on drop of the appropriate dilutions were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

**Preparation of fungal suspension:** The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

**In vitro testing of extracts for antibacterial activity :** The cup-plate agar diffusion method as described by Kavanagh, 2014 (8) was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. Aliquot of 1 ml of the standardized bacterial stock suspension (between 108 and 109 CFU/ml) was thoroughly mixed with 100 ml of molten sterile Mueller Hinton agar (HiMedia, India) which was maintained at 45°C. 20 ml aliquots of the inoculated Mueller Hinton agar were distributed into sterile Petri-dish plates. The agar was left to set and in all of these plates 5 cups (8 mm in diameter) was cut using a sterile corkborer and agar discs were removed. Each cups were filled with 0.1 ml of the methanolic extract using an automatic microliter pipette, and thereafter the extract was allowed to diffuse at room temperature for two hours. The plates were then incubated in an upright position at 37°C for 24h. Two replicates were carried out for each extract against each of the tested microorganisms. After incubation the diameters of the resultant growth inhibition zones were measured and averaged. The mean values were tabulated.

**In vitro testing of extracts for Antifungal activity:** The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25 ºC for two days for the *Candida albicans*.

**Antioxidant activity: DPPH radical scavenging assay:** The DPPH free radical scavenging activity Principle: The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according using the method of Shimada et al., 1992 (9), with slight modification. Active samples can reduce the stable radical DPPH to the yellowcolored diphenyl- picrylhydrazine.

**Assay:** Test samples were allowed to react with 2.2 di (4-tretoctylphenyl)-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37oc in 96-wells plate. The concentration of DPPH was kept at (300µM). the test sample was dissolved in DMSO

while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multi plate reader spectrophotometer. Percentage of radical scavenging activity of the sample was determined in comparison with a DMSO treated control. All tests were conducted in triplicate. $DPPH\ radical\ scavenging$ **(%) = 100−{(**$Ac-At$**)/**$Ac$**} × 100**

**Where**, At= Absorbance value of test compound; Ac=Absorbance value ofcontrol.

**Chromatographic analysis:**

**Gas Chromatography Mass Spectrometry (GC MS) analysis:** GC-MS technique was used in this study to identify the phytocomponents present in the most active fractions. The tested extracts were analyzed by GC-MS using Shimadzu Mass Spectrometer-2010 series. 1 µL of sample was injected in GC-MS equipped with a split injector.

The MS was operated in the electron ionization (EI) mode (70 eV). Helium was employed as the carrier gas and its flow rate was adjusted to 1.2 mL/min.

The analytical column connected to the system was an Rtx-5 capillary column (length-30 m × 0.25 mm i.d., 0.25µm film thickness). The column head pressure was adjusted to 93.9 kPa. Column temperature programmed from 110 °C (7 min) to 200 °C at 10 °C/min and from 200-280 °C at 5 °C/min withhold time 0 and 9 min respectively.

A solvent delay of 4.50 min was selected. Theinjector temperature was set at 250 °C. The GC-MS interface was maintained at 280 °C. The MS was operated in the ACQ mode scanning from m/z 40 to 550.0. In the full scan mode, EI mass spectra in the range of 40–550 (m/z) were recorded at electron energy of 70 eV. Compounds were identified by comparing mass spectra with library of the National Institute of Standard and Technology (NIST), USA/Wiley.

**Results and Discussion: Antimicrobial activity:**

The antimicrobial activity of clove oil was studied in different concentrations (100, 50, 25, 12.5 and 6.2 mg/ml against standard bacterial and fungal. The antibacterial and antifungal potential of clove oil was assessed in terms for inhibition zone of bacterial growth. The results of the antibacterial and antifungal activities are presented in **Table (1)** and **Figure (1)**. Clove oil showed high activity against all types of bacteria under study (*Bacillus subtilis*, *Staphylococcus aurous*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*), and it had high activity against *Aspergillus nigur*, while it had low activity against *Candida albicans* at a concentration of 100 mg/ml. The study obtained by (10) showed that clove oil has an inhibitory and bactericidal effect against S. aureus, E. coli, L. monocytogenes and S. Typhimurium. In addition, the results obtained by (11) showed that the petroleum ether extract of cloves has high efficacy against the standard strain of *Streptococcus oralis, Streptococcus parasanguinis*, *Streptococcus mutans*, and *Streptococcus mutans* that cause dental caries.

