SUDAN MEDICAL LABORATORY JOURNAL

A PEER REVIEWED SCIENTIFIC JOURNAL, ISSUED BY

OMDURMAN ISLAMIC UNIVERSITY

FACULTY OF MEDICAL LABORATORY SCIENCES

وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي

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Preface from the Editor-in-Chief

Medical Laboratory sciences are fast-growing fields, and it is a challenge to keep our journal up to date. The clinical laboratory has a major role in modern medicine, and as new information and concepts emerge and our understanding evolves, the material we teach must also change. Each article in this journal has been revised to encompass this new information.

Each of the authors is an acknowledged trusted in the paper he or she is writing about. The authors, editors and I have tried to eliminate errors that appeared in this issue without interfering with the objective or the meaning of the article. We are grateful to our colleagues, editors and authors for help in identifying mistakes and inconsistencies, and we ask for their continued aid in improving future issues.

Health professionals, health administrators, health policy-makers and nongovernmental organizations, among others, can and should use the scientific method to guide their work for improving the health of individuals and communities. It is hopefully that we gave them useful information through this journal.

I would like to take this opportunity to thank Prof. MB Saad, and Dr. Kamal Abdelsalam for their effort in establishing the journal. Special thanks also go to authors for their interest and encouragement. Once again, welcome to the journal and I hope you find the papers both interesting and thought provoking.

Sincerely,

Dr. Mohammed Abdel Hamid K.Elsid

Editor-in-Chief

Preface from the Editors

We are proud and happy to bring to you this new issue of **Sudan Medical Laboratory Journal (SMLJ)** assembled under our joint editorship.

Laboratory medicine might well claim to be the most popular and the most glamorous of biological sciences today. Accompanied with the fast growing of science and technology, the communication and information exchanges among the worldwide scientists are getting more and more important.

Numerous scientists, researchers, professors, and related experts working in the universities, institutes, hospitals, and pharmaceutical enterprises have been engaged in the research and development from different aspects, such as functional mechanism, toxicology, pharmacology, clinical evaluation, quality control, etc.

So SML journal is initiated to have a very broad scope to reflect medical laboratory scientific researches and other sciences in various aspects of medicine as well as regional and international relevant research. It supports insiders to share their insights on theory, application, and achievements.

In addition, it provides information about the recent tests for anyone who is concerned about human health. We sincerely expect that the increasing number of people in the world would benefit from this journal.

The journal is your friend, my friend, and our friend to connect us together. The journal is your home, my home, and our home to let us share human's intelligence in this field and to develop further.

Dr. Kamal A Abdelsalam
Director Editor
Dr. Mohammed B Saad
Advisor editor

Preface from the College

Welcome to the new issue of **Sudan Medical Laboratory Journal** (SMLJ) the official journal of the **Faculty of Medical Laboratory Sciences** in **Omdurman Islamic University**.

It has been said that over 80% of all medical decisions encompass clinical laboratory data in the decision process! While we know of no evidence to support this statement it is probably not far off the mark. Laboratory medicine plays an integrated role in the diagnosis, prognosis, treatment, and long-term management of disease. Proper selection and interpretation of laboratory tests is critical for quality patient care. In the last few decades, there has been an information explosion in the field of laboratory medicine, making it difficult for health care professionals to remain fluent in all aspects of laboratory testing. Sudan Medical Laboratory journal (SMLJ) aimed to provide a comprehensive overview of modern laboratory medicine in a "real-life" case-based or research-based format.

We hope SMLJ will be an excellent resource for medical technologists, laboratorians, physicians, residents, nurses, physician's assistants, medical students, educators, and many other allied health workers.

Dr. Isam AM Sadig
Vice Dean

Aims and scopes

Sudan Medical Laboratory Journal (SMLJ) is issued by Omdurman Islamic University, College of Medical Laboratory Sciences. This is a peer-reviewed journal published yearly. Its main objective is to reflect medical laboratory scientific research in various aspects of medicine as well as regional and international relevant research. Basic scientific research clinical practice, experiences that help in patient management are also welcome. Review articles, original articles, case reports are welcome. Local research in sciences education and history of laboratory and medicine will be considered for publication.

Manuscripts must be solely submitted to this journal. All authors must sign approving the submitted version. Any conflict of interest must be stated clearly. Ethical clearance must be presented in relevant submission.

Manuscript submission: only electronic version sent to this e mail addressed to the Editor-in-Chief will be considered smljournal@gmail.com.

Directions to contributors

Sudan Medical Laboratory Journal (SMLJ) publishes works, general and clinical articles, reviews, abstracts, society news and matters pertaining to human health and human sciences.

Papers are considered for publication provided that they have not been published before or will not be sent for publication in any other journal.

Articles for publication always should be prepared in a 1.5cm-spaced typewritten on size A4 paper and with 3cm margin on all sides. A covering letter signed by all authors must be forwarded and submitted in 3 paper copies and should be accompanied with a computer CD copy.

Names of authors should be followed by their official address. Names should be written as follow: last name + initials i.e. Cornel, C. E[1]. Ford, E. J. H[2]. Evans, J[3].

All papers should be accepted for publication after being subjected to minor reviewing by an assessor if necessary. No reasons will be given in case of rejecting the article.

One journal issue will be sent free of charge irrespective of the number of authors. Order for extra issues will be considered. Article should be subdivided into **abstract** (not more than 300 words), **introduction** (short concise overview of the current state with background information), **methods** (a clear description of the methodology used), **patient selection** (Inclusion and exclusion criteria), **results** (in form of text, tables and figures, avoid repetition of data), **discussion** (discussing the results obtained) and **references** (Vancouver style).

References should be written as follows: name of author(s) + (year) + title + journal + pages. For example:

One author: Tamir, M. J. (2000). The role of socioeconomic status in plasma concentration. Sud. Med. J. 15-20.

Two authors: Tamir, M. J. and Sami, R. T. (2000). The role of socioeconomic status in plasma concentration. Sud. Med. J. 15-20.

Three authors: Tamir, M. J. Sami, R. T. and Ahmed, S. F. (2000). The role of socioeconomic status in plasma concentration. Sud. Med. J. 15-20.

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Original Article **Sudan Medical Laboratory Journal**

Effect of grand multiparity on HDL-C level in normal pregnant Sudanese ladies.

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Abstract

The objective of this study is to determine maternal serum high density lipoprotein (HDL-C) levels at 26-32 weeks of gestation and the effect of multiparity on it. Study population is normal term pregnant women age 18-42 years who delivered normally with no previous obstetric complication and all previous deliveries were spontaneous, normal term and uneventful. Maternal serum HDL-C levels were determined from 325 normal women. Their mean age was 30 ± 12 years. The nulliparity (P0) was about 30.8 per cent, the first parity –primiparity- (P1) was about 30.8 per cent, whereas, the multiparity -grand and great grand- (PG) were 38.5 per cent. Mean serum high density lipoprotein-cholesterol (HDL-C) levels in P0, P1 and PG were 53 ± 10, 35.8 ± 5.3, 67.2 ± 11 mg/dl, respectively. There was a significant variance between maternal lipid and nulliparity ($p \leq 0.01$) as well as between primiparity and multiparity ($p \leq 0.01$).

Introduction

The role of plasma lipids and lipoproteins in the development of coronary heart disease (CHD) has been extensively studied. There is now a large body of data based on epidemiological studies, experimental research, genetics, and clinical trials that relates elevated total serum cholesterol and particularly LDL cholesterol (LDL-C) as well as low serum HDL cholesterol (HDL-C) to increased risk of CHD. [1, 2] Although the role of TG in the development of CHD has been controversial, it has been shown that high TG in combination with low HDL-C accounts for twice as many cases of CHD as low HDL-C alone. [1, 2]

Pregnancy is associated with profound alterations in the cardiovascular system, the long-term effects of which are unknown. Human epidemiological studies suggest that multiparity (*multiple pregnancies*) increases the risk of cardiovascular disease. The mechanisms underlying these findings remain to be elucidated. The objective of this study was to determine the long-term effects of grand-multiparity on HDL-C level. [3, 4]

High Density Lipoprotein: [HDL]

High density lipoproteins (HDL) form a class of lipoproteins, varying somewhat in their size (8-11 nm in diameter) and contents HDL will usually be

performed as part of an overall lipid profile, wherein LDL and triglycerides will also be measured. The combined information gathered from all of these tests may help determine cardiovascular event risk (risk of heart attack, stroke, and peripheral vascular disease) [5].

HDL particles are the smallest of the lipoproteins. They are the densest because they contain the highest proportion of protein. The main function of HDL appears to be carrying excess cholesterol (and probably other phospholipids and proteins) from the walls of the arteries to the liver for "re-packaging" or excretion in the bile. For that HDL can remove cholesterol from atheroma within arteries, and transport it back to the liver for excretion, HDL is called good cholesterol. [6].

In a review of nine different prospective studies in women, HDL cholesterol emerged as the single most important lipid risk factor in women. Recently, the National Cholesterol Education designates high HDL-C as a negative CHD risk factor, and recommends consideration of HDL-C in choice of drug therapy. [7]

Low HDL-C (*<35 mg/dL*) -as an independent indicator of risk- is equivalent to non-lipid risk factors such as smoking or hypertension. The increased clinical importance attributed to HDL-C concentrations now places additional burden on the clinical laboratory to provide accurate and precise measures of HDL-C. [8]

Normal level:

Normal value ranges may vary slightly among different laboratories.

- Male: 29-62 mg/dl
- Female: 34-82 mg/dl

An HDL 60mg/dL or above helps protect against heart disease. [9]

Multiple pregnancies:Multipara, grand: The term "multipara" applies to any woman who has given birth 2 or more times. A woman who has given birth 5 or more times is called a grand multipara.

For a pregnancy to count as a "birth," it must go to at least 20 weeks' gestation (the mid-point of a full-term pregnancy) or yield an infant that weighs at least 500 grams, irrespective of whether the infant is liveborn or not. [14]

The term "multipara" is composed of two Latin words: "multi-" from "multus", much + "-para" from "pario", to bring forth = to bring forth much (in the way of children).

Uniparous [primiparous]: 1) Having produced only one offspring. 2) Producing only one offspring at a time. [15]

Materials and methods:

The cohort consisted of 600 unrelated normal Sudanese pregnant women recruited from the most hospitals in Khartoum state, Nahr Elnil state and Gadarif state between October 2005 and September 2007. Informed consent, approved by University of Tamyal and Obstetricians committees – Bahry

Teaching Hospital, was obtained from all study participants.

200 of the volunteers are not pregnant, 200 of them were pregnant for the first time, and 200 were pregnant for more than 5 times.

Statistical analyses

Between-group comparison was performed using an ANOVA followed by the parametric *t*-test. Statistical analyses were performed using Microsoft Excel Data and Statistical Package for the Social Sciences (SPSS) computer program.

Sampling

Total serum cholesterol and HDL cholesterol (HDL-C) are the preferred screening tests for most inmates. Blood samples can be obtained at any time in the non-fasting state, since total cholesterol does not change significantly after a fat-containing meal, and HDL-C levels drop minimally. But, because all lipid profile needed to be determined, the 10 – 14 hours fasting samples were collected from all study groups.

