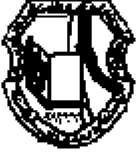

SUDAN MEDICAL LABORATORY JOURNAL

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FACULTY OF MEDICAL LABORATORY SCIENCES

SMLJ

وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْماً

وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي

فَضَّلَنَا عَلَى كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ

النمل15

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Editorial

Dear SMLJ readers,

The latest issue of SLMJ, (*Issue 1, Vol. 5*), was just the rudiment for the electronic versions. To be honest, it was not a forward or rash idea, but plenty of obstacles stood on the way to achieve the anticipated speculations, yet, we have not failed in employing the potentials in availability to publish the edition on-line as well as to ensure a hard copy to reach your hands. It was, indeed, an arduous and grueling crusade to assemble the manpower, the parts and the necessary logistics after the deep slumber the journal has experienced. Perhaps, now you can access the articles on your computer screen, smart cellular phone or whatever other medium accessible. For flawless perfection used to be our ultimate goal, even at the moment, we do not claim to be fully established.

We used to log in via the Omdurman Islamic University which is the www.oiu.edu.sd/, yet now we are attempting to maintain our sole website www.SMLJ.edu.sd/, and so your logon would be rendered pretty simpler. Insofar, despite the technical and administrative discrepancies we are thriving to negotiate the concern. So, hold the dream! Whence, let me guide you into the *contemplated* right approach to our website where you could surf comfortably through the different webpage options. On printing the above-mentioned Url on your search engine, or just type http://www.smlj.edu.sd/ on the address bar and our journal homepage would appear, showing several options to choose. You will behold a menu of titles as Home, About us, Journal, Authors guide, Submit an article or contact us that on click would lead you to the pertaining journal webpage.

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reviewers, and spare us plenty of atrocious and time consuming efforts to maintain the content of the journal well-trimmed and shipshape. Insofar, we went through different hard experiences in this respect, but we made it. So please, give us a hand!

In a nutshell, we speculate forth to see your journal flourish and bloom thanks to your support through contributions and advice.

Sincerely Yours,

Dr. Ali Suleiman Elwakeel

Executive Editor

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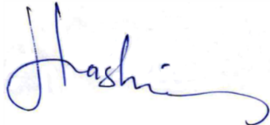
PREFATORY

Firstly I would like to thank our valued readers and authors for their enriching knowledge. Although, I have joined recently the Sudan Medical Laboratory Journal, SMLJ, team. I anticipate that SMLJ will be indexed among international journals. That will provide a solid platform for contributors and readers with useful, accessible developments in laboratory medicine and health care researches.

Best Regards,

Dr. Hashim DafaAllah Badie



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Editorial

A hasty communiqué to readers,

Unfortunately, I was bound to leave abroad just at the time the journal was about to see light. So, you might be luckier to see it in full shape before I do.

I say you see in *full shape,* for I was always present during the elaborate process of making and moulding and conforming it together into shipshape, spic-and-span!

Now, by the time I write this message, I feel happy and elevated for I have left behind a zealous, reliable, knowledgeable and acme staff to man the ship. The journal is in good hands!!

The new distinguished editorial board has been stringently selected to cover the diverse scientific specializations, and is selectively chosen from dignitaries, who hold national and international accreditation. Advisory boards are for opening horizons for all science workers to publish work in this peer-reviewed journal.

Remember that your contributions and efforts are the brick units or the pillars that build the passage to our ultimate target. So, do not forget to be supportive!

Dr. Khalid Omar Abosalif

Editor- in-Chief

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Sud Med Lab J, issue 2, vol 5 (2017): 1-10

OriginalArticle

Sudan Medical Laboratory Journal

Fasting Blood Glucose Level Higher than Post-meal in Healthy Subjects

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2. *Faculty of Pharmacy, New Delhi University, New Delhi, India.*
3. *Department of microbiology, Faculty of Medical Laboratory Sciences, Omdurman Islamic University, Omdurman, Sudan*

ABSTRACT

Aim: The aim of the study is to make attempt to find out reasons behind higher fasting blood glucose value than postprandial blood glucose value in healthy subjects. **Methods:** Fasting and postprandial blood glucose was estimated in 738 subjects. The estimation was done by hexokinase method on Cobas Integra 400 plus. **Results:** Out of these 738 subjects of which 424 were found to have absolutely normal value whereas remaining 314 subjects were having fasting values more than postprandial glucose values. Out of these 314 only 13 were known cases of diabetes mellitus. The abnormal results were studied and some reasons were formulated for such types of results. The reason for such type of results may be the previous night diet, early morning growth hormone and cortisol surge, physiological insulin resistance where body is unable to maintain basal insulin levels during fasting, delay in gastric emptying. Apart from these, increased hepatic output and decrease peripheral utilization, stress, infection, blood pressure medication may be the possible reasons in some of the subjects. **Conclusion:** Fasting blood glucose levels may be higher than the post meal blood glucose in many of the healthy subjects. Such individuals may be said to have physiological insulin resistance and may develop diabetes mellitus as long term complication.

KEY WORDS: *Fasting Blood Glucose, Postprandial blood Glucose, Insulin, Growth Hormone, hyperglycemia, cortisol.*

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INTRODUCTION

The blood sugar concentration or blood glucose level is the amount of glucose (sugar) present in the blood of a human or animal. Glucose is the primary source of energy for the body's cells, and blood lipids (in the form of fats and oils) are primarily a compact energy store. Glucose is transported from the intestines or liver to body cells via the bloodstream, and is made available for cell absorption via the hormone insulin, produced by the body primarily in the pancreas. The mean normal blood glucose level in humans is about 4 mM (4 mmol/L or 72 mg/dL, i.e. milligrams per deciliter); however, this level fluctuates throughout the day. [1, 3, 5] Glucose levels are usually lowest in the morning, before the first meal of the day (termed "the fasting level"), and rise after meals for an hour or two by a few millimolar [5, 20]. Normal human glucose blood test results should be 70 - 110 (mg/dL) before meals and less than 140 mg/dL after meals [7, 15]. A persistently high level is referred to as hyperglycemia; low levels are referred to as hypoglycemia. Diabetes mellitus is characterized by persistent hyperglycemia from any of several causes, and is the most prominent disease related to failure of blood sugar regulation [3, 6, 8, 10]. A temporarily elevated blood sugar level may also result from severe stress, such as trauma, stroke, myocardial infarction, surgery, or illness[9]. Intake of alcohol causes an initial surge in blood sugar, and later tends to cause levels to fall. Also, certain drugs can increase or decrease glucose levels [1]. In clinical and laboratory practices, many of the time a healthy normal subject will present a fasting blood glucose value higher than the post meal blood glucose value. This creates confusion since there is a common perception that in blood, postprandial (PP) glucose level should be higher than fasting, (F), glucose level [16, 20]. The repeated investigation subsequently yields somewhat similar type of result. A review of fasting blood glucose and post meal

blood glucose is been presented here in such cases and possible explanations are formulated for such type of laboratory results.

MATERIALS AND METHODS

Subjects Population

The subjects were instructed accordingly and strict 8 - 10 hour fasting sample was collected. Also they were given sufficient meal and post meal sample was collected 2 hour after the meal. Fasting and post meal blood samples from 738 subjects over a period of 3 months were collected in fluoride vacutainers. The entire subjects included in the study were from master health checkup camps. The age group for the subjects was 21-50 years. Detailed history of each subject was taken before collecting the sample.

Laboratory determinations

The samples were centrifuged and plasma was separated. Glucose estimation was done in fasting and post meal samples. The method used for determination of glucose is hexokinase method (2) and the analyzer used is Cobas Integra 400 Plus. The reference range for fasting and post meal glucose was taken as <110 mg/dl and <140 mg/dl respectively. Fasting blood glucose values between 111 mg/dl to 126 mg/dl were supposed to be pre-diabetic value or impaired fasting glucose. The IQC data for both levels of glucose was within acceptable limit. (±2SD). EQAS result was also within acceptable limit for this period.

Statistical analysis

Data was entered into Microsoft Excel data sheets and was analyzed with paired ”t" test.

RESULTS

Out of the 738 subjects in the study, 424 subjects were found to have absolutely normal values of fasting and postprandial glucose. But, remaining 314 subjects were having fasting glucose values more than post meal glucose value. Though out of these 314 subjects 233 subjects were having both fasting and post meal glucose

values within normal limit their fasting blood sugar was higher than the post meal blood glucose level. Out of these 314 subjects 13 were known cases of diabetes and their values were in diabetic range. 68 of the subjects were having fasting blood glucose values in pre-diabetic range (impaired fasting glucose, 111-126 mg/dl). 233 subjects which were having normal values of fasting and post meal glucose but were having significantly higher fasting glucose value than post meal glucose. The difference between mean fasting glucose value and post meal blood glucose value of the subject is 13.32 mg/dl, (Figure 1).

Table No. 1 Particular physiological conditions and environmental factors

• > 40–45 years of age;[18,19]
• obesity;[18,19]
• body storing fat predominantly in the abdomen, as opposed to storing it in hips and thighs.[19]
• sedentary lifestyle, lack of physical exercise[18,19])
• hypertension;(18)
• high triglyceride level (Hypertriglyceridemia); [18]
• low level of "good cholesterol";[18]
• having developed gestational diabetes during past pregnancies;[18,19]
• giving birth to a baby weighing more than 9 pounds [a bit over 4 kilograms] [18,19]

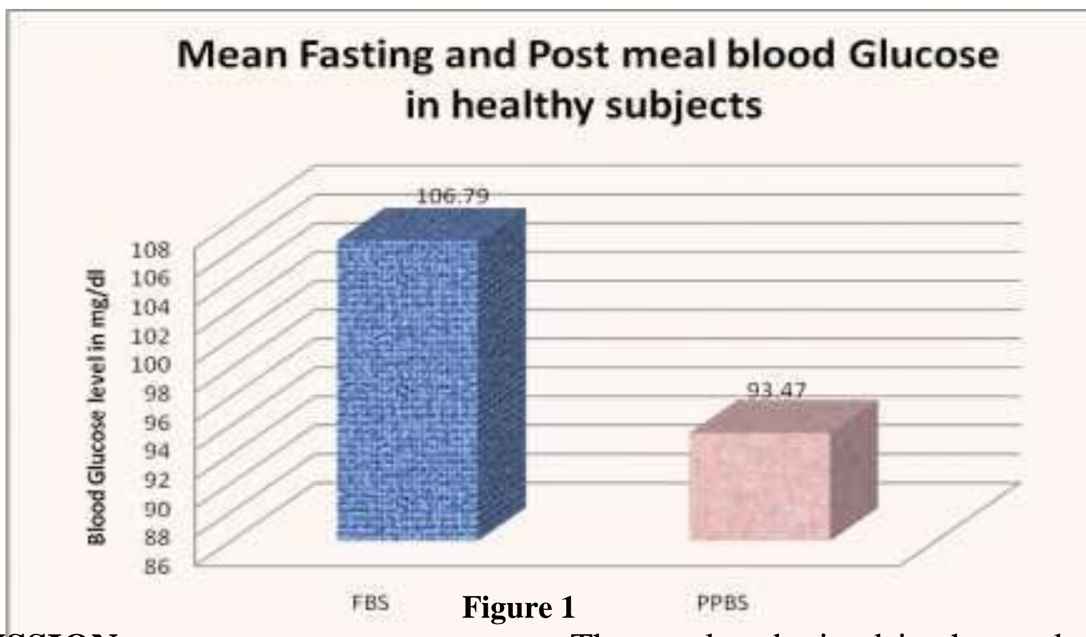

Figure 1

DISCUSSION

The results obtained in the study were

interpreted considering biological variation in morning fasting glucose level. Fasting glucose value higher than post meal glucose value in these subjects may be due to variety of reasons.

Early morning fasting blood sugar is influenced by

1. Post dinner sugar levels,
2. Insulin resistance and
3. Presence of other hormones which stimulate and increase blood sugar level. Glucagon and cortisol are two such hormones. The level of cortisol is highest at around 6-8 AM and it drops gradually to reach the lowest at midnight. Also a good meal inhibits glucagon. All these factors may be the responsible for higher fasting blood sugar and a low postprandial blood sugar.