**Table 1:** Antimicrobial activity of Clove oil against standard organisms

| Sample | Concentrations | Test organisms used Inhibition zones (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *B.s* | *St.a* | *P.s* | *S.t* | *E.c* | *As.n* | *C.a* |
| Clove Oil | 100 mg/ml | 18 | 27 | 27 | 34 | 24 | 23 | 9 |
| | 50 mg/ml | - | 12 | 12 | 22 | - | 9 | - |
| | 25 mg/ml | - | - | - | - | - | - | - |
| | 12.5 mg/ml | - | - | - | 14 | 14 | - | - |
| | 6.2 mg/ml | - | - | - | 15 | - | - | - |

*B.S= Bacillus subtilis; St.a: Staphylococcus aurous; Ps= Pseudomonas aeruginosa; S.t= salmonella typhi ;E.c= Escherichia coli; As.n= Asperiglus nigur; C.a= candida albicans*; M. D. I. Z mm = Mean diameter of growth inhibition zones, in mm.

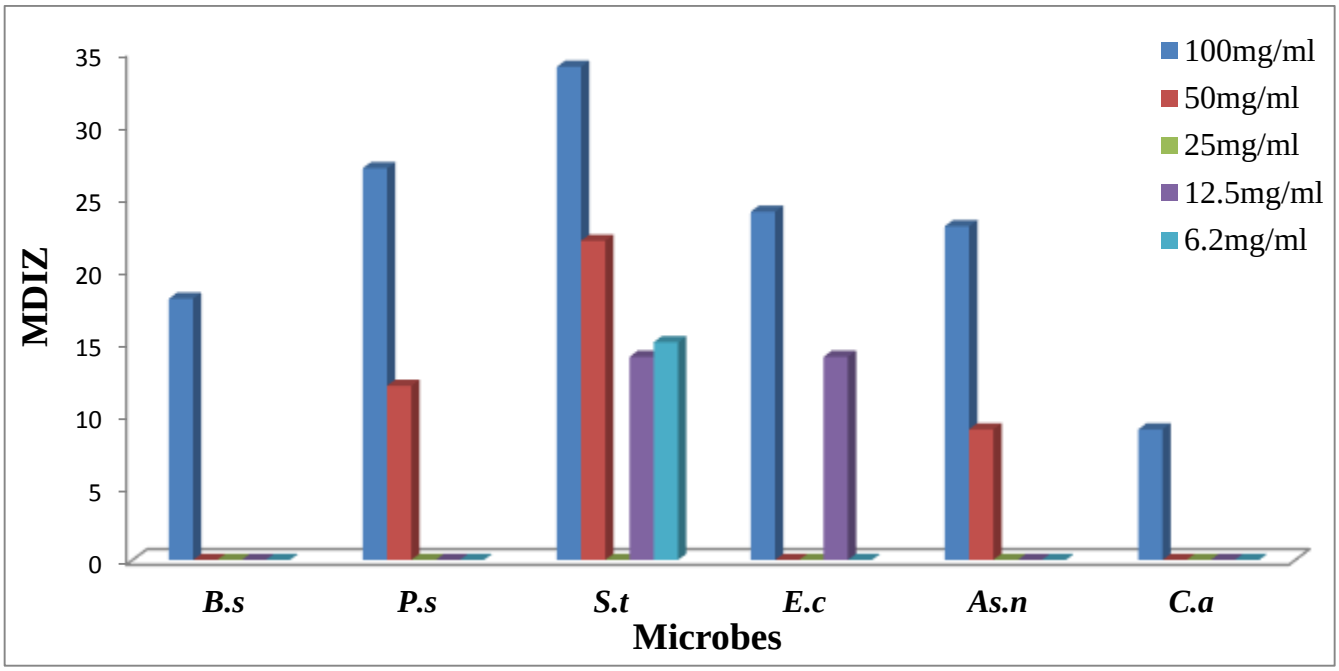


**Figure (1):** Antimicrobial activity of Clove oil against standard organisms

**Antioxidant activity:** DPPH radical scavenging assay is the most common method used in the study of the antioxidant activity of plant extracts (Table 2). Clove oil showed the highest antioxidant activity (94.7±0.003) compared to the standard npropyl gallate (91±0.01). (11) mentioned that petroleum ether extract has strong antioxidant properties. The result obtained by (4) also showed that clove oil has high activity as an antioxidant

**Table (2):** Antioxidant activity of Clove Oil

| **Sample** | **Inhibition %±SD** |
|---|---|
| Clove Oil | 94±0.003 |
| Propyl gallate (Standard) | 91±0.01 |

**Gas Chromatography–Mass spectrophotometer (GC MS) of Clove oil:** GC/MS chromatogram of clove oil showed the presence of four compounds representing 100% of the extract were identified and is given in **Table (3)** and Figure (2).