Venipuncture should be performed after 5 minutes in the sitting position, using the tourniquet as briefly as possible, to minimize the effect of plasma volume and Federal Bureau of Prisons Management of Lipid Disorders posture on cholesterol levels. Recent surgery or trauma, or changes in diet can all affect lipid metabolism and cholesterol levels.

Method of estimation of HDL-C:

Specimen type, collection and storage

HDL-cholesterol measurement is still problematic, especially in samples that have been frozen and in lipemic plasma samples. [11]

Phosphotungstate/ Mg^{2+} method: [12]

Principle:

Phosphotungstate/ Mg^{2+} compound precipitates other lipoproteins than HDL by precipitating apolipoprotein (apo) B-containing lipoproteins. Then estimation of the isolated supernatant for cholesterol is carried.

Hazardous materials This procedure uses phosphotungstate, which is caustic and toxic. Do not swallow, and avoid contact with skin and eyes.

Advantages: sensitive and specific

Disadvantages:

- TG level more than 400mg/dL has a high probability of error when the cholesterol in the supernatant is analysed.
- In some laboratories, a major source of error is the failure to achieve clear supernatant
- more expensive
- **How to prepare patients for the test**
- Fast for 8 to 12 hours before the test.
- Anyone whose HDL level is below 40 mg/DL should consider taking steps to increase their HDL. This is the case even if total cholesterol and LDL cholesterol levels are within the normal range. [13]

Serum or plasma can be used. A fasting blood sample is preferred for lipid profile test. However if HDL-cholesterol alone has to be analyzed, a random sample can also be used. The specimen is stable for a week at 2 - 8ºC and at least for 3 months at -20ºC.

Results

Table1: Describes the results of HDL of all study groups

Type of parity	HDL
Multiparity	67.2
Primiparity	35.8
Nulliparity	53

Table 2: Compares the results of HDL of different number of pregnancy

No of parity	HDL
Nulliparity	53
primiparity	35.8
Multiparity5	65.3
Multiparity6	67
Multiparity7	67.9
Multiparity8	71.1
Multiparity9	71
Multiparity10	72.5

Table 3: Compares the results of lipid profile of HDL basing on the obesity

	Obesity	**HDL**
Multi parity	Non-obese	67.6
	Obese	66.9
Prim parity	Non-obese	36.2
	Obese	35
Null parity	Non-obese	53.2
	Obese	**51.8**

Discussion

Effect of multiparity and grand multiparity on many analytical factors during pregnancy has been reported in several papers as well as the affect of hyperlipidemia on the heard. Serum for high density lipoprotein-cholesterol (HDL-C) levels in nulliparity, primiparity and grand-multiparity were 53 ± 10.5, 35.8 ± 5.3, and 67.2 ± 11 mg/dl, respectively. These results of (HDL-C) levels showed significant decreasing ($p = 0.03$) from nulliparity to primiparity (in as much as 87.7%) and showed significant increasing ($p < 0.02$) from primiparity and grand multiparity (as much as 48%). These findings are in agreement with many researchers such as Johnson JA et al [16], Feskanich D et al [17], Manson JE et al [18] and others who have described the affect of normal pregnancy on lipid metabolism.The hyperlipidaemia during pregnancy is proved in many researches. Cramer DW [19], Cappuccio FP et al [20], Emanuel R [21] and others have described the reference range of lipid profile during the pregnancy, but each one of them has his own parameters. Although there are significant variations between them, they all have the same contentment that the peak level of hyperlipidaemia during pregnancy occurs during 26-32 weeks gestation. Other findings express the results of the grande multiparas according to the number of the pregnancy. All the results of the lipid profile –in the three tables- showed irregular ranging. This means it is undependable results. All the results showed insignificant changes when the lipid results are compared between each two respective numbers. In cholesterol the results showed as irregular results, but showed significant changes when compared the

results between each two respective results. Thus these findings proved that the lipid profile - except for total cholesterol- is not affected by the number of the grande multiparity.

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Original Article

Sudan Medical Laboratory Journal 1 (2011)

Maternal thyroid hormones and human chorionic gonadotrophin concentrations of Sudanese pregnant woman

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Abstract

A prospective study was conducted during the period 2001 to 2003 at Omdurman maternity, Khartoum teaching and Khartoum North teaching hospitals. One hundred and twenty Sudanese pregnant women at different stages of pregnancy were chosen for this study. Forty non-pregnant women of the same age without endocrine disease were chosen as control. Thyroid hormones (free T, and T_3) were measured. In addition hCG was also measured in both groups. The Study group was divided according to the gestational age (weeks) into three subgroups: - first trimester 5 - 12 weeks, second trimester 13 - 27 weeks and third trimester 28 - 40 weeks. Determination of serum hormones concentration was carried out using a highly sensitive specific RIA technique.

The results of this study showed increased levels of thyroid total hormones (T4 and T3) during three trimesters of pregnancy TSH remained within normal range. The results of this study also showed that there was an increase in concentration of free thyroid hormones (T4 and T3). The hCG increased during first trimester, then decreased sharply in the second and third trimesters of pregnancy

Introduction

Thyroid status is frequently assessed during pregnancy both to evaluate suspected thyroid abnormalities and monitor the status of pre-existing thyroid diseases [(1)]. The Production, circulation and disposal of thyroid hormones are all altered during pregnancy. Changes in serum thyroxine binding globulin (TBG) concentration occur in the first trimester, but accelerated thyroid hormone metabolism by the placenta is seen later in pregnancy T_4 is transported bounded principally to thyroxine binding globulin (TBG) and secondarily to thyroxine binding prealbumin (TBP A) and albumin [(2)].

Because of the strong affinity of TBP for T 4 over 99.9% of plasma T 4 is in this form of TBP- T_4 and means plasma (total T_4) effectively in the plasma ,TBP-T_4 [(3)]. In normal pregnancy, plasma total T_4 increased by 30 - 50% than in non-pregnant women [(2)]. Total T_4 may be increased due to estrogen therapy including women taking estrogen containing oral contraceptives[(4)]. Triiodothyrnine T_3 is a hormone synthesized and stored in thyroid gland; more than 99% of T_3 in the blood is bound reversibly to plasma proteins. The concentration of T_3 is much lower than

that of T_4, but its metabolic potency is much grater [5]. T_3 levels increased during pregnancy, oral contraceptive or estrogen treatment paralled thyroxine binding globulin (TBG) increases.

TSH is responsible for the control of synthesis and secretion of thyroid hormones T_3 and T_4. TSH is regulated by thyrotrophin releasing hormone (TRH). Pregnancy is associated with variation in TSH concentrations, but remains within the normal range. Before the development of highly sensitive TSH assay, investigators noted blunted TSH response to TRH in the first trimester of pregnancy, in some women, suggesting thyroid hormone excess and consequent pituitary suppression [6].

Free thyroid hormones (FT4 and FT3) levels

The serum concentration of free thyroxine and triiodothyronine (FT4 and FT3), which are presumably the biologically active form of thyroid hormone [7]. Free thyroxine (FT4) and free triiodothyronine (FT3) concentrations in pregnancy remain within the normal range. Thus measurement of free thyroxine concentration correlates better with clinical status than total thyroxine level [7]. Free thyroxine concentration typically to T_4 uncovers. Patients' actual clinical status (hypo and hyperthyroid conditions), in particular, the free T_4 is not affected by alteration in plasma (TBG); such as occur in pregnancy and women taking estrogen containing oral contraceptives. About 20% of women in the third trimester of pregnancy tend to have low plasma (free T_4), but without any pathological significance necessarily attaching to these findings, reference values appropriate to the stage of pregnancy should be used [3]. There was a close correlation between the reduction in mean-serum TSH concentration and the maximal elevation of hCG in the first trimester. The mean serum free T_4 concentration was also significantly higher in the first trimester compared with the second or third trimester [8].The heterodimeric glycoprotein, TSH and human chorionic gonadotrophin hCG are composed of a common alpha subunits and shared considerable similarity in their unique beta subunits, furthermore TSH and hCG /LH receptors are also quite similar. Excess hCG in the first trimester is thought to result in a hormone syndrome in which high concentration of hCG stimulate TSH receptor [9].

Human chorionic gonadotrophin β-hCG levels

Human chorionic gonadotrophin (hCG) belongs to the glycoprotein hormone with a molecular weight of about 3KD family together with lutinizing hormone (LH), follicular stimulating hormone FSH and thyroid stimulating hormone TSH [10]. hCG is synthesized by trophoplastic cells of the placenta during pregnancy and stimulates the growth of corpus luteum . Thyrotrphin secreted by the maternal pituitary gland, the placenta produces large amounts of human chorionic gondotrophin hormone, which has some thyrotrophin like

bioactivity [11]. The production of human chorionic gonadotrophin occurs during the first week after fertilization and causes a transient increase in serum free T_4 concentrations, which in turn decreases serum thyroid stimulating hormone (TSH) concentrations during the first trimester [12].

Materials and methods

Pregnant women:

One hundred and twenty Sudanese pregnant women were randomly selected from Omdurman Maternity, Khartoum Teaching and Khartoum North Teaching Hospitals. Their ages ranged between 19-45 years, and they were classified according to gestational age per week as follows:

1/ forty subjects in first trimester, gestational age (5-12 weeks)

2/ forty subjects in second trimester, gestational age (13-27 weeks)

3/ forty subjects in third trimester, gestational age (28-39 weeks)

Gestational age was estimated from the date of last menstrual period (L.M.P). Each subject was asked about occurrence of thyroid diseases and examined for the presence of thyroid enlargement and other signs of thyroid abnormalities. Cases of thyroid abnormality were excluded before sample collection.

Non-pregnant women (control)

Forty non-pregnant healthy women from Omdurman Islamic University, with approximately similar ages (18 - 45 years) were selected for this investigation.

Blood samples:

Five ml of venous blood were collected in a dry tube, allowed to clot and immediately centrifuged at 2000 r.p.m for 5 minutes and separated sera were stored at -20°C until analyzed.

Specific reagents:

All radioimmunoassay specific reagents for the measurement of thyroid hormones were obtained from China Institute for Atomic Energy (CIAE), Department of Isotopes (*Beijing China*). The reagents include tracer, standard and antibodies and separating agents for the different hormones.

Measurement of hormones by Radioimmunoassay (RIA) techniques

Principle of RIA: Antigen (Ag) and tracer (Ag*) compete with a limited amount of antibody (Ab) to form Ag-Ab or Ab-Ag* complexes. This reaction counts radioctivity using gamma counter.

Total serum thyroxin (T_4)

All assay tubes were set in duplicates using the following protocol: Add and mix properly after each addition. The assay was carried out using tubes containing the sample, standard, quality control (QC), nonspecific binding (with no antibody) and total count which contained only tracer, were counted in a gamma counter for 60 seconds.

Assay procedure:

1/ Tubes were labeled and arranged in assay rack.

2/ 50µl sample and standard were pipetted into

labeled tubes.

3/ 500µl tracer T4 were dispensed into labeled tubes.