Also the factors for postprandial hypoglycemia excluding the causes for alimentary hypoglycemia, renal glycosuria, hereditary fructose intolerance and galactosemia are:

1. high insulin sensitivity [3],
2. exaggerated response of insulin and glucagon like peptide 1 [1,2,3,4],
3. defects in counter regulation,
4. very lean and /or anxious individuals,
5. after massive weight reduction,
6. women with lower body fat and overweight etc. [5,6]

The first and most important reason for this is physiological insulin resistance. The function of insulin in body is to keep check on blood glucose levels. But in case of physiological insulin resistance, blood sugar may remain elevated even after fasting. This fact may be correlated with higher morning fasting blood glucose level. If the diet consumed is particularly rich in carbohydrate or food with low glycemic index, though considered as good carbohydrate, the blood glucose level rises slowly and may remain elevated for longer period and if the body insulin is not able to counteract these elevated glucose concentration.[7, 8] This may reflect in high morning fasting blood glucose level. The other reasons related

with such types of result may be the hormonal secretion which generally occurs early in the morning. Growth hormone surge and cortisol hormone surge occurs at 4:00 am in the morning. Also, deep sleep stimulates growth hormone secretion. [9] These are gluconeogenic hormone which stimulates the glucose synthesis inside the body as well as increasing the hepatic outflow of the glucose [10], which could not be normalized by circulating body insulin and may be referred as Dawn phenomenon in non-diabetics. This physiological insulin resistance and dawn phenomenon in long term effect may reflect into diabetes mellitus. Delay in gastric emptying may lead to increase in higher fasting morning glucose levels.

Non-formal reason behind higher fasting glucose values may be that the body prepares itself for the coming up day leading to increase in morning blood glucose level. Also, after heavy meal, insulin is released in two stages; this "first phase" of insulin secretion promotes peripheral utilization of the prandial nutrient load, suppresses hepatic glucose production, and limits postprandial glucose elevation. First-phase insulin secretion begins within 2 minutes of nutrient ingestion and continues for 10 to 15 minutes. The second phase of prandial insulin secretion follows, and is sustained until normoglycemia is restored.[11]. This hyper response of insulin may decrease the blood glucose level severely sometimes below fasting level causing fasting blood glucose higher than post meal blood glucose.

High levels of lipids in the bloodstream have the potential to result in accumulation of triglycerides and their derivatives within muscle cells, which activate proteins Kinase C-ε and C-θ, ultimately reducing the glucose uptake at any given level of insulin [12,13]. Stress, Infection [14], Blood pressure medication may be the other possible reason for higher fasting blood glucose level than post meal glucose level. Due

to the physiological insulin resistance body becomes unable to counteract elevated glucose levels. The reasons stated in literature for this physiological insulin resistance are stated in Table no.1.

Fasting blood glucose higher than post meal blood glucose in diabetic may be due to *dawn* phenomenon and *somagyi* phenomenon [15]. Dawn phenomenon states that, up in the morning, body produces a host of counter regulatory hormones like epinephrine, glucagon, growth hormone and cortisol to stimulate the liver to produce glucose.[11, 16] These hormones counter the effect of insulin and blood glucose levels rise to prepare for the start of a working day and somagyi phenomenon states that, if a subject suffers a „hypo" or low blood sugar levels at 2-3 am in the morning, his body is likely to rebound by causing the liver to release extra glucose into.

Moderate consumption of alcohol in the evening may predispose patients to hypoglycemia after breakfast the next morning. This is associated with reduced nocturnal growth hormone secretion [17,18]. Healthy subjects with fasting blood glucose higher than post meal blood glucose should be followed to determine the possibility of getting Type II Diabetes Mellitus in recent or remote future. Also, the lifestyle changes, diet modification should be suggested for such subjects as a step towards primary prevention of diabetes mellitus.

CONCLUSION

The conclusion drawn from the study is that, healthy subject may get fasting blood glucose value higher than post meal blood glucose due to variety of reasons. Such individuals may be having physiological insulin resistance. Further studies are needed to confirm the actual prevalence of this condition in general population and its long term effect if any. This study can

be helpful in clinical and laboratory practices where many of the healthy subjects get fasting blood glucose level higher than the post meal levels.

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I am deeply indebted to mother who spared no effort behind in encouraging me to the last moments.

No less I feel immensely overwhelmed by the unlimited support of my uncle, Professor Ali Elwakeel, without whose assistance this piece of work would have never seen light.

I would like to express my heartiest gratitude to my family and friends for their tolerance, patience, moral support and affection that really gratified and encouraged my person to persevere and carry on. For that I see them ten times taller

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Original Article

Sudan Medical Laboratory Journal

Effect of Campylobacter Coli Lipopolyssccharides on Phagocytosis in Vitro and in Vivo

Haitham E M Elawad[1], Khalid O Abosalif[2],

[1,2]Departmentof Microbiology, Faculty of Medical Laboratory Science, Omdurman Islamic University,

ABSTRACT

Campylobacter coli usually live in the intestinal tract of animals and cause inflammation of the intestine and diarrhea. In this study, *C. coli* were isolated from patients having diarrhea and identified based on morphological and biochemical characteristics. Lipopolysaccharide (LPS) is major outer membrane structural component of gram negative bacteria which induces a broad range of biological response. Here, LPS was isolated with hot phenol-water extraction and purified by gel filtration using Sephadex G-200. Phagocytosis is essential for fighting infections in immune responses to protect organisms from bacteria invasion hence we studied the effect of purified LPS on phagocytosis both *in vitro* and *in vivo*. The results reported in this study may prove to be valuable in analyzing the LPS effect on host immune response.

KEYWORDS: *C. coli*, LPS, Phagocytosis.

Correspondence author: *Dr. Haitham Eltigani. Email: haithamtig@outlook.com*

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INTRODUCTION

Campylobacter species are among the most commonly reported bacterial cause of acute gastroenteritis disease worldwide and especially in the industrialized countries.[1, 2]

Approximately nine million human campylobacteriosis cases are reported annually in 27 European countries.[3] Although most of the human campylobacterios is caused by *C. jejuni*, the importance of *C. coli* study is being recognized due to an increasing resistance of this species to number of antimicrobials.[4] Most Campylobacter research focuses upon *C. jejuni* but still campylobacteriosis caused by *C. coli* infection results in millions dollars of annual cost [5, 6] Livestock wastes from intensive animal production such as manure or slurry contain pathogenic microorganisms including *Campylobacter* species.[7] It has been reported that drinking water may be the source of *C. coli* infection in grandparent breeder farms.[8] This has increased concern about effect of pathogens in animal manure on human and animal health. [9] For *Campylobacter* motility is important for intestinal colonization and invasion of epithelial cells. In *Campylobacter* species flagella function as secretory organelles and recognized as a major virulence factor for this pathogen.[10, 11] It is reported earlier that the flagellar structural protein (FlaA) is immunoprotective antigen,[12, 13] flagellins are heavily glycosylated proteins and are highly immunogenic.[14, 15]

Macrophages play an important role in recognition, uptake and killing of parasites during pathogen infections.[16] It has been reported that LPS activates several signal transduction pathways in macrophages leading to various gene activation and cytokine production.[17, 18] In this study we have isolated LPS from *C. coli* and detected its effect on Phagocytosis using human macrophages: both *in vivo* and *in vitro.*

MATERIALS AND METHODS

Isolation of *C. coli*

C. coli were isolated from diarrhea samples collected from Al-Yarmok Hospital Baghdad, Iraq. The serially diluted samples were inoculated on blood agar containing 5 % SRBCs and other supplement and incubated for 48 hours at 4 ^{0}C under micro aerobic conditions. The single isolated colonies were identified by morphological characteristics, gram Staining and biochemical tests.

Extraction of LPS from *C. coli*

The LPS was extracted from *C. coli* as described previously by Preston and Penner.[19]

Briefly digestive enzymes were used to isolate LPS and hot phenol method was used for extraction process. The extracted LPS were then purified by gel filtration chromatography using Sephadex G-200 as described by manufacturer.

Blood sample preparation

Two milliliter of blood samples was collected from twenty healthy persons in heparinized (50 IU/ml) silicon test tube to prevent the absorption of cells on glass tubes (according to WHO guideline). These Blood samples were directly used to evaluate phagocytosis.

In vitro phagocytosis assay

The *in vitro* phagocytosis was determined by procedure described by Sun.[20] Briefly, monocytes were added into the wells and after 6 h of incubation the floating cells were removed and monocytes were washed twice with PBS. Under the phase microscope, about 300 macrophages per sample including LPS engulfed were counted and the percentage ratio was calculated as the relative number of cells that engulfed LPS per total cells.

In vivo phagocytosis assay

The *in vivo* phagocytosis was estimated as described by Tanaka.[21] Briefly LPS was diluted 10-fold with PBS and injected into the mouse peritoneal cavity by 10 µl/g body weight. After overnight starvation the mice were sacrificed and macrophages were recovered from Peritoneal, Liver and Spleen. For each sample 400 cells were counted,

and the percentage ratio was calculated as the relative number of cells that engulfed LPS per total cells.

RESULTS AND DISCUSSION

C. coli were isolated from patients having diarrhea. The morphological characteristics such as colony morphology and motility were observed and gram staining with biochemical tests such as oxidase and hiturate were performed for identification of *C. coli*. The observed results were summarized in Table 1.

Table 1: Biochemical test and characterization of *C. coli*

Result	Test
Appear small convert grey white colony	Colony on blood agar
Negative (Gram negative bacteria)	Gram staining
Positive	Oxidase test
Hydrolysis negative	Hiturate test
Motile	Motility
Spiral shape	Shape

LPS are heat stable endotoxins and long been known to induce variety of pathophysiologic responses, including fever, coagulant activity, septic shock, and death. [22] Here, we have used hot phenol method to extract LPS from the isolated *C. coli.* To remove the impurities such as protein and RNA, extracted samples were further purified by Size exclusion chromatography using Sephadex G 200. After purification absorbance was measured at 490 nm for collected fractions (Figure 1). The fraction showing maximum absorbance was used for further phagocytosis study.

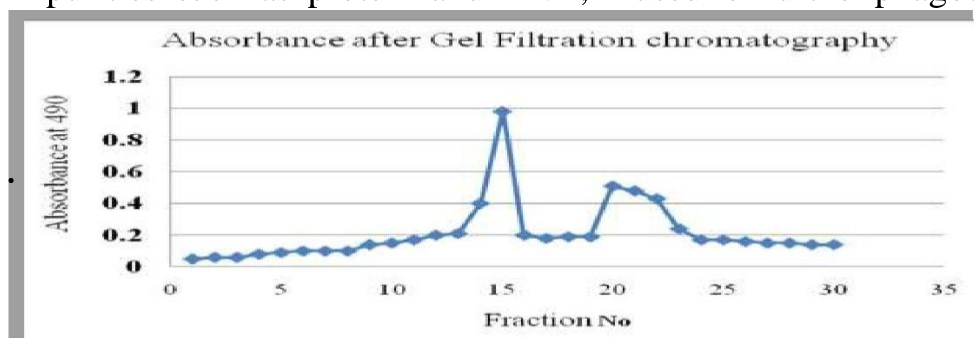

Figure 1: Purification of LPS by gel filtration (Sephadex G-200)

Phagocytosis is controlled by a complex signals located on the target cell surface and their respective receptors which determine phagocytosis process. [23] Many approaches have been designed to study these processes using macrophages as prototypic inflammatory cells, since these cells are potently activated by LPS. [24] In present investigation we studied phagocytosis both *in vivo* and *in vitro.* The percentage ratio was determined for phagocytosed LPS and presented in figure 2 for in *vitro* study and in figure 3 for *in vivo* study.