Quantitatively, the extract was characterized by abundance of Eugenol (58.38%) and Caryophyllene (34.87%). Eugenol is a volatile phenolic compound that is one of the main components of clove essential oil and is responsible for the aroma of cloves (12). Many studies have shown that eugenol has antimicrobial and antioxidant properties (13). caryophyllene is anatural sesquiterpene hydrocarbon present in hundreds of plant species. Caryophyllene has antimicrobial, anticancer, anti-inflammatory and antioxidant properties (14); (15).

**Table (3):** Phytocomponents identified of Clove Oil

| Peak | R. Time | Area% | Compounds Names | Molecular Weight | Molecular Formula |
|---|---|---|---|---|---|
| 1 | 9.929 | 58.38 | Eugenol | 164.20 | $C_{10}H_{12}O_2$ |
| 2 | 10.829 | 34.87 | Caryophyllene | 204.35 | $C_{15}H_{24}$ |
| 3 | 11.253 | 3.58 | 1,4,7,-Cycloundecatriene,1,5,9,9,-tetramethyl-, Z | 204.35 | $C_{15}H_{24}$ |
| 4 | 11.999 | 3.17 | Phenol, 2-methoxy-4-(1-propenyl)-, acetate | 206.24 | $C_{12}H_{14}O_3$ |

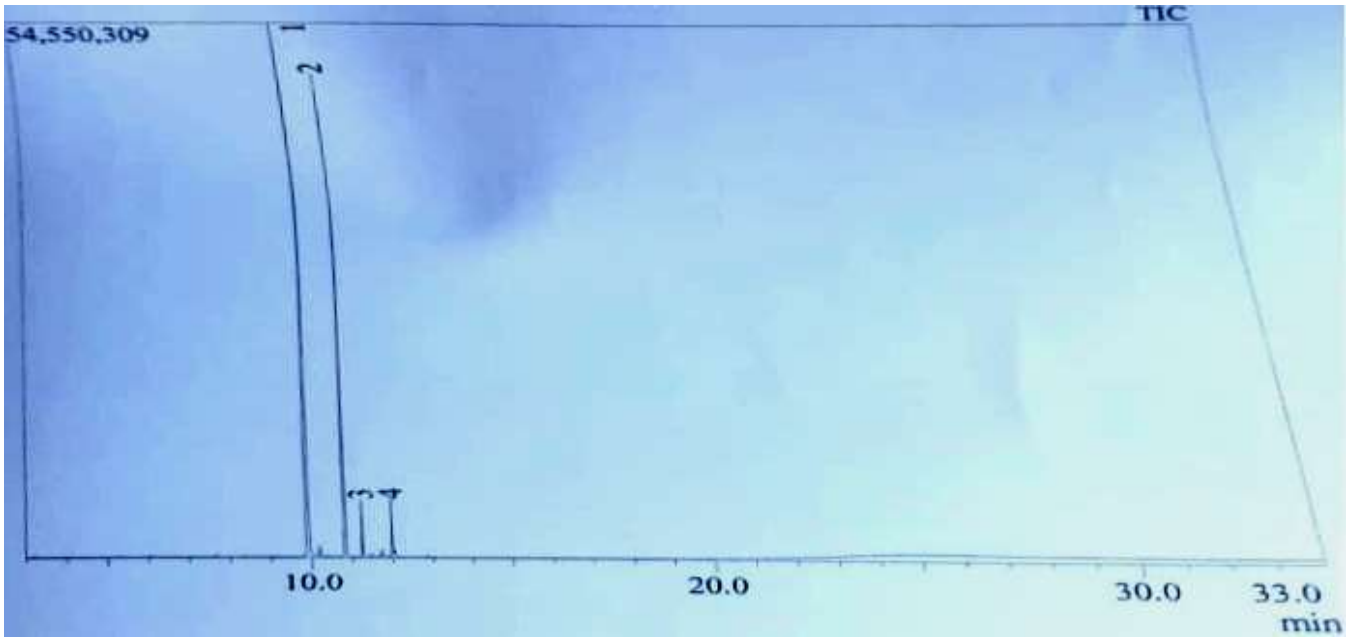


**Figure (2):** GC-MS chromatogram of Clove oil

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