4/ 500µl antibody suspension and NSB reagent were dispensed into labeled tubes

5/ Tubes were vortexed, mixed thoroughly and incubated at 37°C for 45 minutes

6/ Test tubes rack was placed on a magnetic separator and allowed for 10 minutes, then the supernatant was decanted and allowed to drain for minutes.

7/ All tubes were counted in a gamma counter.

Assay procedure for measurement of total and free T3, free T4 was the same as that of total T4.

TSH and hCG were measured using highly sensitive immunoradiometric assay (IRMA)

Specific reagents:

1/ labeled antibody (tracer)

2/ standards

3/ separating agents

4/ IRMA washing buffer

Results

The results of this study covers one hundred and twenty Sudanese normal pregnant women and forty non-pregnant healthy - at Omdurman Islamic university - as control. Their ages ranged between (18 - 45) years at Khartoum and Khartoum north teaching hospital and Omdurman maternity hospital whose serum hormonal levels were analyzed during all trimesters of pregnancy: Total (T_4, T_3, and TSH), for normal pregnant and non-pregnant women were assayed.

Total serum thyroid hormones (T_4 and T_3) concentrations:

Mean serum concentrations of total thyroid hormones (T_4 and T_3) in pregnant and control (non-pregnant women are presented in table (1) and Figs (1 and 2). Serum T_4 and T_3 concentrations in pregnant women increased rapidly and significantly higher ($P< 0.05$) in all trimesters compared to the mean concentration in non-pregnant women. The T_4 concentration continued to increase thereafter, although at slower rate (Fig. 1). The average concentration of T_4 during the second trimester (202 ± 11.7 ng/ml) was significantly higher than that in the first trimester (188.6 ± 9.6 ng/ml) and third trimester (196.4 ± 10.2 ng/ml) (± SEM), but statistically not significantly different. An apparent correlation was observed between increase in gestational age and the increase in T_3 concentration in pregnant women (Fig. 2), the average serum T_3 concentration (3.1 ± 0.13 ng/ml) during the third trimester was significantly higher ($P< 0.05$) than the mean concentrations (2.6 ± 0.21 ng/ml) and (2.3 ± 0.11 ng/ ml) in the first and second trimesters respectively

Serum thyroid stimulating hormone (TSH) concentration

Table (2) and Fig (3) presented the mean concentrations of TSH in pregnant women

throughout all trimesters (1.4 ± 0.18 µU/L), (1.3 ± 0.12 µU/L) and (1.5 ± 0.09 µU/L) respectively, which were similar to the mean concentration of TSH in non-pregnant women (1.7 ± 0.17 µU/L) (P< 0.05). TSH concentration remained within the normal range throughout all gestational age. However, 12.5% of pregnant women in first trimester were observed to have suppressed serum TSH concentration (0.1 - 0.3 µU/L) below the normal concentration (0.4 - 4.0 µU/L).

Free thyroid (FT_4 and FT_3), β-hCG for normal pregnant and non-pregnant women were assayed. The mean ± standard error mean (SEM) of thyroid hormones free (FT_4 and FT_3), hCG were calculated from all different trimester and non-pregnant women.

Serum free thyroid hormones (FT_4 and FT_3) concentrations

The mean concentration of FT_4 in pregnant women (10.0 ± 1.22 pmol/L) in the first trimester was significantly higher (P< 0.05) than the mean concentration of FT_4 (7.9 ± 0.63 pmol/L) in non-pregnant women. However the concentration of the same hormone (5.9 ± 0.34 pmol/L) in the second and (4.2 ± 0.19 pmol/L) in the third trimesters were significantly lower (P< 0.05) than mean concentration (7.9 ± 0.63 pmol/L) in non-pregnant women (table 1 and Fig 1). The increase of FT_3 concentration (3.7 ± 0.23 pmol/L) in pregnant women in the first trimester was not significantly higher than the mean concentration of FT_3 (3.5 ± 0.13 pmol/L) in control (non-pregnant women). However, the concentrations of the same hormone (2.8 ± 0.18 pmol/L) and (1.9 ± 0.11 pmol/L) in the second and third trimesters respectively were significantly lower (P< 0.05) than control concentrations, as in table (1) and Fig (2). Apparent inverse relationship was observed between FT_4 and FT_3 concentrations throughout all trimesters.

Serum human chorionic gonadotrophin (β-hCG) concentration

Mean concentrations of serum hCG in pregnant women showed in table (2). Average hCG concentration for the three trimesters were first trimester (61859.4 ± 14265.9 IU/L), second trimester (28902 ± 3678 IU/L) and third trimester (26246 ± 3439.5 IU/L), the SEM was relatively large. Highly significant increase (P< 0.01) of mean hCG concentrations that limited in the first trimester and decreased significantly and sharply in the second and third trimesters.

Table (1): Serum total thyroid hormones concentrations:

No. of Samples	Trimesters	Weeks of gestation	Concentrations of T_4 (ng/ml)	Concentrations of T_3 (ng/ml)
40	First trimester	5 -12	188.6 ± 9.6	2.6 ± 0.21
40	Second trimester	13 - 27	202 ± 11.7	2.3 ± 0.11
40	Third trimester	28 - 39	196.4 ± 10.2	3.1 ± 0.13
40	Control (non-pregnant)	–	116±2.8	1.8 ± 0.075

Fig (1): Serum free T4 mean concentration during trimesters of pregnancy compared with control (non-pregnant) women.

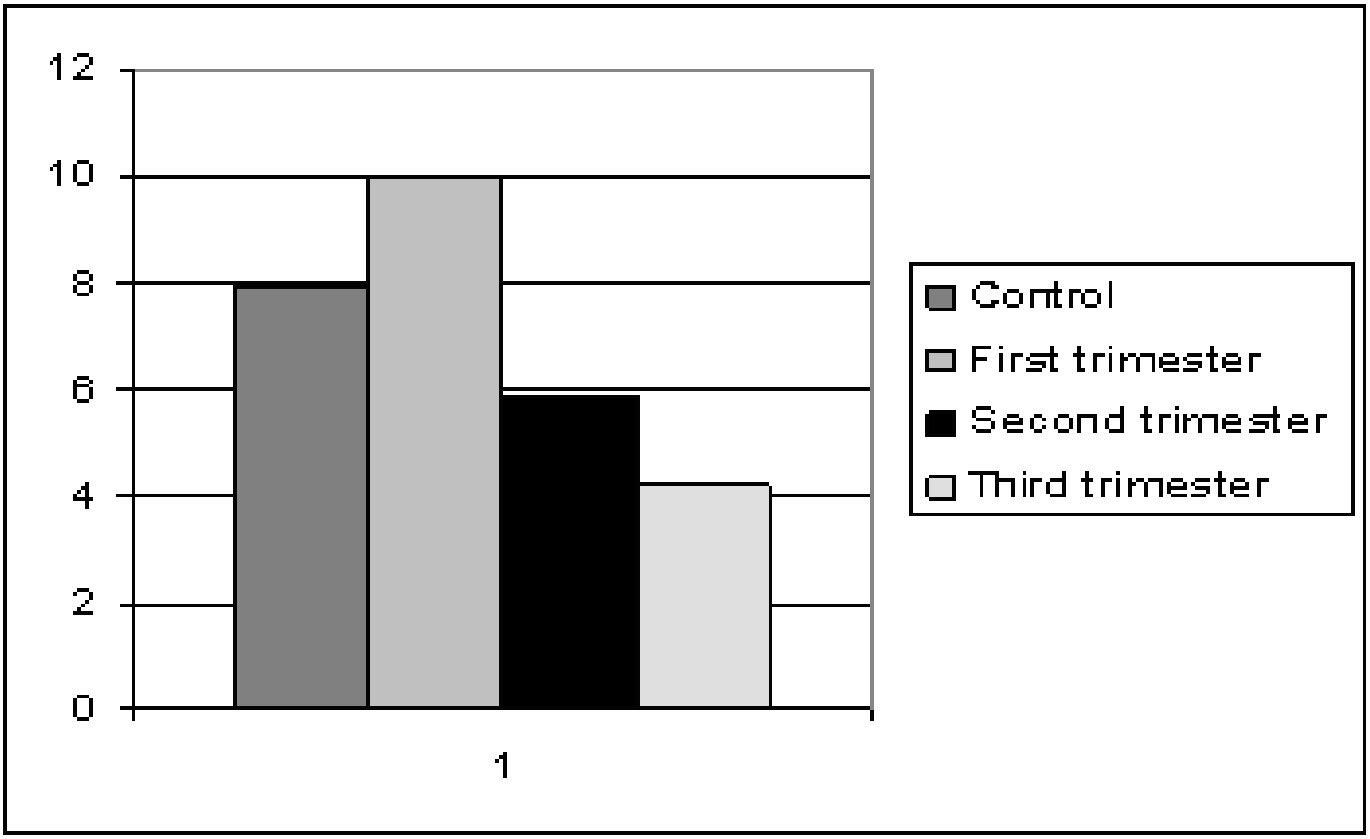
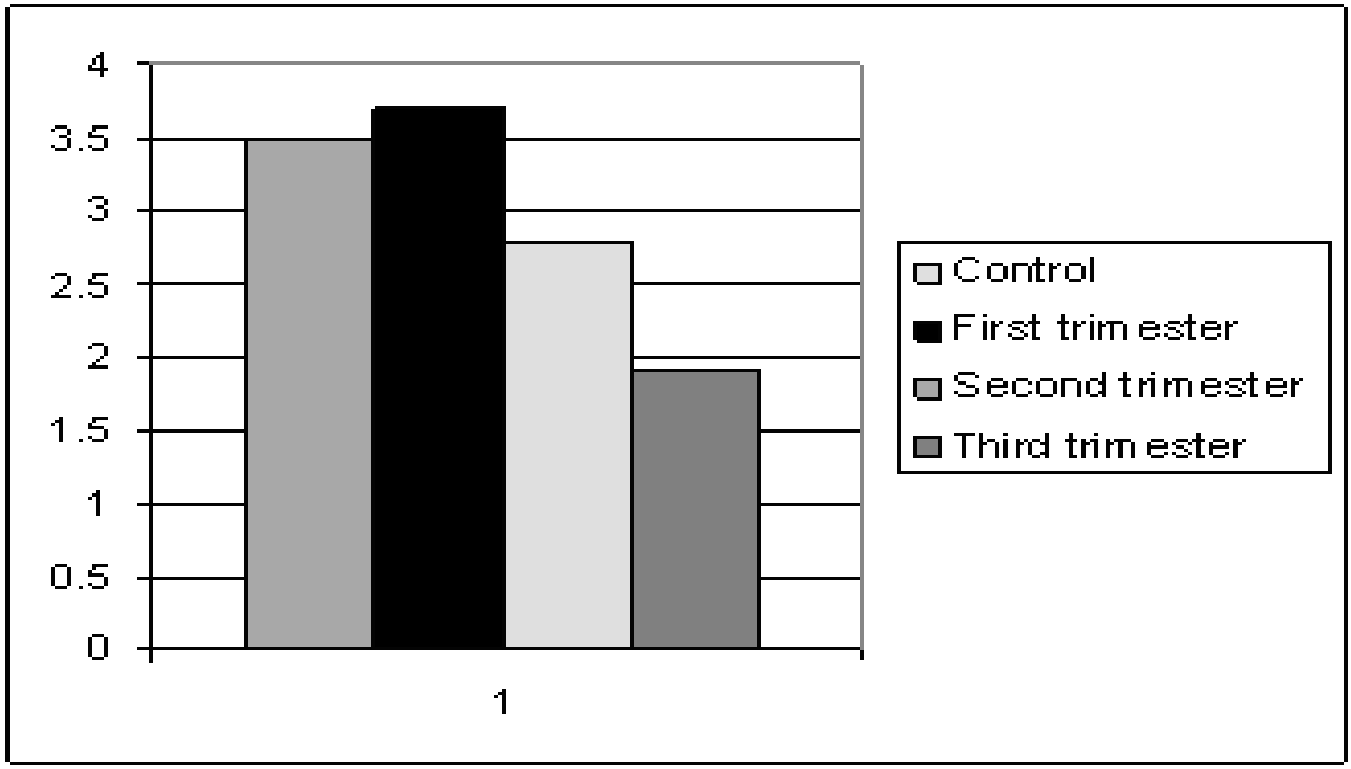