Figure 1: Purification of LPS by gel filtration (Sephadex G-200)

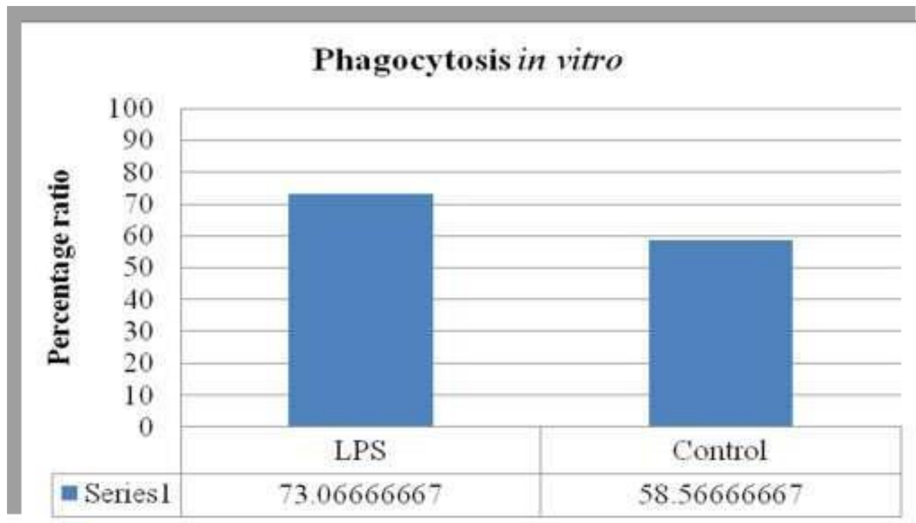

Figure 2: Phagocytosis *in vitro*

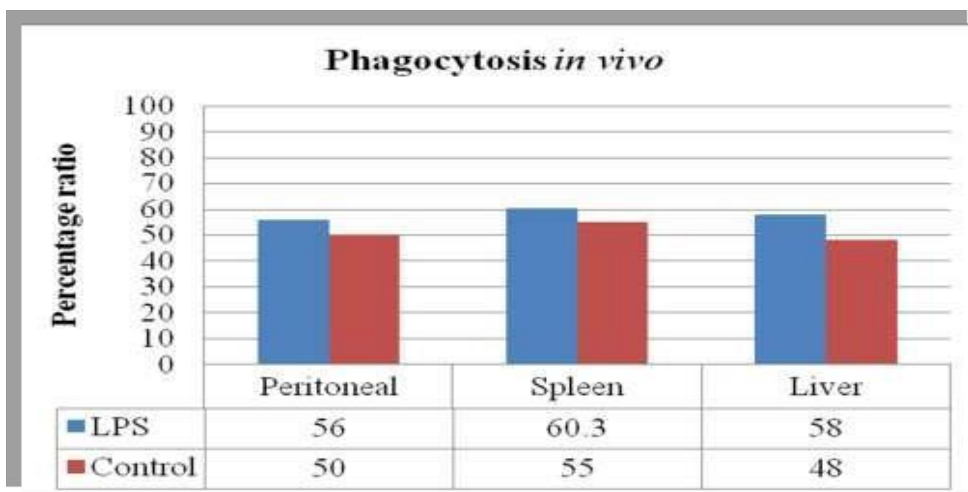

Figure 3: Phagocytosis *in vivo*

In presence of LPS increased phagocytosis was observed in both *in vitro* and *in vivo* studies as compared to control which indicates that LPS exposure required for phagocytosis progress. The increased phagocytosis reported in this study may prove to be valuable in analyzing the alterations induced by LPS on macrophage involved in the host response.

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Effects of Temperature and Delay of Serum Separation in Potassium and Sodium Levels

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ABSTRACT

Background**:** Potassium and sodium estimation are considered two of the important tests to evaluate kidney functions. Clot contact time and temperature have variable effects on the laboratory investigations, leading to misdiagnosis and inappropriate treatment decision. ***Objective*****:** The current study aimed to estimate the maximum acceptable time delay between sample collection and serum separation as well as to analyze the effect of different temperatures on potassium and sodium stability. ***Materials and methods:*** A total of 50 healthy individuals (n=50) of both genders and ages ranging from 17 to 30 years attending to Omdurman Islamic university were included in this cross-sectional study during the period from January to March, 2016. Blood specimens were collected from each participants, the centrifugation of whole blood stored at room temperature was delayed for 2 hours and 4 hours, serum was analyzed immediately, and after stored at 4°C for 24hrs, 48hrs and 96hrs by using ion-selective electrodes, and compared the values with those of matched samples that had been centrifuged within 0.5 hours after whole blood collection. ***Results****:* Storage temperature causes potassium to significantly increase after the entire 24hrs, 48hrs and 96hrs, while sodium was found to have increased significantly at 48hrs and 96hrs. On the other hand Delaying in separation insignificantly affect either the potassium or sodium for a period of up to 4 hrs. ***Conclusion:*** The maximum clot contact time intervals which have no effect on the stability of both sodium and potassium are found to be 4hours, and the stability of both are found to be sensitive to temperature and duration of storage of the samples.

Keywords: Temperature. Delay in separation, Potassium, Sodium.

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Introduction

Potassium (K^+) and sodium (Na^+) are two of the most commonly performed tests in the clinical chemistry laboratory. Potassium plays key roles in in transmission of nerve impulses, contraction of muscles (Hellgren *et al*., 2006), regulation of acid base balance, maintenance of osmotic pressure and volume of intracellular fluid and form a substrate for Na^+/K^+ ATPase (Hall *et al*., 2006). Sodium, on the other hand, is the major contributor to the extracellular fluid. It maintains the osmotic pressure and form a substrate for Na^+/K^+ ATPase (Pohl *et al*., 2013). High serum K^+ concentration over the higher limit of the reference range, called hyperkalemia (Hubbard, 2010), is caused by renal insufficiency, diabetes mellitus, or metabolic acidosis (Desai, 2008), but when serum K^+ levels fall below the lower limit of normal value it called hypokalemia. Yet an increased serum Na^+ concentration called hypernatremia, is caused by excess loss of water relative to Na^+ loss, decreased water intake, or increased Na^+ intake or retention (Kumar and Tomas, 1998), while serum level less than lower limit of normal value is called hyponatremia. There are many pre-analytical factors that can potentially cause, falsely, elevated serum potassium or decrease in serum sodium results, among which (i) Delay in separation of blood sample, can cause pseudo hyperkalemia, the phenomenon of artificial or factitious elevation of serum K^+ results. It is still among the most common and recurrent quality issues faced by clinical laboratories and one of the most frequent sources of physician complaints. There are many potential causes of these. Most of these are related to sample quality issues and occur in the pre-analytical phase of the testing process, i.e. before the sample is actually tested (Carrisoza-Gaytan, 2010). It is a laboratory artifact rather than biological abnormality, typically caused by hemolysis during venipuncture, excessive tourniquet time, or by delay in the processing of the blood specimen. Meanwhile, an elevation of serum potassium does not reflect the level of plasma potassium in

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vivo but the high potassium in serum developed in vitro by leakage from platelets during the clotting process (Pronson, 1966). In addition to that pseudo hyperkalemia, caused by release of K^+ from activated platelets due to clotting or leakage of K^+ from cells as a result of the inhibition of Na^+/K^+ ATPase will disrupt the trans-cellular potassium and sodium gradient. Delay in separation for a period sufficient enough can cause a hemolysis in the blood sample which will lead to false results of K^+ as well as Na^+ levels (Asirvatham, 2013). If the serum sits on the clot too long, there can be changes in test results, including the potassium. The minimum time to form a good clot is usually 20 to 30 minutes. The maximum recommended time between collection and separation of clot and serum is two hours, (CLSI, 2004). Clinically significant increases in potassium occur after three hours at room temperature, (Zhang,1998).(ii) Refrigerated whole blood, in which Potassium levels will be further falsely elevated in refrigerated specimens due to inhibition of the sodium-potassium-ATPase pump causes cells to leak potassium into the plasma or serum that will be used for testing. Cells contain 25 times the level of potassium as serum/plasma. Refrigeration can lead to falsely elevated potassium levels in serum/plasma in a relatively short time period, (Arbique, 2008). Beside pseudo hyperkalemia, pseudo hyponatremia, the phenomenon of artificial or factitious reduction of serum Na^+ results, may be seen with in vitro hemolysis, is considered the most common cause for a false decrease. When the RBCs lyse, Na^+, K^+, and water are released. Na^+ concentration is lower in RBCs, resulting in a false decrease. Care must be exercised to control all of these variables in order to obtain accurate and clinically meaningful test results and precisely evaluate the correct extracellular K^+ and Na^+ levels, to avoid misdiagnosis and, eventually, mistreatment. This review aims to help all health care interested individuals to be more cautious about the test performance.

Materials and Methods

cross-sectional study conducted on healthy individuals, randomly selected from Omdurman Islamic university in Khartoum State in the period from January to March, 2016. Venous blood samples were collected from healthy individuals (n=50) of both genders and ages ranging from 17 to 30 olds.

Data collection

A structured questionnaire was designed to obtain demographic data. Clinical and laboratory investigation data from blood analysis for K^+ and Na^+ were also recorded in the same form. Venous blood samples (7.5 ml) were collected from all participants in 3 serum separator tubes, each containing 2.5 ml of blood. The centrifugation was for 10 min at 3000×g, of the whole blood stored at room temperature and was delayed for 2 hours and 4 hours. The serum was analyzed immediately, and then stored at 4°C for 24hrs, 48hrs and 96hrs. Then using ion-selective electrodes, the values obtained were compared with those of matched samples that had been previously centrifuged within 0.5 hours upon the whole blood collection.

Estimation of Potassium and sodium levels

Potassium and sodium levels were measured by using ions-selective electrodes. The Kits were obtained from the Medica Corporation, (USA). There were four different electrodes in the electrolyte analyzer (sodium, potassium, chloride and a reference electrode). Each electrode had an ion selective membrane that undergoes a specific reaction with the corresponding ions contained in the sample being analyzed. The membrane was an ion exchanger, reacting to the electrical charge of the ion causing a change in the membrane potential and reading the voltage which is built up in the film between the sample and the membrane. A galvanic measuring chain within the electrode determined the difference between the two potential values on either side of the membrane. The galvanic chain was closed through the sample on one side by the reference electrode, reference electrolyte and the (open terminal). The membrane, inner

electrolyte and inner electrode close the other side. A difference in ion concentration between the inner electrolyte and the sample causes an electro chemical potential to form across the membrane of the active electrode. The potential is conducted by a highly conductive inner electrode to an amplifier. The reference electrode is connected to ground as well as to the amplifier. The ion concentration in the sample is then determined by using a calibration curve determined by measured points of standard solution with precisely known ion concentration.

Statistical analysis

Statistical analyses were performed with Student's t-test. Correlation between different parameters was performed using Pearson's. For all statistical comparisons a P-value of ≤ 0.05 was considered statistically significant. All statistical procedures were performed using SPSS software, version 16.

Ethical consideration: The study was approved by Omdurman Islamic university, and the signed informed consent was obtained from each participant.

RESULTS

Effect of delayed separation of blood (0.5 h, 2h and4h) on sodium and potassium levels

Table (1) shows that the difference between potassium mean separated at 0.5 h (4.05 ± 0.40) and at 2h (4.02 ± 0.43) was considered insignificant (p>0.05), as well as the difference between sodium mean separated at 0.5 h (137.4±1.69) and 2h (137.4±1.87) (p>0.05). While (table 2) shows that the difference between potassium mean separated at 0.5 h (4.05± 0.40) and 4h (4.04 ± 0.48) as considered insignificant (p >0.05), as well as the difference between sodium mean separated at 0.5 h (137.4±1.69) and 4hrs (137.3±1.81) (p>0.05).

Table (1):Comparison between 0.5 h and after 2 h specimens for sodium and potassium

Variables	0.5 h (mean ±SD)	2 h (mean ±SD	*P*. value
Potassium (mmol/l)	4.05±0.40	4.02±0.43	0.482^{ns}
Sodium (mmol/l)	137.4±1.69	137.4±1.87	0.99^{ns}

ns : no significant difference

Table (2): Comparison between 0.5 h and after 4 h specimens for sodium and potassium

Variables	0.5h(mean ±SD)	4h (mean ±SD)	*P*. value
Potassium (mmol/l)	4.05 ±0.40	4.04 ±0.48	0.83^{ns}
Sodium (mmol/l)	137.4±1.69	137.3±1.81	0.524^{ns}

ns : no significant difference

Effect of storage of serum at 4◦C for (24h, 48h and 96h) on sodium and potassium levels

Table (3) shows that the difference between potassium level separated at 0.5 h (4.05±0.40) and storage at 4◦C for 24h (4.09±0.39) was significant (*P*<0.01), whereas the difference between sodium level separated at 0.5 h (137.4±1.69) and storage at 4◦C for 24h (137.5±2.06) was found to be insignificant (*P*>0.05). While (table 4) shows that the difference between potassium level separated at 0.5 h (4.05±0.40) and storage at 4◦C for 48h (4.15±0.45) was found to be highly significant (*P*< 0.01), as well as the difference between sodium level separated at 0.5 h (137.4±1.69) and storage at 4◦C for 48h (138.9±2.5) (*P*< 0.01).While (table 5) shows that the difference between potassium level separated at 0.5 h (4.05±0.40) and storage at 4◦C for 96h (4.22±0.47) was found to be significant (*P*< 0.01), as well as the difference between sodium level separated at 0.5 h (137.4±1.69) and storage at 4◦C for 96h (140.1±3.69) (*P*<0.01).

Table (3): Comparison between 0.5 h as control and after 24h specimens for sodium and potassium

Variables	0.5h(mean ±SD)	24h (mean ±SD)	*P.* value
Potassium (mmol/l)	4.05±0.40	4.09±0.39	0.008**
Sodium (mmol/l)	137.4±1.69	137.5±2.06	0.679ns

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

Table (4): Comparison between 0.5 h as control and after 48 h specimens for sodium and potassium

Variables	0.5h(mean ±SD)	48h (mean ±SD)	*P.* value
Potassium (mmol/l)	4.05±0.40	4.15±0.45	0.007**
Sodium (mmol/l)	137.4±1.69	138.9±2.5	0.000**

** : significance at 0.01 level of probability

Table (5): Comparison between 0.5 h as control and after 96 h specimens for sodium and potassium

Variables	0.5h(mean ±SD)	96h (mean ±SD)	*P.* value
Potassium (mmol/l)	4.05±0.40	4.22±0.47	0.000**
Sodium (mmol/l)	137.4±1.69	140.1±3.69	0.000**

** : significance at 0.01 level of probability

DISCUSSION

Sodium and potassium estimation are considered to be two of the important tests to evaluate kidneys function .This study was primarily designed to estimate sodium and potassium levels in healthy Sudanese individuals in an attempt to spot the effects of delaying in separation time and storage temperature on sodium and potassium levels. In the present study stability of sodium as well as potassium is insignificantly altered after few hours if there is a delay in centrifugation.