Table (2): Serum free thyroid hormones (FT_4 and FT_3) concentrations

No. of Samples	Trimesters	Weeks of gestation	Concentrations of free T_4 (pmo1/L)	Concentrations of free T_3 (pmol/L)
40	First trimester	5 -12	10 ± 1.22	3.7 ± 0.23
40	Second trimester	13 - 27	5.9 ± 0.34	2.8 ± 0.18
40	Third trimester	28 - 39	4.2 ± 0.19	1.9 ± 0.11
40	Control (non-pregnant)	–	7.9 ± 0.63	3.5 ± 0.13

Values are mean ± SEM

Fig (2): Serum total T_3 mean concentration during trimesters of pregnancy compared with control (non-pregnant) women.



ISSN*: 1858-6147*

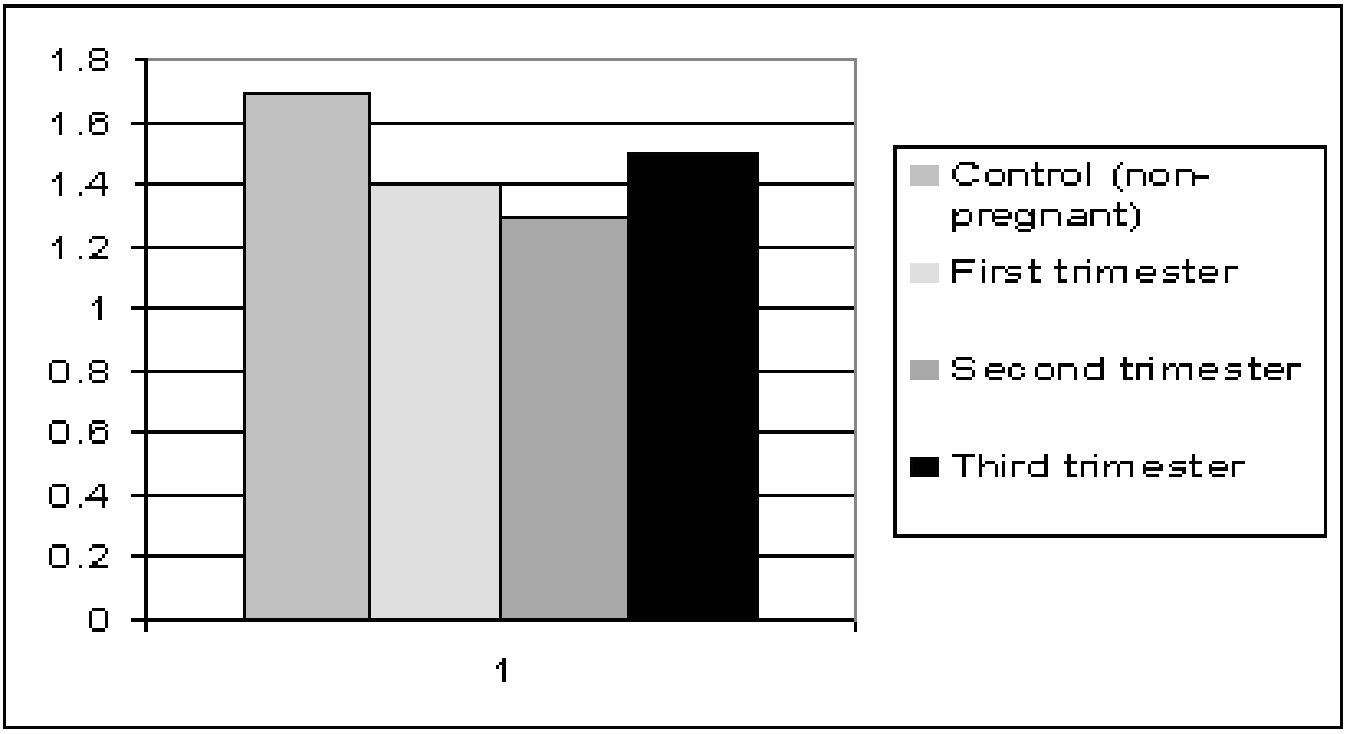
Table (3): Serum human gonadotrophin (β-hCG) concentrations

No. of Samples	Trimesters	Weeks of gestation	Concentrations of β-hCG (IU/L)
40	First trimester	5 -12	61859.4 ± 14265.9
40	Second trimester	13 - 27	28402 ± 3678
40	Third trimester	28 - 39	26246 ± 3439.5

Table (4): Serum thyroid hormone concentration (TSH)

No. of Samples	Trimesters	Weeks of gestation	Concentrations Of TSH (Mu/L)
40	First trimester	5 -12	1.4 ± 0.18
40	Second trimester	13 - 27	1.3 ± 0.12
40	Third trimester	28 - 39	1.5 ± 0.09
40	Control (non-pregnant)	–	1.7 ± 0.17

Fig (3): Serum TSH mean concentration during trimesters of pregnancy compared with control (non-pregnant) women.



Discussion:

The present study showed that the increase in thyroid total hormones (T_4 and T_3) concentrations that occur during pregnancy did not proceed at steady rate. Serum T_4 and T_3 concentrations rose rapidly and were significantly higher than non-pregnant women during all trimesters of pregnancy. T_4 concentration continued to increase, thereafter although at slower rate. In contrast T_3 increased in early pregnancy and slightly decreased in the second trimester and then continued to increase until the end of pregnancy. The increase of T_4 and T_3 may be due to the progressive increase of thyroxine binding globulin (TBG) in the first trimester of pregnancy. As a result of reduced clearance by the liver, due to increase of estrogen secretion throughout pregnancy [13]. These results were similar to these reported by [1, 6 & 14]. The increase of T, after first trimester of pregnancy was associated with increased requirement of T_4 and T_3 during pregnancy, these mostly common in women with hypothyroidism who were treated with T 4 therapy if they become pregnant. In those women the dose of T_4 has been increased by 25 - 50 % to maintain normal serum thyroid stimulating hormone (TSH) level during pregnancy [15 & 16]. The specific reason for this increase need for T_4 is unknown. The increase of T_4 to about 30mg which is largely achieved during first trimester, since the increase of T, requirement continues until delivery.

In this study, TSH remained within the normal range (0.4 - 4.0 mU/L) during all trimesters of pregnancy. Although pregnant women had serum TSH level was suppressed below the normal in the first trimester (12.5%), but within the normal range of TSH. The increase in T4 level in pregnancy is probably due to non-pituitary stimulation of thyroid hormones, on the other hand the negative feedback control system of hypothalamic pituitary thyroid axis functions normally during pregnancy. The result of the present work was similar to that previously reported by [17]. The results showed suppressed TSH in first trimester, which might be due to severity of morning sickness in women with hyperemesis gravidareum in which hCG level was higher than normal. These results were in accoord with those reported by [6]. This was associated with a decrease in serum TSH, which suggested a physiological activation of thyroid gland [6].

The maternal free thyroid hormone concentration has been variously reported to be unchanged [19] or decreased [20] or increased [21 and 22].

Serum free thyroid hormones (FT_4 and FT_3) changes

Figs (I and 2) showed that there was increase of free T_4 and free T_3 only in the first trimester and then decreased gradually in the second and third trimesters, although the increase of free T_4 was limited in the first trimester is statistically significant, while the increase of free T 3 is not significantly different compared to non-pregnant women. While level of FT_4 and FT_3 in second and third trimesters remain within the normal range. The reason of the decrease of free thyroid hormones is

not clear, but the interrelationship of TSH, estrogen and TBG may be important. These findings agreed with [14 and 18]. Thyroid hormone test in the first trimester has been slightly different from that in the second or third trimesters, therefore, the stage of pregnancy should also be considered when evaluating thyroid function in pregnancy, these results similar to reports by [6, 8 and 21].

Serum human chorionic gonadotrophin (hCG):

In the present study it has been shown that serum hCG concentration increased only in the first trimester of pregnancy (Table 2) and then decreased progressively in the second and third trimesters of gestation. The difference between the first and second trimesters values were significant as was the difference between the first and third trimesters. It was well known that excess hCG has a thyroid stimulating effect when TSH was within the normal level. The significant correlation between serum hCG and FT_4 concentrations suggested that thyroid gland is physiologically activated by hCG in early pregnancy. These results confirmed those reported by [6, 18 and 23] who considered that hCG has intrinsic thyrotrophic activity. There is considerable evidence that hCG has intrinsic thyrotrophic activity [24]. These results reflected the effect of hCG on thyroid hormones in the first trimester.

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Original Article

Sudan Medical Laboratory Journal

Cholesterol of normal immature neutrophils: comparison with acute myeloblastic leukemia cells and normal neutrophiIs

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Abstract

The lipid composition of immature myeloid cells from the bone marrow of normal persons and myeloblasts from patients with acute myeloblastic leukemia was studied and compared with the lipid composition of normal mature human neutrophils. Total cholesterol, phospholipid, and fatty acid composition was determined on each cell type. The leukemic cells showed decreased total cholesterol and cholesterol-to-phospholipid ratio, increased phosphatidylcholine and phosphatidylinositol, decreased phosphatidylethanolamine, and an increased percentage of unsaturated fatty acids when compared to normal mature neutrophils. A nearly identical pattern was seen in the normal immature myeloid precursors from normal bone marrow. We conclude that the altered lipid composition of acute myeloblastic leukemia cells is related to unexplained factors related to cell age and not to malignancy per se.-Klock, J. C., and J. K. Pieprzyk. Cholesterol, phospholipids, and fatty acids of normal immature neutrophils: comparison with acute myeloblastic leukemia cells and normal neutrophils. *J. Lzptd Res.* 1979. 20: 908-91

Introduction

Acute myeloblastic leukemia (AML) in humans is a proliferative disorder in which the progeny of certain primitive myeloid cells fail to differentiate and accumulate in the body of the host. Such cells do not share the morphologic or knctional characteristics of their mature counterparts, the neutrophils. When compared to neutrophils in vitro, leukemic myeloblasts show an increase in cellular rigidity (l), a low adhesiveness to glass and plastic, a low propensity to aggregate, a slow rate of cell spreading, and a very diminished ability to form pseudopodia and to move and ingest particles [(2)]. These same in vitro characteristics of AML cells have also been observed in normal immature marrow myeloid cells [(3, 4)].

AML cells differ from normal neutrophils in their lipid composition, having decreased cholesterol content, a decreased cholesterol-to-phospholipid molar ratio, a decrease in phosphatidylcholine (PC), and an increase in sphingomyelin (SM) [(5-7)]. If the abnormal lipid pattern of AML cells is peculiar to these cells and is not shared by their normal counterparts, then it is possible that the

alteration in lipid content may be a determining characteristic in their abnormal growth and development. With these questions in mind we have developed methods for the isolation of normal immature myeloid cells from bone marrow and have examined their lipid composition and compared it with myeloblasts from patients with AML and with normal mature neutrophils.

Materials and methods

Cell isolation

Normal mature neutrophils were isolated by dextran sedimentation and Ficoll- Hypaque gradient centrifugation as previously described [(8)]. The cells were over 95% pure neutrophils. AML cells were purified from the blood of ten patients with acute myeloblastic leukemia. The patients were 22-54 years of age, and all had peroxidase-positive leukemia cells and peripheral blast counts over 30,0OO/p1. The blood was anticoagulated in heparin (10 U/ml) and the AML cells were isolated by centrifugation on Ficoll-Hypaque as described above. Cells were over 90% myeloblasts and promyelocytes. Normal bone marrow myeloid precursors were isolated from normal bone marrow anticoagulated with heparin (20 U/ml). Adenosine diphosphate (Sigma Chemical Co., St. Louis, MO) was added to a final concentration of M to aggregate platelets, and the mixture was poured into an inverted plastic syringe which was packed with nylon wool fiber (3 denier, Fenwal Laboratories, Deerfield, IL). The syringe was placed at 37°C for 30 min, and the nonadherent cells were eluted with 10 volumes of 0.15 MNaCl. Eluted cells were placed on a Ficoll-Hypaque gradient as above and centrifuged at 440g at room temperature for 45 min. Cells at the Ficoll-NaC1 interface were removed by aspiration, and cell counts were performed. Differential counting of cells revealed 65% of the cells were myeloblasts, promyelocytes, and myelocytes. Approximately 25% were metamyelocytes, and the remainders were megakaryocytes, lymphocytes, and monocytes.

Lipid studies

Spectral grade solvents from Fisher Chemicals, Pittsburg, PA were degassed and stored under nitrogen. All material studied was extracted overnight in 20 volumes of chloroform-methanol 2: 1. Extracts were filtered through glass wool and washed three times with chloroform-methanol 2: 1. The aqueous phase was removed and water-soluble salts and water were removed from the organic phase by flash evaporation in the presence of Sephadex G-25 [(9)]. Extracts were stored in Teflon-capped vials under nitrogen at -20°C until analyzed. Cholesterol determination was done according to Wybenga et al. [(1 0)] using a solution of *64%* (w/v) ethyl acetate- 15 N HzS04 containing 30% FeC13 (w/v). After mixing with the sample and heating for 90 sec at 100°C, absorbance was read at 595 nm. Phosphorus analysis was done using a modification of the method of Ames and

ISSN*: 1858-6147* S*ud Med Lab J, issue 1, vol 1 (2011)*

Dubin [11]. Samples were ashed in HC104-H2S04 solution for 2 hr at 180°C with added Hz02 and continued heating if color persisted. Three ml of a solution of 2.5 mM ammonium molybdate, 0.1 M sodium acetate, and 0.05 M ascorbic acid was used as color reagent, and absorbance was determined at 790 nm after color development at 80°C. Known phospholipid standards (Applied Science Laboratory, State College, PA) were co-chromatographed with samples for identification.

Two-dimensional thin-layer chromatography of phospholipids was performed using a modified method of Broekhuyse [12]. Extracts containing 10 pg of lipid phosphorus were dried to 5O-pl volumes and spotted onto precoated thin-layer plates (Silica Gel H, Applied Sciences). Plates were then developed two-dimensionally in chloroform-methanol-ammonium hydroxide 130: 80:15 and chloroform-acetateacetic acid-methanol-water 100:40:20:20: 10. After drying, the plates were exposed to I2 vapor and the spots were scraped and analyzed for phosphorus.

Fatty acid analysis was done on methylated samples after reaction of 20-50 *pg* of lipid with 1% sulfuric acid in methanol at 70°C for 8 hr and extraction with hexane. Samples were dried under N2 and dissolved in CS, for injection into the gas chromatograph. The gas chromatographic column was 5% DEGS-PS (Supelco, Inc, Bellefonte, PA); carrier gas was N2, and the column temperature was 180°C. Quantitation was done by measuring peak areas; identification of fatty acids was done by comparing retention times with those of the following fatty acid standards: 14:0, 14:1, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 20:5,22:0,22:1,22:5,22:6,24:0,24:1 (Supelco, Inc.). Student's *t* test was used for tests of significance.

Results

Results of lipid analyses of neutrophils (PMN), AML cells, and normal immature myeloid cells (BM) are shown in Figs. 1 and 2. Fig. 1 shows that total lipid phosphorus was higher in AML and BM cells ($P > 0.$ l), and total cholesterol was lower in AML and BM cells ($P < 0.01$) when compared to PMN cells. Cholesterolto-phospholipid molar ratios were 0.52 f. 0.1 for PMN and 0.30 * 0.12 for AML and BM cells, respectively ($P < 0.05$). Less than 10% of the cholesterol was in the esterified form (data not shown).

Fig. 2 shows the phospholipid composition of the three cell types. AML and BM cells had significantly increased proportions of PC when compared to PMN ($P < 0.025$). AML and BM cells also showed increased phosphatidylinositol (PI) and decreased phosphatidylethanolamine (PE), but with these small numbers the values are not significant ($P > 0.2$). Specific analyses for plasmalogens and lysobisphosphatidic acid were not done [13]. Fatty acid analyses on the total phospholipid extract are shown in Table 1. The major finding is that most of the fatty acids in PMN are saturated (58%), whereas AML cells and

BM cells had 49% and 41% saturated fatty acids, respectively ($P < 0.05$). This was mostly due to an increase in 16:O in PMN PE and a decrease in 20:4 and 18:2 in PMN SM, and an increase in 18:2, 18:3, and 20:4 in AML and BM SM, PS, and PI as shown in Fig. 3.

Discussion

This work describes a method for the purification of an enriched fraction of immature normal myeloid precursors from normal bone marrow which has allowed us to study the significance of the altered lipid composition of AML cells. The data on AML cells and PMN agree with previously published data [5-7, 13, 15]. Gottfried [6] previously suggested that the alterations in lipid composition of leukemia cells might be related to their maturational age. In contrast to those studies in which analyses were done on normal transformed lymphocytes and myeloid leukemic cell lines maintained in long-term culture, we have performed analyses on the normal immature counterpart of the leukemic cell. This study has confirmed that the alterations in neutral and phospholipids in AML cells are shared by normal immature myeloid cells, and the data are in keeping with recent results of similar studies in lymphocytes [14]. The reason for the altered lipid patterns in the immature myeloid cells (AML and BM cells) is not clear from these studies.

It is possible that the differences are reflections of subcellular organelle and membrane composition, e.g., the increase in saturated fatty acids in PMN may be explained on the basis of an increase in the number and kind of intracellular granules which are known to be enriched in such fatty acids [13, 15]. We do not know whether the altered cholesterol, phospholipid, and fatty acid composition of the young neutrophil has anything to do with its decreased deformability, decreased adhesion to surfaces, low propensity to aggregate, and diminished locomotion and ingestion. However it seems unlikely that the lipid changes in leukemic cells are related to their abnormal growth and development. It was initially felt that the abnormal lipid composition in leukemic cells was directly related to their malignant potential [16, 17]; however, recent studies now suggest that some of these changes may be related to the growth cycle of the leukemic cell [18]. Differences in glycoprotein and/or glycolipid composition of these cells might also explain behavioral differences; nevertheless, the results of our studies underscore the necessity for correlating abnormal biochemical findings in AML cells with the results obtained from similar studies in normal immature myeloid cel1s.

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Original Article **Sudan Medical Laboratory Journal**

The rate of anti-*Toxoplasma* antibodies in females in Umdawanban village, Sherg Elnil (Khartoum North)

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Abstract

The present study was carried out Umdawanban village, where serum samples taken from 100 pregnant and 100 non-pregnant women were examined for anti-Toxoplasma antibodies by latex agglutination and ELISA tests. They were 14-45 years old.

The overall rate of anti-*Toxoplasma* antibodies was 6% by ELISA (IgM) and 22.5% by the latex agglutination test.

The results showed that the highest prevalence rate was reported among the 15-20 age group (28.6%) when using the latex agglutination test and 20% among the 41-45 age group when using ELISA test. Drinking milk was found to be of no significance in the transmission cycle. However, different rates were reported among those who consumed different types of meat. A statistically significant difference was reported between different trimesters in pregnant women. Contacts with cats have been shown to be of great importance in the transmission cycle.

There was no correlation between the history of abortions and occurrence of toxoplasmosis.

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Introduction

Toxoplasmosis is a universal zoonotic disease caused by protozoan *Toxoplasma gondii.* The parasite infects most warm-blooded animals e.g. human being, cattle, sheep, goats, camels, cats, dogs, rats, mice, pigeons and chickens **(1)**, but the primary host is the field (cats), family, in which all the stages of this accidium, including the highly resistant and infective oocyst, have been positively identified **(2)**. Animals are infected by eating infected meat, through both direct and indirect contant with cat faeces or by transmission from mother to fetus. The consumption of unwashed vegetables or undercooked meat or unpasteurized milk from infected animals is potentially sources of infection **(3)**.

Toxoplasmosis in Sudan:

In Sudan, the first report of human toxoplasmosis dates back to 1966 **(4)** when Carter and Fleck, using the dye test carried out a survey in Khartoum and Gezira. They reported a prevalence of 27.8% in the general population excluding children less than 10

years of age. Later, Abdelhameed [5] investigated the disease in Gezira from where he reported a prevalence of 41.7% with females showing a higher prevalence rate than males. He observed that there was no correlation with animal contact and no cases of active toxoplasmosis were detected as indicated by the negative IgM test. [4]

In 1994, a cross-sectional survey was carried out in Khartoum by Al Hindy [6]. He collected samples from 5 sources and examined them for *Toxoplasma* specific IgG and IgM antibodies by ELISA. He reported that 17.5% of males and 30.1% of pregnant women had positive IgG reaction. The difference in prevalence rates between the two sexes was not significant.