Potassium level is affected at 2h or more at room temperature. This result is in parallel with a studies done by Seamark *et al*., and Oddoza *et al*., but is contrary to a study done by Donnelly *et al*., who found that the potassium remain stable for 24 h at room temperature. While the sodium level remains stable at 2h at room temperature. This finding was in agreement with the finding reported by Donnelly *et al*. On the other hand sodium level was affected after 4h. This finding was in agreement with the finding reported by (Mohammed, 2009). Another study done by Bobby *et al*., found that the sodium and potassium remain stable up to 56 h at room temperature. Similarly Heins *et al*., found that the sodium and potassium remain stable after 24h at room temperature. But Tanner *et al*., found that the potassium stability was altered within 24 h, yet sodium remained stable up to 24h at room temperature. Possible explanation is that, improper temperature maintenance in laboratory leading to significant evaporation from sample cups as well as the climatic conditions hold responsible for this evaporation as well as the falsely high serum electrolyte values. In the estimation of potassium levels, we noticed that, it decreased after 2h followed by an increase at 4h. The change in the potassium levels was, perhaps, due to effect of glycolysis which moves the potassium into the cells or diffuse out of the cells (Goodman,1957) and (Datta *et al*.,2014). An initial, glycolysis is very fast,thus the serum potassium concentration decreased. However as time went on,glucose in the serum wsas depleted, and potassium passively diffused from cells and increase the levels after long serum clot contact. This finding is in agreement with the result reported by (Zhang *et al*.,1998), (Datta *et al*.,2014) and (Kalasker and Sudhamadhuri, 2015). The sodium level, an initial no change at 2h duration was noticed followed by a decrease in the levels at 4hours duration. The decrease in sodium levels at 4h might be due to the effect of passive diffusion into the cells. The

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current study also reported that, temperature and duration of storage of potassium and sodium at 4◦C for one day or more, affect the potassium and sodium levels. These results agreed with (Stankoric and Smith, 2004), but were contrary to Donnelly *et al*., who found that sodium and potassium remained stable for 24h at 4°C.

CONCLUSION

The stability of both potassium and sodium was found to be sensitive to sample separation delayed up to 4 h. On the other hand the stability of potassium and sodium was found to be statistically affected to prolonged serum storage at 4◦C for 24h or more. Thus it is essential that the blood samples obtained from the patients be processed timely and the analysis carried out immediately.

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Lipid Profile among Sudanese Narghile (Shisha) and Cigarette Smokers

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ABSTRACT

Introduction: Smoking is now increasing rapidly throughout of the republic of Sudan especially in the States and is one of the biggest threats to current and future health, **Aim**: to study the effect of cigarette and shisha (water pipe) smoking on lipid profiles among the East Region people of Sudan. **Methodology:** this cross–sectional study done on 500 participants, of whom 250 were smokers of (cigarettes and shisha) whereas 250 were non-smokers. A questionnaire was well-designed to provide date, blood samples and was collected and kept in safely until the analysis at the clinical chemistry laboratory. **Results:** The mean±SD of total cholesterol , triglycerides and LDL were increased and P. value less than 0.05 considered significant ($P < 0.05^*$) while HDL not effected , and positive correlation exist between LDL / TC and LDL / TG ($r = 0.960^{**}$, $P < 0.01$) ($r = 0.709^{**}$, $P < 0.01$) and negative correlation exist between LDL / HDL , HDL / TC and HDL / TG ($r = -.814^{**}$, $P < 0.01$) ($r = -.831^{**}$,$P < 0.01$) ($R=-.618^{**}P < 0.01$) were consumed numbers of cigarettes and shisha per day , also the results explain insignificant correlations between lipid profile and duration of cigarette and shisha smoking , and exist influence in concentrations of lipid profiles levels with numbers of cigarettes and shisha were consumed. **Conclusion** : This study revealed that there are significant differences in the means of parameters of TC,TG,LDL, and HDL between case and control groups, and insignificant correlations between lipid profile and duration of cigarette and shisha smoking.

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Introduction

Oxford university estimates that there are about a third of the male adult global population, who smokes (1) ; about 15 billion cigarettes are sold daily or 10 million every minute. Smoking related-disease kill one in 10 adults globally or cause 4 million deaths. If current trends continue , by 2030 smoking will kill one in each six people, every eight seconds someone dies from tobacco use (2) . Smoking is on the rise in the developing world but falling in developed nations .`

A study in Khartoum State estimates that the prevalence of smoking among school adolescents was 13.6% (3). Among children and adolescents (4 – 17 years) prevalence of tobacco use was quite low (2% , range 1-2%) but there was an abrupt increase up to 25% in late adolescence . Among the adult population aged 18 years and older, the prevalence of cigarette smoking 12% among males was significantly higher than among females in urban areas (4).

Nicotine increases the amount total cholesterol TC, low density lipoprotein LDL and triglyceride TG) circulating in the blood vessels and the amount of good fat (high density lipoprotein HDL) availability . These observations are clinically important because of the wide spread use of smokeless tobacco products such as nicotine containing chewing gum and nicotine lozenge in smoking cessation therapy . In fact, 1-5 cigarettes per day present a significant risk for a heart attack. Nicotine and other toxic substances from tobacco smoke are absorbed through the lungs in to the blood stream and circulated throughout the body

(5). Some studies done in Iraq and Saudi Arabia on the effect of smoking on lipid profile conclude that total cholesterol, low density lipoprotein, very low density lipoprotein and triglyceride were significantly higher in smokers as compared with non-smokers (control group), while high density lipoprotein in smoking group was significantly lower than in non-smokers the number of cigarette per day (5,6). Shisha is known by a number of different names, narghile, goza, hookah and hubble-bubble. Its origin is often traced to India, although there are theories that it was first used in South Africa, Persia , Ethiopia or other countries (7). It has been claimed that > 100 million people worldwide smoke water pipes daily (7,8). It is a common practice in the Arabian Peninsula, Turkey, India, Pakistan, Bangladesh and some regions of China. In some areas water pipe smoking is more prevalent than cigarette smoking. Among Arab women in many countries , there is less of a stigma associated with shisha than with cigarette smoking and therefore less of a gender differential (9). The composition of the tobacco used in water pipe smoking (WPS) is variable and not standardized. The nicotine content of water pipe tobacco has been reported to be 2% to 4% in comparison with 1% to 3% for cigarette (10). Study of carbon monoxide in water pipe and cigarette smoke found that carbon monoxide concentrations of 0.34% to 1.4% for water pipe smoke and 41% for cigarette smoke . The carbon monoxide concentration in water pipe smoke was significantly greater for smoker water pipe size and for commercial as opposed to domestic charcoal (11). Water pipe smokers are at risk for the same

kinds of diseases caused by cigarette smoking , these include oral cancer, lung cancer, stomach cancer, cancer of the esophagus, reduced lung function and decreased fertility (12,13). Relative to a single cigarette, completed in about 5 minutes (14), a single water pipe use episode typically lasts for about an hour (15).

The aim of this study is to assess the effect of smoking on lipids profile (cholesterol , triglyceride , HDL ,LDL), among cigarette and shisha smokers in the east region of Sudan

Materials and Method

study Area:: This cross–sectional study was conducted in East of Sudan, in the main cities (Portsudan, Sauakin, Kasslla, New Halfa and Gadarif) during the period from December 2015 to November 2016

Study population: Five hundred and fifty subjects had been enrolled as target study population, 250 smokers (cigarettes 150 , shisha 100) as the case study group, and another 250 subjects of non-smokers as case control group. The ages were (20-60) years in both groups. Cigarette and shisha smokers (case study) were divided into 5 subgroups depending upon intensity of cigarette smoking, and the number of sessions for smoking shisha in a day. Group I - Mild smokers (n =76), smoking 5- 14 cigarettes / day) Group II - Moderate smokers (n=53), smoking 15-20 cigarettes / day, and Group III Heavy smokers (n= 21), smoking >20cigarettes/day), and shisha smoking (Number of sessions for smoking shisha per day) group IVa (n=57) 3 - 5 sessions per day and group Vb (n=43) more than five sessions per day.

Data collection: Data were collected by carefully designed questionnaire**.** Venous blood

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sample (`5mL) was taken from each participant using disposable syringe .The blood samples were allowed to clot at room temperature and then serum was obtained after centrifugation at 3000 rpm .The clear serum was withdrawn by means of pipettes and transferred to plan container and stored at -70°C. Serum triglyceride was taken in fasting (10-12 hours.)

Serum level of total cholesterol , triglyceride , low density lipoprotein , and high density lipoprotein were measured by using commercial kits (BioSystem S.A) from Costa Brava30 ,0803 Barcelona (Spain Quality system certified according to EN ISO 13485 and EN ISO 9001 standards.)

Statistical analyses: the statistical analyses were done using Microsoft Excel Analyse-it software, SPSS Statistics19. The bias comparisons were carried out and shown as mean and 95% confidence intervals (95% CI). Serum cholesterol, triglycerides, LDL,HDl expressed as mean, standard deviation. Significance for the differences between groups was analyzed using the Wilcoxon matched-pairs signed rank test and differences between prevalences by chi square test. We considered a P value less than 0.05 as statistically significant..

Ethical Consideration : The ethical authorization obtained from Ethical committee of Omdurman Islamic university for clinical and health research and verbal consent from the participates.

Results:

Table 1 shows that two hundred and fifty smokers were distributed as cigarette smokers(150), 60% and shisha smokers (100), 40%

Table1: distribution of Smokers (Cigarette, shisha)

Habit of Smoking	Frequency	Percent
Cigarette	150	60
Shisha	100	40
Total	250	100

Table (2) shows that distribution of case group, depending on basis of ages and noticed that large numbers of Smokers were in the range of (41 - 50) years old; a total of 103 smokers .

The same table shows that , two hundred and fifty were non-smokers as control and its distribution was on the basis of age. High frequency between (41 – 50) years old , and low frequency of control between (51 – 60) years old.

Table 2: Distribution of age among smokers groups and non-smokers.

Ages	Freq (%) **smokers group**		Freq (%) **Non-smokers group**	
20 year	1	0.4%	00	00%
21 - 30 Year	50	20%	59	23.6 %
31 - 40 Year	48	19.2%	79	31.6 %
41 - 50 Year	103	41.2%	86	34.4%
51 - 60 year	48	19.2%	26	10.4%
Total	250	100%	250	100%

Table :3 Comparisons of lipid Profile parameters among smokers and non-smoker groups.

Variable		Mean ± SD	P. value
Cholesterol LDL	Smokers (N=250)	191.4 ± 34.4	P<0.001*
	Non-smokers (N=250)	59.2 ± 25.4	
Cholesterol HDL	Smokers (N=250)	23.8 ± 6.03	P<0.001*
	Non-smokers (N=250)	68.9 ± 17.6	
Triglyceride	Smoker (N=250)	174.8 ± 23.7	P<0.001*

	Non-smokers (N=250)	109.8 ± 21.9	
Total Cholesterol	Smokers (N=250)	249.4± 31.6	P<0.001*
	Non-smokers (N=250)	149.7 ± 17.7	

The present study shows that there were significant difference between case, and control group when compared means of level serum cholesterol serum cholesterol mean±SD (249.4±31.6, 149.7±17.7 mg/100) p value <0.001, serum triglycerides mean±SD (174.8 ± 23.7, 109.8 ± 21.9 mg/100) p value <0.001, serum HDL mean±SD(23.8 ± 6.03, 68.9 ± 17.6 mg/100) p value <0.001,serum LDL mean±SD (191.4 ± 34.4, 59.2 ± 25.4 mg/100) p value <0.001

Table 4: comparison of lipid profile parameters among smokers According to numbers of cigarettes/day and number of sessions /day for smoking shisha.