During the period from June to December 1996 across-sectional survey was carried out in Khartoum hospital and Omdurman maternity hospitals by Alhadi [7]. In this study, serum samples were collected from 487 pregnant women. Screening test for toxoplasma specific IgG antibodies was done using ELISA. IgG sero positive prevalence rate was found to be 34.1%. Also 35 subjects with IgG were re-examined by ELISA for IgM antibodies. He found that 14.3% has positive IgM antibodies indicating active recent infection. [5]

A study in 2001 was conducted by abdel Rauof in Khartoum where serum samples were taken from different groups including males, pregnant women, aborters, patients with sleenomegaly, patients with vision defects and mentally retardation patients. Screening for anti-*Toxoplasma* antibodies was done using latex agglutination and specific IgG and IgM using ELISA. The overall rate of anti-*Toxoplasma* antibodies was 17.3% by ELISA and 13.4% by latex agglutination test. Found that there was no correlation between abortion and high specific *Toxoplasma* antibodies titers. [8]

In study carried by Bushra [9], the overall rate of anti-*Toxoplasma* antibodies was 5.7% by ELISA IgM and 23.9% by latex agglutination test in pregnant women. He reported that positive was more expressed in the age group 20-40 (36.3%).

As a continuation to elucidate more on the situation to toxoplasmosis among women, this study was designed to investigate the disease in Umdawanban village in sherg Elnil district in Khartoum North.

Materials and methods:

Study area:

The study was conducted in Umdawanban village that located about 45 kilometers from Khartoum center.

Types of study:

An observation case finding hospital-based study (comparative)

Study population:

The study was conducted on female representing pregnant and non-pregnant ladies

Sample size:

Samples were taken from 200 females comprising 100 pregnant ladies and 100 non-pregnant ladies. The females were categorized according to their

ISSN*: 1858-6147*

age groups (15-20, 21- 25, 26-30, 31-35, 36-40, and 41-45 years).

Samples collection:

5ml of serum was collected from each female and was stored separately at -20°C. When required, aliquots were thawed to room temperature by using water-bath.

Data collection:

A questionnaire was designed for data collection. It includes questions on milk, meat pregnancy stage, contact with cats and history of abortion.

Techniques:

Direct agglutination test:

Commercial kits produced by Linear chemicals SI was used

Procedure:

- Before using the kit, components were allowed to reach room temperature
- Components were gently shacked (r. Toxo-latex) to disperse the latex particles
- The reagent was checked against positive and negative controls
- 50µl of serum sample is placed into one of the circles on the card. One drop of the positive control and one drop of the negative control were dispersed into two additional circles.
- 25µl of toxo-latex were added next to the serum
- Both drops were mixed by spreading them over the surface of the circle
- The slide then was rotated by means of a mechanical rotator at 100 R.P.M for about 5 minutes. The presence or absence of visible agglutination was read.

Interpretation of the results:

A homogenous appearance (negative reactions) should be interpreted as absence of Toxoplasma antibodies or titer less than 10 IU/ml.

A clear agglutination (positive reaction) should be interpreted as presence of Toxoplasma antibodies which may reflect either a past infection or an evolving Toxoplasma infection.

Enzyme linked immune sorbent assay (ELISA):

Index Toxo IgM EIA kit:

Enzyme immunoassay (EIA) procedure for the determination for IgM antibodies to Toxoplasma gondii, for in vitro diagnosis use only.

Assay procedure:

1. The required numbers of micro-wells were placed in the micro-wells holder. One end of each strip was marked for orientation
2. The sample dilutions were prepared by mixing 1/100 using serum diluent (10 µl serum to 1ml diluent fluid). The calibrators were not diluted as they were ready for use. The diluted samples were incubated for 30 minutes at room temperature
3. 100µ of negative control, low positive standard (cut-off), high positive standard and serum specimens were added to subsequent wells

4. The micro wells were incubated at room temperature for 15minutes
5. The micro wells were washed by inverting and flicking into a sink, completely filled with wash buffer and washes will be repeated three times, refilled with wash buffer and soaked for 5 minutes wells and blot were emptied with absorbent paper. Using an automatic washer the wells were filled and aspired five times without soak.
6. 100µl of enzyme conjugate were dispensed into each well and incubated at room temperature for 15 minutes.
7. At the end of incubation period the contents of the well were discarded and washed as outlined in step five.
8. 100µl TMB substrate was added to each well and incubated at room temperature for 10minutes.
9. The reaction was stopped by adding 100µl stop solution to each well. This produced colour. Immediately, the absorbance of each well was measured at 450n.m.

Calculation and interpretation results:

For each test and control serum the average optical density (OD) obtained during the test run was determined.

- The average OD of the low positive control was calculated. This was the cut-off value of the assay.
- The sample OD was divided by the value obtained in (1) above.
- A ratio greater than 1.0 indicates a positive sample. A ratio lower than 0.9 indicates negative sample. A ratio between 0.9 – 1.1 indicates equivocal result. An equivocal sample was re-tested with a fresh new sample. In case that the same equivocal result is obtained, the test was repeated with a new sample after 2-4 weeks.

Statistical analysis:

Data were analyzed by using SPSS soft ware program.

Results:

Out of 200 serum samples (100 pregnant and 100 non-pregnant women) which were examined by latex agglutination test, 45 samples were found positive for anti *Toxoplasma* antibodies (table 1). This constituted an overall rate of 22.5%.

When the same samples were examined by ELISA test, 12 samples were found to be positive for anti *Toxoplasma* antibodies. This constituted an overall rate of 6%. As all these cases detected by ELISA were IgM related, latex detected only 3 of them representing 6% and 2%, detection rate respectively (table 2). This difference was found to be statistically insignificant (p=0.734).

When age was considered, the percentage with positive anti *Toxoplasma* antibodies by ELISA test ranged between 3.3% in 21-25years and 20% in 41-45years age group. The peak rate of antibodies was observed in the age group 41-45years (table

3). This difference was found to be statistically insignificant (p=0.295).

The percentage of positive anti *Toxoplasma* antibodies using latex agglutination test ranged between 16% in 31-35 years and 28.6% in 15-20 years. The peak rate of antibodies was observed in the age group 15-20 years (table 3). This difference was found to be statistically insignificant (p=0.428).

Out of the 123 samples of women drinking cow milk, 8 samples (6.5%) were found to be positive for anti *Toxoplasma* antibodies by ELISA test (table 4) and out of 56 samples of women drinking cow and goat milk, 4 samples (7.1%) were found to be positive for anti *Toxoplasma* antibodies.

When the same samples were examined by latex agglutination test for women drinking cow milk, 24 samples (19.5%) were found to be positive. Out of the 56 women who consumed cow and goat milk, 16 samples (28.6%) were found to be positive. Out of the 15 samples of women who consumed goat milk, 2 samples (13.3%) were found to be positive. In 6 women who did not consume milk, 3 samples (50%) were positive (table 4). There was no significant difference between cow milk and other types of milk (p=0.162)

Among 7 serum samples of women who ate beef, when ELISA test was applied, one sample (14.3%) was found positive (table 5), and 2 samples (28.6%) were positive for anti *Toxoplasma* antibodies by latex agglutination test. Out of the 78 women who consumed mutton, the percentage of positives was 7.7% by the ELISA test (table 5), and 24.4% by the latex agglutination test (table 9 and figure 9).There was no significant difference between the types of meat consumed (p=0.793).

The results showed that the highest rate (16%), was reported in the first trimester when using ELISA and the lowest 0.0% was reported in the third trimester while the second trimester showed 7.9% detection rate (table 6). There was insignificant difference between pregnancy trimesters when using ELISA test (p=0.065). For latex agglutination test, the highest rate was reporting in the first trimester (40%) and the lowest (13%) was reported among the non-pregnant women, the second and third trimesters reported rates of 28.9% and 29.7% respectively (table 6). This difference was found to be statistically significant (p=0.009).

The result revealed that anti *Toxoplasma* antibodies appeared in 6.1% of those who are in contact with cats and in 5.8% of those who have no contact with cats when using ELISA (table 9).This difference was statistically insignificant (p=0.930). When using latex agglutination test anti Toxoplasma antibodies appeared in 19.1% of those who are in contact with cats and 29% of those who have no contact with cats (table 7). This difference was statistically insignificant (p=0.111).

The history of previous abortion revealed that the highest detection rate of anti Toxoplasma antibodies when using ELISA 18.2% was reported

in one abortion group while the group with a history of four abortions showed no antibodies (table 8). The differences was found to be statistically insignificant (p=0.113). When using latex agglutination test the rate was higher in both groups 22.7% and 100% respectively (table 8). This difference was found to be statistically insignificant (p=0.374).

Tables:

Table 1: The rate of anti *Toxoplasma* antibodies in the study group obtained by ELISA (IgM) and latex agglutination test (IgM and IgG)

Test	Number Examined	Positive	Percentage
ELISA	200	12	6%
Latex	200	45	22.5%

Table 2: Detection rate of anti *Toxoplasma* antibodies (IgM) using ELISA and latex agglutination test

	Number Examined	IgM detected	Detection rate
ELISA	200	12	6%
Latex	200	3	2%
P. value	0.734		

Table 3: The rate of anti *Toxoplasma* antibodies according to age groups

Age groups (years)	Number Examined	Positive	
		ELISA	Percentage
15-20	56	4	7.1
21-25	60	2	3.3
26-30	33	3	9.1
31-35	25	1	4
36-40	16	0	0
41-45	10	2	20
Total	200	10	6
P.value		0.295	

Table 4: The rate of anti *toxoplasma* antibodies according to types of milk

Type of milk	Number Examined	Positive			
		ELISA	Percentage	Latex	Percentage
No milk	6	0	0	3	50
Cow	123	8	6.5	24	19.5

Goat	15	0	0	2	13.3
Cow + goat	56	4	7.1	16	28.6
Total	200	12	6	45	22.5
P.value		0.676		0.162	

Table5: The rate of anti *Toxoplasma* antibodies according to types of meat consumed

Type of milk	**Number Examined**	**Positive**			
		ELISA	**Percentage**	**Latex**	**Percentage**
Cow	7	1	14.3	2	28.6
Goat	5	0	0	1	20
Sheep	78	6	7.7	19	24.4
Cow + Sheep	60	4	6.7	8	13.3
Sheep + goat	6	0	0	2	33.3
Cow + goat +sheep	40	1	2.5	11	27.5
Cow + goat	4	0	0	2	50
Total	200	12	6	45	22.5
P.value		0.676		0.162	

Table 6: The rate of anti *Toxoplasma* antibodies according to pregnancy trimesters

Stage	**Number Examined**	**Positive**			
		ELISA	**Percentage**	**Latex**	**Percentage**
Not-pregnant	100	5	5	13	13
1st trimester	25	4	16	10	40
2nd trimester	38	3	7.9	11	28.9
3rd trimester	37	0	0	11	29.7
Total	200	12	6	45	22.5
P.value		0.065		0.009	

Table7: The rate of anti *Toxoplasma* antibodies according to contact with cats

Type of milk	**Number Examined**	**Positive**			
		ELISA	**Percentage**	**Latex**	**Percentage**
Contact with cats	131	8	6.1	25	19.1
Not contact with cats	69	4	5.8	20	29

P.value		0.930		0.111	

Table 8: The rate of anti *Toxoplasma* antibodies according to pregnancy trimesters

No. of abortions	Number Examined	Positive			
		ELISA	Percentage	Latex	Percentage
No abortion	162	7	4.3	37	22.8
1	22	4	18.2	5	22.7
2	9	1	11.1	1	11.1
3	6	0	0	1	16.7
4	1	0	0	1	100
P.value		0.113		0.374	

Discussion

From the result, the overall prevalence rate of anti *Toxoplasma* antibodies using latex agglutination test was found to be 22.5%. This rate was found to be lower than the rate reported by abdel Hameed [(5)] who reported rates of 41.7% However, our rate was higher than the rate reported by abdel Rauof [(8)] who reported rates o 13.4%. It is to be mentioned that our rates was almost similar to that of Bushra [(9)] who reported rates of 23.9%.

When detecting IgM, our results revealed that ELISA detected 12 cases while latex agglutination test detected only 3 cases. Despite the fact that the difference between the two methods was statistically insignificant, one would properly suggest that ELISA test is comparatively highly sensitive in detecting IgM (table 2). Presence of IgM is an indication that these women have acquired recent *Toxoplasma gondii* infection. For pregnant women, the presence of IgM is an opportunity for the parasite to move trans-placental to the fetus, thus increasing the possibility of congenital toxoplasmosis.

The results clearly indicate that the higher prevalence of toxoplasmosis was reported among 41-45 years group (20%) by ELISA and among 15-20 years age group (28.6%). Both rates are less than rate reported by Frenkl and Ruiz [(2)] who reported 61.4% prevalence rate among 15-25years age group.

As far as milk consumption is concerned and its association with the occurrence of toxoplasmosis, and in our opinion, this fact might probably be neglected as it has not that much significance in th transmission cycle.

In this investigation, the rates reported among those who consumed different types of meat (beef and mutton) were found to be 14.3% and 7.7% respectively when ELISA was used and 28.6% an 24.4% respectively when latex agglutination test was used. As shown above, the role of eating raw or undercooked beef or mutton is of great

importance in the transmission of cysts containing bradyzoites. This concept was high lightened by Basalamah and Serebour [10] in France where raw meat is a government item who not surprisingly reported Toxoplasma serology rate as high as 60-87%. Our finding is strengthen by Abdel Hameed [5] who related the high rate of toxoplasmosis to the consumption of raw or partially cooked liver, marrara, undercooked meat, shaya and spleen in Sudan.

This investigation revealed that there is no statistically significant difference between those who are in contact with cats and those who are not. This result contradiction with the results of Feldman [11] who did not find antibodies to *Toxoplasma gondii* in the Pacific Island when cats are absent suggesting strong evidence that cats are very important in the transmission cycle.

For pregnant ladies, the study showed that the highest prevalence rate 40% and 16% for latex agglutination and ELISA tests respectively were reported in the first trimester. For the second the third trimesters, the relatively high prevalence rates were reported (28.9% and 29.7%) respectively by latex agglutination test. This result might probably suggest the possibility of maternofetal transmission. this type of transmission as suggested by Pratlong et al [12] increases in the second trimester of pregnancy to 25-53% and in the third trimester, it reaches 65% and up to 80% at term as the highest incidence of congenital toxoplasmosis. As mentioned by Russo and Galanti, [13]; Klapper and Morris, [14].

As far as the number of abortions is concerned, the prevalence rate was found to be 18.2% and 22.7% for ELISA and latex tests respectively. If these rates were compared with those with no history of abortions, the rates reported were 4.3% and 22.8% for ELISA and latex agglutination test respectively. The difference was found to be statistically insignificant suggesting that *Toxoplasma* infection may have not contributed to the cause of abortions. This finding was not in agreement with the finding of Griffin and Williams [15] who reported a prevalence of 42.3% in who has a history of abortion indicating that Toxoplasma infection may in this case have contributed to the cause of abortions.

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Original Article

Sudan Medical Laboratory Journal

The prevalence of *Schistosoma haematobium* among the population of Keryab village, Sharg El Nil, Khartoum North with emphasis on secondary bacterial infection

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Abstract

The study was conducted in Keryab village to investigate the association, if any, between *Schistosoma haematobium* infection and urinary bacteria. The study revealed that the overall infection rate was 25% (78 out of 312 urine samples examined). The prevalence in females was higher than that in males (34% and 22% respectively).

The highest infection rate (30%) was reported among the 11-20 years age group, while the infection was 0.0% among 21-30years age group. Bacteria were isolated from 12 urine samples of those with *S.haematobium* infection. These compromised the following: *E. coli, Enterococcus faecalis* and *Proteus species*.

On the other hand, 36 urine sample out of the 234 individuals with negative S. haematobium infection showed bacterial growth comprising the following: *E. coli* isolated from 12 samples, *Enterococcus faecalis* from 4 samples, *Salmonella typhi* from 2 saples *Staphylococcus aureus* from6 samples, Staphyllococcus saprophyticus from 2 samples, *Proteus* species from4 samples, *Klebsiella* species from 2 samples and *Pseudomonas* from 4 samples.

Introduction

Human Schistosomiasis is the major health roblems in many countries including Sudan. the disease is a chronic, debilitating and remains one of the most prevalent parasitic infection in tropical and subtrobical environment (WHO, 1993).

Urinary schistosomiasis was reported in Darfur and Kordufan provinces. Wright (1973) estimated that, urinary schistosomiasis ranged between 9% in Darfur and 35% to 47% in Kordufan. These figures were confirmed by other studies (Eltom 1976, Dafa Allah and Suleiman 1988). In khartoum state, the prevalence of both types of human schistosomiasis has been reported (Malek 1985). However, a marked increase in their prevalence was reported after the extensions in the irrigated areas around Khartoum (Hilali1992). Among certain villages in Sharg El Nil, Sitelbanat (2006) reported an overall prevalence rate of 9.9% which reflects the impact of the extension of irrigated areas around Khartoum North.

Literature on secondary bacterial infection in urinary schistosomiasis was meager. We did not come through any of the literature concer4ned with conducting similar studies in Sudan

In *Schistosoma* infections and despite that Melhem and Loverde (1984) reached the conclusion that the stool and often urine culture ate *Salmonella* negative which makes the disease difficult to diagnose, Lambertucci et al (1985) showed that *Salmonella* are easily recovered from h blood and in 25% of cases; they have been isolated from the faeces or urine. Nevertheless, Melhem and Loverde demonstrated a direct association by culture and immunofluorescence of *Salmonella paratyphi* A with a surface tegument of *Schistosoma mansoni.*

In Nigeria, Adeyeba and Ojeaga (2000) studied the prevalence of urinary schistosomiasis and concomitant urinary tract pathogens among 1600 pupils. their study revealed that 920 (57.5%) who had the ova of *Schistosoma haematobium* also had pyuria; 75.4% of which had concomitant bacteruria. The bacteruria isolated included *Klebsiella* species, *Escherichia coli*, and *Staphylococcus aureus* with *E. coli* occurring more frequently than the rest.

In 1970, Lehman et al reported bacterial urinary tract infection, commonly due to salmonella among 75 hospitalized Egyptian farmers with urinary schistosomiasis.

This study aimed to:

- access occurance *Schistosoma haematobium* infection and relate it gender and age groups
- isolate and identify bacteria incriminated in *Schistosoma haematobium* infection

Materials and methods:

Study area:

The study was conducted in Keryab village in Khartoum North. The village is situated 25 kilometers far away from Khartoum center.

Types of study:

Cross sectional study

Study population:

The study was carried out on S. haematobium positive cases that were detected in the area. Negative controls were also considered in the study. The populations were categorized according to gender (223 male and 89 female) and was divided into 4 age groups (1-10, 11-20, 21-30, and over 30 years).

Study period:

The study commenced in March 2008 and ended in September 2008.

Samples collection:

312 mid stream urine specimens were collected as aseptically as possible, in a sterile wide mouth container. Since urine itself is a good culture media, all specimens were processed by the laboratory within 2 hours of collection, or were kept refrigerated at 4°C until delivery to the laboratory and were processed no longer than 18

hours after collection. Wherever possible, urine samples for culture were collected in the morning.

Methodology:

Diagnosis of *Schistosoma haematobium*:

Sedimentation method (centrifugation technique):

Diagnosis of urinary schistosomiasis was conducted using the centrifugation concentration technique. 10 ml of urine sample was centrifuge at 2000rpm for 5 minutes, and the sediment was then examined for each individual microscopically under (10×) the low power field lens. Cheesbrough 1998.

Filtration method:

10 ml of urine sample was collected in plastic disposal syringe, and was then passed through the filter that is held in filter holder, and then the filter holder removed and unscrewed by using blunt ended forceps to remove the filter and was then covered with a cover glass and examined microscopically under (10×) the low power field lens. Cheesbrough 1998.

Isolation and identification of bacteria:

Media:

Blood agar:

Blood agar is widely used in medical bacteriology laboratory. In addition to being an enriched media, it is an indicator media showing the haemolytic properties of bacteria such as streptococcus pyogenes.

Deoxycholate citrate agar (DCA):

The media is particularly suitable for isolation of dysentery bacilli, food poisoning salmonella and paratyphi B, and less so, but superior to MacConky agar.

MacConky agar:

This media is recommended for the selection and recovery enterobacteriacae and related enteric gram negative bacilli.

Biochemical tests:

- urea agar
- citrate agar
- semi solid agar
- Kligler ion agar (KIA)
- peptone water

Gram's stain:

This is the routine bacterial stain used to differentiate between gram positive bactria (dark purple in colour) and gram negative bacteria (red colour), using crystal violet, Gram's iodine, acetone or alcohol and safranine as a counter stain.

Oxidase disc.

Statistical analysis:

Statistical analysis was done using SPSS computer program.

Results:

Parasitological examination; Out of 312 urine sample examined, 78 were found positive for *Schistosoma haematobium* infection. This

constituted over all prevalence rate of 25% (table 1).

Table 1: The overall prevalence rate of *S. haematobium* among the population in Keryab village

Number of examined	**Positive**	**Prevalence**
312	78	25%

The results showed that the prevalence in female was higher than that of males (34% and 22% respectively) (table 2). This difference was found to be statistically significant at p=0.05.

Table 2: The prevalence rate of S. haematobium among the population in Keryab village according to gender

Gender	**No. examined**	**No. positive**	**prevalence**	**P.value**
Male	223	48	22%	0.025
Female	89	30	34%	0.05
Total	312	78		

The highest infection rate (30%) was reported among the 11-20years age group and the lowest infection rate (0) was reported among the 21-30 year age group (table 3). This difference was found to be statistically insignificant at p=0.5.

Table 3: The prevalence rate of S. haematobium among the population in Keryab village according to age groups

Age group	**No. examined**	**No. positive**	**Prevalence**	**P. value**
1-10	182	42	23%	0.144
11-20	112	34	30%	
21-30	10	0	0%	
>30	8	2	25%	
Total	312	78		

For the males, the highest prevalence rate (34%) was reported among 11-20 years age group and the lowest prevalence rate (0) was reported among 21-30 year and over 30 years age group (table 4). This difference was found to be statistically significant at p=0.05.