Parameters	**Cigarettes smoker**			**Shisha smokers**	
	Group–I mild (n=76)	Group-ii moderate (n=53)	Group-iii Heavy (n=21)	Group-iva 3-5 sessions of smoking/day (n=57)	Group-ivb 6≥ sessions of smoking/day (n=43)
LDL-Cholesterol Mean ± SD(mg%) P value	166.9 ± 35.9	189.2 ± 20.5	207.6± 15.8 P<0.001†	200.8± 26.8	229.4 ± 15.2 P<0.001*
HDL-Cholesterol Mean ± SD(mg%) p.value	27.3 ± 6.3	24.8 ± 3.8	20.9 ±3.5 P<0.001†	22.2±5.2	20.9 ±3.5 P<0.001*
Triglyceride Mean ± SD(mg%) P value	157.8+22.4	171.3±8.1	180.3±8.06 P<0.001†	181±17.4	174±23 P<0.001*
Total Cholesterol Mean ±SD (mg%) P value	225.6 ± 30,7	246.5± 15.7	246± 13.6 P<0.001†	259.8 ±`25	287.2 ± 15.6 P<0.001*

* t- test was used to calculate p.value when compared tow means

† One way Anova was used To calculate p. value. Mor than tow means

P. value less than 0.05 considered significant.

The results of the present study as.is shown by table 4 gives the

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levels of total cholesterol, TG, HDL-C, and LDL-, in mild smokers (Group-I), in moderate smokers (Group –II) , in heavy smokers (Group –III) and shisha smoking (Number of sessions for smoking shisha per day) group Iva (n=57) 3-5 sessions per day, and group Vb (n=43), (more than five sessions per day). In the comparison of the parameters in different test groups (Group- I, II. III and Iva, Vb), there were statistically significant (P< 0.001).The mean and standard deviation of total cholesterol, (TC) , triglyceride (TG), and low density lipoprotein (LDL) were increased with the number of cigarettes and shisha consumed per day , while HDL was not affected . The rising values of concentrations were found higher in shisha smokers than in cigarette smokers.

Discussion:

Smoking is an escalating health problem especially in the developing countries such as Sudan. The prevalence of smoking in India varies from about 15% to over 50% among men. However, smoking is less common among women with a prevalence of 4% (or) less. Cigarette smoking has been found to alter the lipoproteins levels. Plasma lipoproteins abnormalities are major risk factors for the occurrence of atherosclerotic vascular disease. It also increases oxidative modification of LDL, circulating products of lipid peroxidation and autoantibody titres to oxidized LDL. The increase is significant among smokers .

No doubt, lipids have important roles in human life; providing energy storage, preventing heat lost, helping the structural components in cell membrane, but their excessive concentrations are associated with many metabolic

disorders.

The results in this study significant showed differences in the total cholesterol level among cigarette and shisha smokers ($P<0.05$), compared to non-smokers. This indicates the cigarette and shisha smokers have increased serum concentration of cholesterol. This result agrees with Adedeji and Etukudo, who reported that a higher concentration of serum cholesterol among smokers group n compared with the non-smokers (16). On the other hand, it disagrees with Waheed and Alharbi, on the influence of cigarette smoking upon lipid profile in male students. There was no significant difference between means of serum cholesterol, in smokers and non-smokers group. (17). The increase of cholesterol in smokers due to the activity of hepatic HMG–CoAreductase (18), the main rate limiting enzyme in cholesterol synthesis. The duration of smoking has not always demonstrated positive significance between serum concentration of Cholesterol and the numbers of cigarettes and shisha that were consumed per day. Example ; smoking for a long period, of light content cigarettes (less than 14 / day) differs in comparison with smoking for a short period of heavy content cigarettes (more than 24 / day). Also the influence of smoking cholesterol becomes obvious when the duration of smoking increases. From this current results of lipid profile the level of serum of triglyceride in smokers increased compared with triglyceride in non-smokers, and that was statistically significant, ($P = 0.05$), the reason, why triglyceride increased as reported by Khurana M, Latha MS and Goh EH *et al* that

found the induction of lipogenic enzyme by nicotine (19-20) and also nicotine stimulated the release of adrenaline from the adrenal cortex leading to increase serum concentration of free fatty acids which further stimulated hepatic synthesis and the secretion of cholesterol (21) as well as hepatic secretion of very low density lipoprotein (VLDL) and hence increased triglyceride (22). ***** HDL of smokers was not affected or low significance when compared with non-smokers. This result in line with the report of Robbert KM , Dary KG, *et al* (23) that HDL concentrations varies directly with the activity of hepatic lipase as well as LCAT. The results findings of high level LDL–C in smokers when compared with non-smokers revealed an increase of LDL due to the down regulation of LDL receptors and failure of receptor mediating endo-cytosis by metabolites of cigarette smoke. The numbers of shisha smokers were less than the cigarette smokers; (in this research shisha smokers are one hundred, but cigarette smokers were one hundred and fifty). That was because the health authorities enforced the shisha cafes to close, and that smokers became conscious of the risk factors of smoking and the hazard of shisha smoking. Many smokers denied to donate the samples, for they felt afraid of the expected results, because shisha smoking transmitted many diseases through multi users. Notice the rising values of lipid profiles concentrations in smokers per day, which mean that smokers are at much greater risk of developing atherosclerotic and different heart diseases than non-smokers.

Conclusion. The study found that there are significant differences in

means of parameters of TC,TG,LDL, and HDL between cigarette and shisha smoking, and non-smokers groups, and insignificant correlations between lipid profile and time duration of cigarette and shisha smoking,

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and apo11 fractional catabolic rate in patient with LDL cholesterol level with or without hyper triglyceridaemia. Journal of clini.1 vest, 199 ; 87 (2):536 -544.

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OriginalArticle

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Frequency of Methicillin and Vancomycin Resistant *Staphylococcus aureus* among the Patients with Chest Infections

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Abstract

The aim of this study was to evaluate methicillin and vancomycin resistant *Staphylococcus aureus* among the patients with chest infections, in Al-Shaab hospital during the period from May to June 2011. In this study 147 sputum samples were collected from patients, from all ages, suffering from chest infection, who were previously investigated by physician. All the samples were subjected to bacteriological examination in order to isolate *Staphylococcus aureus*. Then the samples were subjected to antimicrobial sensitivity tests by disc diffusion method, using Methicillin and Vancomycin discs. Various strains of *Staphylococcus aureus* were isolated as causative agents, 25(17%). On subjecting to antimicrobial sensitivity these strains gave different results about 3(12%) were found resistant to Methicillin and all the strains were susceptible to vancomycin. The study showed that the prevalence of the MRSA was greater in ages between 15 to 30 years and more in males than in females.

Keywords: *S. aureus* – Pneumonia – MRSA - VRSA

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Introduction

Staphylococcus aureus normally causes pneumonia only after preceding infleunzae viral infection, [1] and in association with right-sided staphylococcal endocarditis, which is a serious complication of intravenous drug abuse. [2] Complication of staphylococcal pneumonia includes staphylococcal pericarditis, meningitis, osteomyelitis and multiple metastatic abscesses in soft tissues. [3] Furthermore, these serious infections have emerged more frequently among non-hospitalized patients and are associated with strains that produce the Panton-valentine leukocidin toxin. Also worrisome is that these serious infections are frequently mediated by methicillin-resistant *S. aureus*. [4]

S. aureus is normal flora, spreads of patient's endogenous strain to normally sterile site by traumatic introduction, and may transmitted by fomites, air, or unwashed hands of health care workers, especially in the nosocomial setting. may be transmitted from infected skin lesion of health care worker to patient. [4] MRSA, representing 12.7% of all patients colonized with *S. aureus*. [5] Many of MRSA strain are not type-able with standard and additional or experimental phages. [6] The control and prevention of MRSA involves early and reliable detection in the laboratory through surveillance, patients isolation when admitted to hospital. [6] Health care workers should strictly adhere to infection control policies by wearing gloves and wash hands before and after patients contact. [7] and the sensible use of antibiotics such measures have been very successful. [6]

Serious MRSA infections such as those involving the blood stream , respiratory tract and bones or joints more difficult to treat than

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infections caused by methicillin-susceptible isolates. Methicillin resistance is mediated through the mecA gene ,which encodes a unique penicillin-binding protein. Community-acquired strains have been describe that can cause soft tissue infections, these strains often produce the panton-valentine leukocidin. They can be distinguished from endemic hospital strains, from which it is believed that have arisen. [6]

S. aureus are variably sensitive to many antimicrobial drugs, Resistant to penicillin depends on production of the enzyme penicillinase, a β-lactamase that open β-lactam ring. [6] Penicillin-resistant strain of *S. aureus* are treated with flucloxacillin, methicillin and the cephalosporins independent of β-lactamase production. MRSA arise due to the mec A gene, which encodes nafcillin resistance resides on the chromosome, and the gene encodes a low-affinity penicillin binding protein (PBP2 or PBP2a). [7] MRSA strains resistant to all β-lactam agent, and often other agents such as aminoglycoside, and fluoroquinolones. glycopeptides (vancomycin or teichoplanin) are the agents of choice in the treatment of systemic infection with MRSA. [6] In the United States, *S. aureus* is considered to be susceptible to vancomycin if the minimum inhibitory concentration (MIC) is equal or less than 2mg/ml, of intermediate susceptibility if the MIC between 4-8mg/ml, and resistant if the MIC is equal or more than 16mg/ml. Strains of *S. aureus* with intermediate susceptibility to vancomycin have been isolated in Japan, the united states, and several other countries, these are often known as vancomycin intermediate *S. aureus*, or VISA. They have generally been isolated from patients with complex infection who have received prolonged

vancomycin therapy. Often there has been vancomycin failure. The mechanism of resistance is associated with increased cell wall synthesis and alterations in cell wall and is not due to van A gene found in enterococci. *S. aureus* strains of intermediate are susceptible to vancomycin usually are nafcillin resistant but generally are susceptible to oxazolidinones and to quinopristin/dalfopristin. Since 2002, several isolates of vancomycin-resistant *S. aureus* (VRSA) strains were isolated from patients in the United States. The isolates contained vancomycin resistant gene van A from enterococci and nafcillin resistant gene mec A. Both of the initial VRSA strains were susceptible to other antibiotics. Vancomycin resistance in *S. aureus* is a major concern worldwide. [7]

Because of the misuse and abuse of antibiotics, the treatment options for MRSA in near future are going to shrink further. This study was carried out to evaluate antibiotics resistant *Staphylococcus aureus* among the patients with chest infections as one of causative agents.

Materials and Methods

Sample collection:

A total of 147 sputum samples were collected from patients with chest infections (different ages and both genders) during the period from May to June 2011 at Al-Shaab teaching hospital.

Methods

All collected samples were tested first for direct Gram stain then by using routine culture media for isolation *S. aureus* (Mannitol salt agar, Nutrient agar, blood agar), indirect Gram stain and biochemical tests (Catalase test, DNA-ase medium) then susceptibility to 5microgram Methicillin and 30 microgram vancomycin discs were tested by Kirby-buar method using (Muller-Hinton agar). American

Type Culture Collection) ATCC 25933 strain was used as control for the diagnostic process of potency of antimicrobial discs.

Results

Twenty five (17%) *S. aureus* were isolated including 14(56%) from males and 11(44%) from females. Three (12%) methicillin resistant *S .aureus* were detected (1:2 female: male) using phenotypic detection by oxacillin 5 micg, there is no resistance to vancomycin 30micg. (*vide* Table1)

Table 1: The effect of age in MRSA-chest infection (age in years).

Age-group	**No of *S. aureus* infection**	**No. of MRSA infection**	**No of males**	**No of females**
14-19	6	1 (17%)	5	1
20-29	7	1 (14%)	3	4
30-39	4	1 (25%)	4	0
40-49	4	0	0	4
=/>50	4	0	2	2
Total	25	3 (17%)	14	11

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Discussion

Most Staphylococcus *aureus* bacteria can be treated with vancomycin and methicillin antibiotics, some bacteria have developed a resistance and can no longer be treated. MRSA and VRSA are types of antibiotic resistant *Staphylococcus aureus*. In the present study the percentage rate of MRSA was 12% and no VRSA (0%). However this percentage was less than 67.2% which was reported by Al-Zahra Hospital, Iran, from patients who contracted nosocomial infection [8]. The results of our study were less than the value reported by Khan *et al* in medical college, during the period from August 2005 to July 2007, who found that (143) 33.25% isolation rates for MRSA[9]. The results correlated with those of , Jernigan, J. Pullen et al Manian, F. Senkel, Mylotte, J. M [5,10,11], but not Khorvash *et al* and Khan *et al* [8,9]. Pullen *et al* observed that acute rehabilitation units were considered high-risk populations of MRSA colonization infection because the majority of those patients were transferred from an acute care hospital or nursing home. However other factor might be possible; that community-acquired MRSA was dependent on a geographic factor or specific high-risk population, such as, children in day care, inmates, sport teams, and other similar minorities.

Conclusions:

All *Staphylococcus aureus* which were isolated from chest infection showed resistance to Methicillin while they showed sensitivity to vancomycin. In conclusion, the results obtained suggest that there is an emergence of a new

Staphylococcus aureus resistant strain.

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OriginalArticle

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An *in Vitro* Antimicrobial Potential of Various Extracts of Commiphora Myrrha

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Abstract

Objectives The aim of this study was to evaluate the antimicrobial activity of various extracts obtained from the resin of medicinal plant *Commiphora myrrha* on standard microorganisms. ***Methods*** The agar well diffusion technique was followed to perform the antimicrobial activity of the candidate extracts against Gram-positive bacteria (*Bacillus subtilis* NCTC8236, *Staphylococcus aureus* ATCC 25923), Gram- negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 53657, *Proteus vulgaris* ATCC6380, *Pseudomonas aeruginosa* ATCC 27853), as well as, two fungal species (*Candida albicans* ATCC 7596; *Apergillus niger* ATCC 9763). ***Results:*** Methanolic and aqueous extracts of the resin of *C. myrrha* at concentration of 100 mg/ml was found to be more active against Gram- negative bacteria (*Proteus vulgaris* ATCC 6380; *Klebsiella pneumoniae* ATCC: 53657; *Escherichia coli* ATCC: 25922 and *Pseudomonas aeruginosa* ATCC: 27853) and Gram- positive bacteria (*Bacillus subitilis* NCTC: 8236), as well as, they showed high antifungal activity against (*Candida albicans* ATCC :7596 and *Aspergillus niger* ATCC:9765),while the chloroform extract of the resin showed moderate activity against Gram- positive and Gram- negative bacteria ,as well as against *Candida albicans* ATCC :7596,whereas the same extract revealed high antifungal activity against *Aspergillus niger* ATCC:9765. ***Conclusion*** Methanolic, chloroform and aqueous extracts of the resin of *C. myrrha* revealed that the selected plant had a significant potential effect capable to inhibit the growth of both bacterial and fungal standard species.

Keywords**:** *Commiphora myrrha*; antimicrobial activity; Methanol extract; chloroform extract; standard strains.

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Introduction

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For a long time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies[1]. Currently a large and ever expanding global population base prefers the use of natural products in treating and preventing the attack of some illnesses. This has influenced many pharmaceutical companies to produce novel antimicrobial formulations extracted from plants or herbs. Virtually all cultures around the globe have relied historically, and continue to rely on medicinal plants for primary health care. There is currently a worldwide upsurge in the use of herbal preparations and the active ingredients isolated from medicinal plants in health care. Most of modern drugs were derived from natural sources, using either the natural substance or a synthesized version[2]. Some antibiotics have become almost obsolete because of the drug resistance and consequently new drugs must be sought for. Herbal treatment is one possible way to treat diseases caused by multidrug resistant bacteria. The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in therapeutic treatments. In the past few years, a number of studies have been conducted in different countries to prove such efficiency[3]. *C. myrrha* belonging to family *Burseraceae* is a shrub or small l tree (5 m tall); it is native to Arab countries, Northern Africa and Somalia[4, 5]. *C. myrrha* was used as a wine preservative, aromatic for funerals and insect repellents by the ancient Egyptians. Ancient Greek and Roman physicians used it to treat wounds and prescribed it as a digestive aid, menstruation promoter and analgesic[6]. It used as a remedy for numerous diseases, including intestinal disorders, wound infections and some helminths infections[7, 8]. It is used today as an aid to nervous system disorders, rheumatic complaints, tooth decay, gum disease and as anticancer [9]. *C. myrrha* possesses secondary metabolites like flavonoids,

alkaloids, tannins, glycosides, steroids, saponins, tannins and terpenoids. Bioactive compounds like flavonoids, glycosides are rich in methanolic extract[10, 11]. The antimicrobial activity and many applications including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies of *C. myrrha* resin extracts has been formed[12, 13]. The purpose of this study was to investigate the antimicrobial activity of various extracts obtained from the resin of *Commiphora myrrha* against standard microorganisms.

Materials and Methods

Plant Materials

Fresh resin of *C. myrrha* was purchased from Omdurman Local Market, Omdurman, Sudan. The laboratory work has been carried out at Microbiology Department, Medicinal and Aromatic Plants Research Institute (MAPRI).The resin was washed thoroughly three times with running water and once with distilled water and it was then air- dried under shade. Voucher specimens were deposited at the herbarium of the institute.

Preparation of Crude Extracts

Each of the coarsely powdered plant material (50 g) was exhaustively extracted with methanol and chloroform in Soxhlet apparatus. The extracts were filtered and evaporated under reduced pressure using a rotary evaporator until they become completely dry. The residues were stored at 4 ˚C for further need. Each residue was weighed and the yield percentage was determined and kept in refrigerator until used. For aqueous extract 100 g of powdered plant material was infused in 500 ml hot water for 4 hours then filtered through Whattman filter paper. The residue was weighed and the yield percentage was determined and kept in refrigerator until used.

Test Microorganisms

Eight different standard strains examined in this study were obtained from

National Collection of Type Culture (NCTC), Colindale, England and American Type Culture Collection (ATCC) Rockville, Maryland, USA. Those strains include Gram- positive bacteria (*Bacillus subtilis* NCTC8236, *Staphylococcus aureus* ATCC 25923), Gram- negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 53657, *Proteus vulgaris* ATCC6380, *Pseudomonas aeruginosa* ATCC 27853), as well as, two fungal species (*Candida albicans* ATCC 7596; *Apergillus niger* ATCC 9763).

Identification of Standard Strains

All examined strains were inoculated on blood agar and nutrient agar plates, incubated aerobically and the obtained growth were then purified by streaking on plates containing the appropriate selective and differential culture media, Mannitol salt agar and MacConkey's agar. Microscopic examination and biochemical tests of the purified microorganisms were done for identification and confirmation of these organisms. The biochemical tests that carried out include Fermentation tests, Methyl red tests, Voges- Proskauer test, Citrate utilization test, Indole production test, Hydrogen sulphide production test, Catalase test, Coagulase test, Oxidase test and Urease test[14].

Preparation Test Microorganisms

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 10^8- 10^9 CFU/ ml. The suspension was stored in the refrigerator at 4° C till used. 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.

Testing of Extracts for Antimicrobial Activity

The cup-plate agar diffusion method[15] was adopted with some minor modifications to assess the antibacterial and antifungal activity of the prepared extracts. One ml of the standardized bacterial and fungal stock suspension 10^8 –10^9 CFU/ ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45 ºC. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates 4 cups (10 mm in diameter) was cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

Determination of minimum inhibitory concentration (MIC)

The principle of the agar plate dilution is the inhibition of growth on the surface of the agar by the plant extracts incorporated into the medium. Plates were prepared in the series of increasing concentrations of the plant extract. The bottom of each plate was marked off into 4 segments. The organisms tested were growing in broth over night to contain 10^8 CFU/ml. Loop-full of diluted culture is spots with a standard loop that delivers 0.001 ml on the surface of segment. The endpoint (MIC) is the least concentration of antimicrobial agent that completely inhibits the growth. Results are reported as the MIC in mg/ml.

Results

The average of the diameters of the growth inhibition zones produced by methanol, chloroform and aqueous extracts of the resin of *C. myrrha* are presented in Table 1. Table 2 and 3 showed on the other hand, the anti-microbial activity of the reference chemotherapeutic agents on the standard bacterial and fungal strains tested. The results were interpreted as sensitive, intermediate and resistant. According to results that presented in Table 2 and 3 extract resulting in 15 mm or more growth inhibition zone are considered to be active and those resulting in less than 15 mm are inactive[14].

MIC of Resin Methanolic Extract of *C. myrrha* Against Standard Strains

The minimum inhibitory concentration for methanolic extract of the resin of *C. myrrha* exhibited various degrees of activity against the test microorganisms, it was 50 mg/ml for *Escherichia coli*, 25 mg/ml for *Pseudomonas aeruginosa* and *Proteus vulgaris*, 12.5 mg/ml for *Klebsiella pneumoniae*, whereas it was 6.25 mg/ml for *Bacillus subitilus*, *Candida albicans* and *Aspergillus niger* (Table 4).

Table 1: Antimicrobial activity of *C. myrrha* resin extracts against standard strains.

Solvent system	Yield %	Standard strains */MDIZ mm							
		E.c	***Ps.a***	***Kl.p***	***P.v***	***B.s***	***S.a***	***C.a***	***Asp.n***
Methanol	3.5	18.5	19.5	20.5	20.0	16.0	-	21.5	29.5
Chloroform	2	-	14.5	15.0	15.0	15.0	-	16.0	20.0
Aqoeous	2.4	17.5	16.5	17.0	16.0	14.0	-	17	18

Key: concentration used 100 mg/ml; Standard microorganisms (*E.c: Escherichia coli, Ps.a: Pseudomonas aeruginosa, Kl.p: Klebsiella pneumoniae, P.v: Proteus vulgaris, B.s:Bacillus subtilis, S.a: Staphylococcus aureus, C.a :Candida albicans* and *Asp.n :Apergillus niger* .
MDIZ: Mean diameter inhibition zone; (-) Not determined

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Table 2: Antibacterial activity of reference antibiotics against standard strains.

Antibiotic	Conc.used (µg/ml)	Standard strains /MDIZ mm					
		E.c	***Ps.a***	***Kl.p***	***P.v***	***B.s***	***S.a***
Ampicillin	40	18	-	18	-	15	25
	20	16	-	15	-	14	20
	10	13	-	13	-	13	18
	5	-	-	12	-	12	15
Tetracyclin	40	24	16	27	16	23	31
	20	19	13	25	-	21	27
	10	-	12	21	-	20	25
	5	-	-	18	-	18	17

Table 3: Antifungal activity of reference antifungal drugs against standard strains.

Drug	Concentration mg /ml	Tested fungi	
		C.a	***Asp.n***
Nystatin	25	14	26
Clotrimazole	20	24	34

Table 4: M ICs of the resin methanol extract of *C. myrrha*.

Part used	MIC of Standard microorganisms mg/ml						
	E.c	***Ps.a***	***Kl.p***	***P.v***	***B.s***	***C.a***	***Asp.n***
Resin	50	25	12.5	25	6.25	6.25	6.25

Discussion

Methanol, chloroform and aqueous extracts of the resin of *C. myrrha* were investigated for their antimicrobial potential against eight standard microorganisms; two are Gram- positive bacteria (*Bacillus subtilis and Staphylococcus aureus*); four are Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus vulgaris*) and two fungal species (*Candida albicans* and *Aspergillus niger*). It is clear from

Table 1 that the resin methanolic extract showed high activity against *Klebsiella pneumoniae* (20.5 mm), *Proteus vulgaris* (20 mm), *Pseudomonas aeruginosa* (19.5 mm), *Escherichia coli* (18.5 mm) and *Bacillus subtilis* (16 mm). This finding agreed in points with that reported by Al-Daihan *et al*.[16] in Saudi Arabia, and Rahman *et al*.[17]. In our study, *Staphylococcus aureus* was not found to be sensitive to any one of the candidate extracts; this result is in agreement with the study of Al-Daihan *et al*.[16]. On the other hand, the same extract exhibited high antifungal activity against *Candida albicans* (21.5 mm) and *Aspergillus niger* (29.5 mm). This result is parallel to that study reported by Omer *et al*.[18].The chloroform extract of the resin of *C. myrrha* showed moderate activity against most of the tested bacterial strains and the mean diameter of inhibition zones were ranged from 14 mm to 15 mm for all tested bacteria, except *Escherichia coli* in which there is no activity for chloroform extract, while the same extract exhibited high antifungal activity towards *Candida albicans* (16 mm) and *Aspergillus niger* (20 mm). This finding is not agreed with those results of Masoud and Gouda[19]. The resin aqueous extract of *C. myrrha* showed high antimicrobial activity against most of the tested microorganisms and the mean diameter inhibition zones that obtained by the tested microorganisms were 17.5 mm, 16.5 mm, 17 mm, 16 mm and 14 mm for *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Proteus vulgaris*, and *Bacillus subtilis*, respectively, whereas the antifungal activity of the same extract was 17 mm and 18 mm for *Candida albicans* and *Aspergillus niger*, respectively. These results are corresponded to that reported by Masoud and Gouda[19].

The comparison of observation given in Table 1, 2 and 3 demonstrated that the resin methanolic extract of *C. myrrha* showed high activity (20.5 mm) against *Klebsiella pneumoniae*, which is almost similar to the activity of 10 μg/ml Tetracycline and more than activity of

40 µg/ml Ampicillin. It also inhibits *Proteus vulgaris* (20 mm) which is higher than activity of 40 µg/ml Ampicillin. *Pseudomonas aeruginosa* being exhibited (19.5 mm) which is higher than activity of 40 µg/ml Tetracyclin. On the other side, the same extract showed high activity against *Candida albicans* (25.5 mm) which is more than activity of 25 mg/ml Nystatin and less than activity of 20 mg/ml Clotrimazole, whereas it inhibit *Aspergillus niger* (29.5 mm) which is lower than activity of 20 mg/ml Clotrimazole and higher than activity of 25 mg/ml Nystatin. The chloroform extract of *C. myrrha* exhibited the least antimicrobial activity towards the tested microorganisms. It was about 15 mm for *Klebsiella pneumoniae, Proteus vulgaris and Bacillus subtilis* that is almost similar to the activity of 20 µg/ml Ampicillin for *Klebsiella pneumoniae*, less than activity of 40 µg/ml Tetracycline for *Proteus vulgaris* and less than activity of 40 µg/ml Ampicllin for *Bacillus subitilis*. Whereas the same extract showed high antifungal activity against *Aspergillus niger* (20 mm) which is less than activity of 25 mg/ml Nystatin. The aqueous extract of the same plant revealed moderate activity against *Escherichia coli* (17.5 mm) which is higher than activity of 20 µg/ml Ampicillin, and *Pseudomonas aeruginosa* (16.5 mm) which is more than activity of 40 µg/ml Tetracycline. On the other hand, the antifungal activity of the aqueous extract against the tested fungi was 17mm and 18 mm for *Candida albicans* and *Aspergillus niger* which is more than activity of 25 mg/ml Nystatin.

Conclusion and Recommendation

It was observed that all extracts obtained from *C. myrrha* found to be active against almost all of the tested organisms, except *Staphylococcus aureus* which was not affected by candidate extracts. Methanol extract of the resin of *C. myrrha* was found to be more active mainly against Gram-negative bacteria compared to the reset test extract. The most interesting finding in our study is that *Klebsiella penumoniae* was inhibited by the three candidate extracts, as well as it was the most sensitive organism compared with the rest microorganisms. In the present study *C. myrrha* resin extracts had a significant potential effect toward most of the tested organisms in spite of the difference in the solvent systems; this may be due to the highly antimicrobial ingredients of the resin. Further researches and investigations are required to elucidate the mechanism of action of *C. myrrha* resin as antimicrobial agent.

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OriginalArticle

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Comparative Study Serum and Cutaneous Total Bilirubin among Newborns with Hyperbilirubinemia in Khartoum State

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Abstract

The standard methods used in measuring serum bilirubin require blood specimens taken by heel pricks or intravenous blood sampling but transcutanse bilirubin is a new method for measuring bilirubin in neonate. So this study was conducted to compare the estimates of serum bilirubin using a recently introduced device called (MBJ20), which is TransCutaneous Bilirubinometer (TCB) index with the standard direct spectrophotometric measurement of Total Serum Bilirubin (TSB). This cross-sectional study was conducted in neonatal intensive care unit at Soba University Hospital, where one hundred and fifty three, newborns with hyperbilibrubina were enrolled. Both TSB and, TCB readings were evaluated.

According to the date of jaundice onset 91.9% developed jaundice during period of 2-7 days; 5.9% in the first day and 2.6% after a week. TSB level among jaundiced neonates less than 12 mg/dL was found in (29.4%), 12 – 2 mg/dL were detected in (67.9%), and More than 20 mg/dL was recorded in (2.6%). On the other hand TCB level was less than 12 mg/dL found in (20.9%), 12 – 2 mg/dL was detected in (77.1%) and more than 20 mg/dL cited for (1.9%). At P≤0.05 there were no significant differences in TSB and TCB before phototherapy with Mean (13.40±2.83, 13.49±3.00) mg/dL for TSB and TCB respectively (P value .539). And after photo therapy Mean of TSB and TCB index were (13.00 ±4.40, 13.06±2.57) mg/dL respectively (P value 0.769). Neonatal birth weight showed extremely low birth (2.6%), low birth weight in (54.9%), normal weight (37.3%) and Macrosomic (5.2%). The Gestational age of jaundiced neonates showed pre-term (48.4%), term (49%) and Post-term (2.6%). The study revealed that TCB was an accurate method, as TSB before and after phototherapy. No difference when TCB was used for measuring TSB in term or preterm babies. So TCB device could be used in every Nursery as reliable.

Keywords: Sudan, bilirubin, neonates, TSB, TCB

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Introduction

Hyperbilirubinemia is a common condition in newborn affecting over 50-60% of all babies. About 50% of term baby and 80% of preterm babies developed jaundice in the first week of life. [1]. It is a common phenomenon but relatively few affected infants require intervention. Whatever the cause, total serum bilirubin (TSB) levels, above defined thresholds warrant treatment to prevent the development of kernicterus [2]. Neonatal jaundice is a yellowing of the skin and sclera of the newborn infant; a bilirubin level of more than 85umol (5mg/dl) [3]. Delay of management leads to serious central nervous system complications like kernicterus and acute bilirubin encephalopathy. However, the preventability of these complications has led to recommendations to screen all neonates for hyperbilirubinemia. [4, 5, 6, 3] Phototherapy is the primary treatment in neonates with unconjugated hyperbilirubinemia. It is a common treatment in neonatal medicine and is used to prevent the neurotoxic effects of bilirubin. Studies have assessed the optimal wavelength of phototherapy light as well as the importance of irradiance and spectral power, and the types of light source, including the use of single versus multiple light sources. Outcome measures have been duration of need for phototherapy or rate of reduction of serum bilirubin over a given time. [7, 8] The standard methods used in measuring serum bilirubin require blood specimen taken by heel pricks or intravenous blood sampling, which involves pain of the newborn, and it is time-consuming. A non-invasive transcutaneous measurement of bilirubin concentration is developed to be an alternative method, reliable to detect hyperbilirubinemia, and by this new method, and through it, addition to (TSB) reached the etiological factors of neonatal jaundice methods of management and short term outcome. [9] Neonatal Transcutaneous Jaundice meter MBJ20 (TCB) is a handhold

instrument used in the dynamic clinical examination of neonate jaundice. It non-invasively and instantly tests transcutaneous concentration of bilirubin correlative with serum bilirubin concentration. It relieves the neonates from any discomfort brought by blood drawing. Moreover, it reduces test cost, increases the working efficiency of clinicians and nurses, decrease hazard, prevent parents' anxiety, early detection and rapid start of management. Since TCB requires relatively lower thresholds with false-positive results for having a sensitivity of 100%, it cannot be recommended as a complete substitute for serum bilirubin measurements. [10]

Although Jaundice is one of the common problems among neonates worldwide, no similar studies were conducted in Sudan, the thing that necessitates assessing such problem in Sudan. Identifying neonatal jaundice will help verification for the disease aspects. We can make use of the fact that most of causes are preventable; most of short term outcomes can be detected earlier and are treatable and the recent method for measuring serum bilirubin by transcutaneous bilirubinometer (TCB) will provide early detection of jaundice that helps quick action of treatment. So this study was conducted to compare bilirubin level using a recently introduced device called (MBJ20). It is the transcutaneous bilirubin index with the standard serum bilirubin assessment before and after phototherapy in addition to detect neonatal weight, term and gestation period in susceptibility to hyperbilirubinemia.

Materials and Methods

This study was carried out in the neonatal intensive care unit (NICU) at Soba university hospital. One hundred and fifty three neonates, who had been admitted to intensive care unit (NICU), with clinical neonatal Jaundice, or who developed Jaundice later during period of admission at (NICU) were considered as study samples. Data was collected during 1st of April to the August 2014.

Ehical consideration

This study was performed upon the approval by The Ethical Committee of Omdurman Islamic University , delegated by the hospital, for clinical and health research. Informed consent was obtained from mothers before collection of blood from newborns.

Samples processing

Blood samples were obtained from neonates collected into heparin-containing, containers. The samples were immediately sent to the hospital laboratory, where the plasma was separated and then immediately assayed for bilirubin by a diazo-method in an AU640 (Olympus) analyzer.

The transcutaneous bilirubinometer (± 30) minutes for measuring bilirubin level, (TCB) measurement was performed over forehead.

Technique of MBJ 20 (TcB)

This is a hand-held, portable and rechargeable device that when pressure is applied to the photoprobe, a xenon tube generates a probe light, and this light passes through the subcutaneous tissue. The reflected light returns through the second fiber optic bundle to the spectrophotometric module. The intensity of the yellow color in this light, after correcting for the hemoglobin, is measured and instantly displayed in arbitrary units.

Tools

Data were collected through questionnaire, investigation, diagnosis, management and outcome.

Statistical Analysis

The data was analyzed using Statistical Package for Social Sciences (SPSS), Windows version8x, 1997 SPSS, Inc, Chicago, IL, and USA.

Student's ''t'' test used to assess the significance percentage was also calculated.

Results

Classification according to the date of onset of jaundice revealed that 91.9% developed jaundice during period of 2-7 days, 5.9% in the first day and 2.6% after a week (Table 1).

Table 1: Onset of jaundice

Days	First day	2-7 days	more than 7 days
Percentage	5.9	91.5	2.6

Serum total bilirubin level among jaundiced neonates less than 12 mg/dL was found in (29.4%), 12 – 2 mg/dL were detected in (67.9%),and more than 20 mg/dL was recorded in (2.6%). While, the total Cutaneous Bilirubin less than 12 mg/dL found in (20.9%), 12–2 mg/dL was detected in (77.1%) and More than 20 mg/dL were cited for (1.9%), (Tables 2, 3).

Table 2: Serum total bilirubin Level among jaundiced neonates

	Frequency	Percent
Less than 12 mg/dL	45	29.4%
12 – 20 mg/dL	104	67.9%
More than 20 mg/dL	4	2.6%

Table 3: Total Cutaneous Bilirubin level among jaundiced neonates

	Frequency	Percent
Less than 12 mg/dL	32	20.9%
12 – 20 mg/dL	118	77.1%
Greater than 20 mg/dL	13	1.9%

At P≤0.05 there were no significant differences in total serum bilirubin (TSB) and total cutaneous bilirubin (TCB) before and after phototherapy. Mean of serum total bilirubin, and total cutaneous bilirubin index were (13.40±2.83, 13.49±3.00) mg/dL respectively (P value=.539). Meanwhile after phototherapy the mean of TSB and TCB index were (13.00 ±4.40, 13.06±2.57) mg/dL respectively (P value 0.76), (Table 4).

Table 4: Total serum and cutaneous bilirubin before and after photo therapy

	Mean±SD	**P value**
First TSB	13.40±2.83	0.539
First TCB	13.49±3.00	
Second TSB	13.00±4.40	
Second TCB	13.06±2.57`	0.769

Neonatal birth weight showed extremely low birth (2.6%), low birth weight in (54.9%), normal weight (37.3%) and Macrosomic (5.2%).The Gestational age of jaundiced neonates showed pre term (48.4%), Term (49%) and Post-term (2.6%) (Tables 5, 6).

Table 5: Neonates birth weight

Birth weight	**Number**	**Percentage**
Extremely low birth(less than 1 kg)	4	2.6
Low birth (1 - 2.5 kg)	84	54.9
Normal (2.5 - 4 kg)	57	37.3
Macrosomic (greater than 4Kg	8	5.2

Table 6: Gestational term

Gestational period	Number	Percentage
Preterm (less than 37 weeks)	74	48.4
Term (37-42 weeks)	75	49
Post-term (greater than 42)	4	2.6

Discussion

In this study hyperbilirubinemia more predominates among low birth neonates. This is supported by a study done by Korejo, *et al*. and Martínez-Cruz, *et al* [11-12]. Bus Korejo, reported that low birth weight was a risk factor for neonatal hyperbilirubinemia.

Total serum and cutaneous bilirubin before and after phototherapy showed no significant difference. These results were in concert to those reported by Janj and Tans [13] who mentioned the non-significance of such relationships between TSB and TCB. Thus, the recent TCB method may be considered a reliable diagnostic method of hyperbilirubinemia in neonates. It helps early detection of neonatal hyperbilirubnemia and, eventually, the start of early of management, and minimizes the period of staying at the hospital. The obtained results provide the opportunity to use this recent method (TCB) in both the term and preterm neonate. Moreover, Yasuda [14] and Samar, *et al.,*[15] recommended TCB as an accurate method, in term and preterm upon obtaining similar results.

Transcutaneous bilirubinometer (TCB) could be used not only as a screening device but also as a reliable substitute of TSB determination. At higher levels of TSB, in which phototherapy might be considered, TCB performed slightly better than the other laboratory detection. The accuracy and precision of the TCB measurement is comparable to the standard of care laboratory test, Janjindamai, *et al.,*[13] and Charuruks *et al*., [16] & Maisels. [17] Other studies found that there is close correlation between TSB and TCB measurement, David, *et al.* [18] Panburana, *et al.* [9] found that the TCB levels can accurately predict TSB with the different cutoff points at various postnatal ages before phototherapy, Felc [19] and Zecca, *et al*., [20]. Recently,

modifications introduced improvements on conventional transcutaneous bilirubinometery that gave results almost identical to those of bilirubin determination by the laboratory methods.

Concerning skin bilirubin measurement during phototherapy in preterm and term newborn infants, it was found that the difference between TSB and TCB was not more than 0.2, (*i. e. not significant!*) This statistical result showed a good congruence between TSB and TCB. Also it could be safely used for the evaluation of bilirubin levels in newborn infants under phototherapy. Its reliability on patched skin of the forehead is high enough to consistently reduce blood draws and to ascertain when to discontinue phototherapy. Because of the individual variance, any clinical decision has to be taken on the basis of the transcutaneous bilirubin trend more than on a single value, Stillova, *et al.* [21], and Slusher. [22] In African newborns TCB measurements are a useful and reliable index for estimating STB levels in pigmented neonates, including those with hyperbilirubinemia and kernicterus. In the absence of reliable TSB measurements, the relatively simple and non-invasive TCB measurements can be an important adjuvant in directing phototherapy and exchange transfusions, thereby preventing bilirubin-induced morbidity and mortality in low-technology clinical environments Tan. [10] The prevalence of neonatal jaundice was high among preterm neonates. This was supported by a study done by Brouillard, [23], who reported infants, especially preterm infants that have higher rates of bilirubin production than adults, because they have red cells with a higher turnover and a shorter life span. Together, these limitations led to physiologic jaundice that yielded high serum bilirubin

concentrations in the first days of life in full-term infants (and up to the first week in preterm infants and in some full-term Asian infants), followed by a decline during the next several weeks to the values commonly found in adults. The average full-term newborn infant has a peak serum bilirubin concentration of 5 to 6 mg per deciliter (86 to 103 μmol per liter).

Conclusion

There is no variance found in bilirubin level either by using blood sampling or transcutaneously by MBJ20 device. Term and neonates' weight play a major role in susceptibility to neonatal jaundice.

Recommendations

Further longitudinal studies with large sample sizes to be carried out to achieve better results more than this cross-sectional study, are suggested.

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Review Article

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Application of Molecular diagnostics in Microbiology: a Review

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ABSTRACT

In microbiology, routine laboratory diagnosis involves conventional methods, but in protracted diagnostic methods and there are certain limitations associated with them. Currently, molecular biology techniques are increasingly used to diagnose bacteria and viruses structures in order to enhance the diagnosis of diseases due to fastidious, slow growing, nonviable or non-cultivable organisms which cannot be detected by conventional culture techniques. The aim of the present study was to review the main current and new diagnostic techniques for confirmation of bacteria and viruses infections, namely: polymerase chain reaction (PCR), real-time polymerase chain reaction (RT-PCR), loop-mediated isothermal amplification (LAMP), Transcription mediated amplification (TMA)/ Nucleic Acid Sequence Based amplification (NASBA), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and restriction fragment length polymorphism (RFLP). This review will focus on various molecular techniques which are performed in clinical microbiology laboratories and their clinical applications and therefore help in the management of infectious diseases. The objectives behind this study are:

1- To increase the efficiency and accuracy of research in microbiology field.
2- To Advise and draw the attention of Sudanese physicians and Laboratory Medicine doctors to use Molecular techniques towards improving clinical outcomes for their patients.

***Keywords**: Diagnosis, monitoring, infectious diseases, molecular techniques.*

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INTRODUCTION

Detection and identification of the causative infectious agent is a highly relevant issue in microbiological diagnostics. Molecular methods have been increasingly incorporated in laboratories, particularly for the detection and characterization of isolates and for the diagnosis of diseases due to fastidious, slow growing, non-viable or non-cultivable organisms which cannot be detected by conventional culture techniques [1]. Molecular methods are based on the basic chemistry of DNA replication. Their advantages are rapid turn-around time, sensitivity and specificity [2]. The beginning of molecular diagnostics started in 1985 during the invention of Polymerase Chain Reaction (PCR) by *Kary Mullis*. Molecular diagnostics is a broad term describing a class of diagnostic tests that assess a person's health literally at a molecular level, detecting and measuring specific genetic sequences in deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) or the proteins they express. These tests can diagnose and monitor infectious diseases by identifying gene, RNA, and protein to detect and quantify the presence of specific viruses, and bacteria, etc. [3].

Some Molecular Methods for the Detection of Infectious Agent Structures

Molecular Methods are Broadly Classified as

1] **Hybridization methods**: which are better for identification; yet not as sensitive as amplification methods.

2] **Amplification methods**: which are more sensitive due to the amplification step [4].

Nucleic acid hybridization methods

Hybridization methods are based on the ability of two nucleic acid strands that have

complementary base sequence, i.e. they are homologous, to specifically bond with each other and form a double-stranded molecule, (the hybrid). Since the hybridization requires sequence homology, a positive hybridization reaction between two nucleic acid strands, each derived from a different source, indicate genetic relatedness between the two organisms. Hybridization assays require that one nucleic acid strand is from the known organism while the other is derived from the organism to be identified or detected. The results of hybridization are expressed as percent hybridization/ percent similarity or percent relatedness [5, 6].

Clinical applications of hybridization techniques

1. Direct detection of pathogens e.g. in Pharyngitis (gp-A streptococci), genital tract infections (*N. gonorrhoeae, C.trachomatis*), by Gen Probe
2. Speciation of microorganisms
3. Identification of culture isolates e.g. identification of dimorphic fungi and mycobacteria etc. by Accu-probe.

Several molecular tests have been developed in the last decade. Their specificity and sensitivity have gradually increased, and the infectious agents that were previously difficult to diagnose, using conventional techniques, began to be identified by molecular techniques. As a result, currently, these agents can be easily treated before causing major harm to the infected population.

In this review I have focused on Amplification methods because of their higher sensitivity than hybridization methods.

Next, several techniques used in molecular detection of infectious agents will be described.

Polymerase Chain Reaction (PCR)

The PCR makes it possible to perform selective amplification from complex genomes. This technique is based on the process of denaturing a double-stranded genomic DNA template using heat. Next, the temperature is lowered to ensure that primers can anneal to their complementary sequences into the template. Thus, the elongated DNA template follows in both directions from the primer site by means of enzymatic catalysis with a thermo-stable DNA polymerase, generating double-stranded products .

PCR-based techniques have revolutionized many areas of study because the enzymatic amplification of DNA can be performed *in vitro* from small amounts of material. PCR-based methods have also been combined with other techniques such as RFLP or nested PCR to genotype organisms [7].

Real-Time Polymerase Chain Reaction (RT-PCR)

This technique was developed in the early 1990s and enabled the monitoring of PCR amplification in real time. The specificity and sensitivity of the original method were maintained and combined with direct detection of the target of choice. Fluorescently labeled primers, probes or dyes are applied to a continuous collection of fluorescent signals from one or more reactions over a range of cycles. A digital camera and a fluorometer are coupled to the reaction tube in order to detect these signals. The technique has been modified to include other safer dyes. RT-PCR is a simple, fast, closed, and automatized amplification system responsible for decreasing the risk of cross-contamination typical of conventional PCR. [8]

Nested-PCR

This modality of PCR increases both the sensitivity and specificity. This technique uses two pairs of amplification primers and two rounds of PCR. In the first round, it uses one primer pair for 15 to 30 cycles. The product of the first round of amplification is submitted to a second round of amplification with the second pair of primers. [9]

Multiplex PCR

In the same reaction mixture, two or more primer sets, designed for amplification of different targets are used. More than one target sequence in a clinical specimen can be co-amplified in a single tube. However, the primers used must be carefully selected in order that they have similar annealing temperatures and lack complementarily. This kind of PCR is less sensitive than PCR with the single primer set. Multiplex PCR assays for viral respiratory pathogens and for detection of viral infections of the central nervous system have been developed and commercialized [10, 11].

Transcription Mediated Amplification (TMA)/ Nucleic Acid

Sequence Based Amplification (NASBA)

These are isothermal RNA amplification methods rather than DNA. The advantage of this technique includes performance of the test in isothermic conditions and no requirement of a thermocycler. The technique uses three enzymes in the reaction mixture; reverse transcriptase, RNase H and DNA dependent RNA polymerase.

Clinical applications of TMA/ NASBA

1. To determine viral load in the sample e.g. HIV, Hepatitis B virus and Hepatitis C virus.
2. To monitor HCV patients.

3. To direct detection of M. tuberculosis in clinical samples.
2- To differentiate enteroviruses from other viruses. [4, 12]

Loop Mediated Isothermal Amplification (LAMP)

LAMP is a method of nucleic acid amplification with extremely high sensitivity and specificity to discriminate single nucleotide differences [13]. It is characterized by the use of a DNA polymerase that has low sensitivity to inhibitors and a set of four primers specially designed to recognize six different sequences on the target gene. Amplification occurs only when all primers bind, thus forming a product. It is a technique that can amplify a few copies of genetic material up to 10^9 in less than an hour [14].

Random Amplified Polymorphic DNA (RAPD)

RAPD has been extensively used for description of strains in epidemiological studies. The surveying of genomes of microorganisms is enhanced by the advantage that RAPD is a very simple, fast, and inexpensive technique that does not require either prior knowledge of the DNA sequence or DNA hybridization. In contrast with other genotyping techniques, RAPD makes use of random amplifications of the genome. The fragments are separated by gel electrophoresis and the resulting banding pattern is used in genomic profiling. The technique generally involves the use of primers of ten bases in a PCR protocol with low selectivity; the primers anneal to the numerous homologous sites in the genome to generate a large number of DNA fragments by the subsequent amplifications. [3]

Amplified Fragment Length Polymorphism (AFLP)

The AFLP technology is also a technique that allows the detection of a DNA

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polymorphism without prior information on the sequence. This technique is highly efficient because of the possibility of analyzing a large number of bands simultaneously, with extensive coverage of the genome. This method of molecular diagnosis employs PCR to selectively amplify the groups of restriction fragments of a totally digested genomic. It consists of four steps: DNA digestion, ligation, amplification and gel analysis. Polymorphisms are identified based on the presence or absence of DNA fragments by polyacrylamide gel analysis [15].

Ligase Chain Reaction (LCR)

LCR amplification is based on sequential rounds of template dependent ligation of two juxtaposed oligonucleotide probes. LCR allows the discrimination of DNA sequences differing in only a single base pair. Single stranded target DNA is incubated with oligonucleotide probes that bind to the target in an end-to-end fashion. A thermostable DNA ligase then ligates (or joins) the two probes together. The resulting duplex is heated to separate the target DNA and the ligated probes. Both the separated target sequence and the ligated probes now act as targets for the probes, which bind in an end-to-end fashion. These steps are repeated several times resulting in geometric probe amplification.

Clinical applications of Ligase chain reaction

1. For the detection of certain bacteria like, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.
2. For the isolation and identification of viruses e.g. Human papilloma,

virus, Herpes simplex virus, HIV etc. [16].

Restriction Fragment Length Polymorphism (RFLP)

The RFLP technique is currently one of the most commonly used molecular methods for diagnosis of *M. tuberculosis*. This technique was first used to detect variations at the DNA level. The reaction is based on the digestion of the PCR products by restriction enzymes or endonucleases. These enzymes cleave DNA into fragments of certain sizes, whose analysis on agarose or polyacrylamide gel results in different patterns of fragment sizes, enabling the identification. The RFLP technique is suitable for environmental samples because it permits the detection of multiple genotypes in the same sample [3, 17].

Current Applications of Molecular Techniques in Microbiology

1. Diagnosis of non-culturable agents: e.g. Human papilloma virus, Hepatitis B virus etc.
2. Fastidious, slow-growing agents: e.g. *Mycobacterium tuberculosis*, *Legionella* etc.
3. Highly infectious agents that are dangerous to culture: e.g. *Francisella*, *Brucella*, and *Coccidioidis immitis* etc.
4. In situ detection of infectious agents: e.g. *H. pylori*, *Toxoplasma gondii* etc.
5. Organisms when present in small volume in the specimen: e.g. detection of HIV in antibody negative patients, intra-ocular fluid, forensic samples etc.

Future of Molecular Diagnostic Techniques

Despite being expensive, rapid diagnosis will result in decreased cost in the future. Detection of Multidrug resistant *M. tuberculosis* will lead to more timely public health measures. Increased specificity and sensitivity of molecular testing will become the standard of practice in immunology and microbiology. Testing will continue to become more rapid as assays are automated which will also bring down the costs.

CONCLUSION

The ultimate goal of the diagnosis and monitoring by using these techniques, to provide molecular information that will combine with and complement information related to patient history. In addition to that, clinical laboratory results, histopathological findings, and other diagnostic information can provide a more sensitive, precise, and accurate determination of disease diagnosis and thus lead toward appropriate medical decisions.The main advantages of molecular techniques are its higher sensitivity and specificity compared with other diagnostic methods such as serological assays and culture methods, as well as its rapidity and possibility of automation. Many of these techniques are now available commercially in an automated or semi-automated format**.**

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