Table 4: The prevalence rate of S. haematobium among the male population in Keryab village according to age groups

Age group	**No. examined**	**No. positive**	**Prevalence**	**P. value**
1-10	129	20	16%	0.03
11-20	82	28	34%	
21-30	8	0	0%	
>30	4	0	0%	
Total	223	48		

For the females, the highest prevalence rate (50%) was reported among over 30 years age group and the lowest prevalence rate (0) was reported among 21-30 year age group (table 5). This difference was found to be statistically insignificant at p=0.5.

Table 5: The prevalence rate of *S. haematobium* among the female population in Keryab village according to age groups

Age group	**No.examined**	**No. positive**	**Prevalence**	**P. value**
1-10	53	22	42%	0.141
11-20	30	6	20%	
21-30	2	0	0%	
>30	4	2	50%	
Total	89	30		

Bacteriological examination:

Out of 78 urine sample positive for *Schistosoma haematobium* infection, 12 samples showed

bacterial growth. On the other hand, 36 samples out of 234 *Schistosoma haematobium* negative samples showed bacterial growth. This constitutes 15% and 15% respectively (table 6).

Table 6: The overall bacterial growth among the positive and negative S. haematobium infection

S. haematobium result	Number of examined	Positive	Percentage
Positive	78	12	15%
Negative	234	36	15%
Total	312	48	15%

Bacterial culture for those who were positive for *S. haematobium* infection (78) reflects growth of *Enterococcus faecalis*, *Proteus* species and *E. coli* in 12 urine samples. This constitutes a growth rate of 15%. *E. faecalis* was isolated from 4 samples (2males and 2 females) which constitutes 33.3% of the positive cultures. Proteus species from 4 samples (all males) which constitutes 33.3% of the positive cultures (table 7)

Table 7: Ehe growth rates of different bacterial isolated in the positive cultures of positive *S. haematobium* infection

Organism	No. culture positive	No. isolated in culture	Percentage in growth culture
E. coli	12	4	33.3%
E. faecalis	12	4	33.3%
Proteus	12	4	33.3%

Bacterial culture of those who were negative for *S. haematobium* infection (234) showed growth of different microbes in 36 cultures. The different microbes isolated comprise the following (table 8 and figure 4).

1. *E. coli* were isolated from12 samples (4males and 8 females) which constitutes 33.3% of the total growth.
2. *E. faecalis* were isolated from 4 samples (2 males and 2 females) which constitutes 11.1% of the total growth.

ISSN*: 1858-6147*

3. *Klebsiella* were isolated from 2 samples (all females) which constitutes 5.6% of the total growth.
4. *Proteus species* were isolated from 4 samples (all males) which constitutes 11.1% of the total growth.
5. *Pseudomonas* was isolated from 4 samples (2 males and 2 females) which constitutes 11.1% of the total growth.
6. *Staphylococcus aureus* were isolated from 6 samples (3 males and 3 females) which constitutes 16.7% of the total growth.
7. *Staphylococcus saprophyticcus* were isolated from 2 samples (all females) which constitutes 5.6% of the total growth.
8. *Salmonella typhi* were isolated from 2 samples (all females) which constitutes 5.6% of the total growth.

Table 7: The growth rates of different bacterial isolated in the positive cultures of negative *S. haematobium* infection

Organism	**No. culture positive**	**No. isolated in culture**	**Percentage in growth culture**
E. coli	36	12	33.3%
S. aureus	36	6	16.7%
E. faecalis	36	4	11.1%
Proteus spp	36	4	11.1%
Pseudomonas	36	4	11.1%
S. saprofeticus	36	2	5.6%
Klebsiellaspp	36	2	5.6%
S typhi	36	2	5.6%

Discussion:

The study showed that the overall prevalence rate of *S. haematobium* infection was 25%. This rate was lower than the rate reported by Abosalif (2004) (45.9%) in Khartoum state. However, it was higher than that reported by Sitelbanat (2006) (9.9%), in Hillat Kuku in hartoum North and was

ISSN*: 1858-6147*

also lower than that rate reported by Kamal (2005) (39.2%) in Keryab Village.

In this study, the highest prevalence rate (30%) was reported among the age group 11-20 years and the lowest (0%) was reported among the age group 21-30 years. This finding was consistent with the prevalence reported by Sitelbanat (2006) among the 10-12 years age group (25%).

In this investigation, females showed higher prevalence rate (34%) than that of males (22%). The difference was statistically significant (p=0.025).

Our results contradicted the results of Abusalif (2004) and Kamal (2005) who reported the higher prevalence rate among the males rather than the females.

As there are no published works in Sudan, this study represent the first preliminary report on the association between urinary schistosomiasis and secondary bacterial infection.

As far as the bacteriological investigation is concerned, the study showed that urine cultured were negative for *Salmonella* in those individual who were positive for *S. hematobium* infection. This fining is in fact in line with Molhem and Loverde (1984) who reached the conclusion that in schistosomes, the stool and often urine culture are *Salmonella* negative which in their opinion makes the disease difficult to diagnose. However, Lamertucci et al (1985) showed that *Salmonella* are easily recovered from the blood and in 25% of cases; they have been isolated from the faeces or urine.

Lehman et al (1970) reported that bacterial urinary tract infection commonly due to *Salmonella* was found in one third of the *S. haematobium* patients in their study which was carried out on 75 hospitalized Egyptian farmers.

On the other hand, *Salmonella typhi*, in this study was isolated from 2 samples in those who were negative for *S. haematobium* infection. This finding might be strengthened by the fact that when there is haematogenous spread to the urinary tract, *S. typhi* and *staphylococcus aureus* may be found (Mims et al 1998). The results showed that 12 out of the 78 who were positive for *S. haematobium* infection were bacteriuric which constituted a growth rate of 15%. This rate was higher than that rate reported by Laughlin et al (1978) who reported 5.1% growth rate among school boys in Egypt but was extremely lower than the growth rate reported by Adeyeba and Ojaeya (2000) (75.4%).

As far as the bacterial isolates from urine cultures of those who were positive for *S. haematobium* infection are concerned, it was obvious that only *E. coli*, *E. faecalis* and *Proteus species* were isolated.

It is worth mentioning that bacterial cultures for those who were positive for *S. haematobium* infection revealed the growth of *Enterococcus faecalis*, *Proteus species* and *E. coli* in 12 urine samples. Our finding is in agreement of that of Adeyeba and Ojaeya (2000) who studied the

prevalence of urinary schistosomiasis and concomitant urinary tract pathogenic among 1600 pupils and revealed that *E. coli* occurs more frequently than other bacteria isolated in their study.

In this study, it was obvious that the bacteria isolated in those with *S.haematobium* infection (*Enterococcus faecalis, E. coli* and *Proteus*) were also isolated from those who were negative for *S. haematobium* infection. The above mentioned bacteria as described by Mackie and MacCartney (1996) are involved in urinary tract infection (UTI) with *E. coli* the commonest cause followed by *Proteus* and *Enterococcus* which may also cause biliary tract infection. Hagberg et al (1981) ascertained the role of *E. coli* as a cause of pyelonephritis.

Other bacteria isolated in this study in those who were negative for *S. haematobium* infection was *S. saprophyticus* which is considered by Machie and MacCartney (1996) as an important cause of UTI in younger sexually active women.

Klebsiella was also isolated from negative *S. haematobium* individuals. Machie and MacCartney (1996) stated that "this organism which is commonly isolated from water and human and animal faeces is the most frequent encountered in clinical specimens especially from hospitalized patient in whom it causes infections of surgical wounds and urinary tract.

It is to be noticed that in this study, Pseudomonas was also isolate from those individuals who were negative for *S. haematobium*. Mims et al (1998) reported that this bacterium is more frequently found in hospital acquired UTI because their resistance to antibiotics favors their selection in hospital patients.

In this investigation, not a single case of *Salmonella* infection in urine of *S. haemoatobium* infected individuals was reported. This contradicts a previous work (Crewe and Haddock 1985) who revealed that schistosomiasis predisposes to chronic urinary excretion of *S.typhi*, and patients may have recurrent *Salmonella* bacteraemia.

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Original Article **Sudan Medical Laboratory Journal**

High density lipoprotein in familial apoprotein AI deficiency

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Abstract

Our aim was to characterize HDL in a kindred with familial apolipoprotein AI (apoA-I) deficiency. Sequencing of the apoA1 gene revealed a nonsense mutation at codon –2, Q[–2]X, with two documented homozygotes, eight heterozygotes, and two normal subjects in the kindred. Homozygotes presented markedly decreased HDL cholesterol levels, undetectable plasma apoA-1, tuboeruptive and planar xanthomas, mild corneal arcus and opacification, and severe premature coronary artery disease. In both homozygotes, analysis of HDL particles by two-dimensional gel electrophoresis revealed undetectable apoA-I, decreased amounts of small α-3 migrating apoA-II particles, and only modestly decreased normal amounts of slow α migrating apoA-IV- and apoE-containing HDL, while in the eight heterozygotes, there was loss of large α-1 HDL particles. Our data indicate that isolated apoA-I deficiency results in marked HDL deficiency with very low apoA-II α-3 HDL particles, modest reductions in the separate and distinct plasma apoA-IV and apoE HDL particles, tuboeruptive xanthomas, premature coronary atherosclerosis, and no evidence of fat malabsorption.

Introduction

Decreased plasma HDL cholesterol levels (<40 mg/dl in men and <50 mg/dl in women) have been associated with an increased risk of coronary heart disease (CHD) (1). Marked HDL deficiency states (HDL cholesterol < 5 mg/dl) and undetectable plasma apolipoprotein A-I (apoA-I) levels have been reported in humans as a result of mutations at the APOA1/C3/A4 gene locus (2–4). Such patients lack apoA-I-containing HDL in plasma, with normal or decreased triglyceride levels, normal LDL cholesterol levels, and often strikingly premature CHD (5–8). Other patients with marked HDL deficiency have mutations affecting the apoA-I sequence that can affect the activity of lecithin:cholesterol acyl transferase activity (9–12). In this regard, they differ from patients with homozygous Tangier disease caused by mutations in ABCA1, who have defective cellular cholesterol efflux, detectable plasma apoA-I in preβ-1 HDL only, hypertriglyceridemia, and decreased LDL cholesterol (13-15). Previously, defects involving the apoA1/C3/A4 gene cluster, the contiguous APOA1 and APOC3 genes, and the APOA1 gene in isolation have been described (2–6). Here, we

ISSN: 1858-6147 Sud Med Lab J, issue 1, vol 1 (2011)

report a kindred with isolated apoA-I deficiency, with precise lipoprotein and clinical characterization and characterization of fat-soluble vitamin levels, and document differences between this type of apoA-I deficiency and those combined with other apolipoprotein deficiencies in humans. These data provide us with important insights about the function of these apolipoproteins in human health and disease as well as about HDL particle subspecies.

Methods

The index case presented to the Heart Clinic in El Shaab hospital, Sudan.

He was a 44 year old male with striking tuboeruptive xanthomas on his buttocks and lower back, and biopsy of these lesions confirmed lipid-laden macrophages. He also had palmar and planar xanthomas, as indicated by yellow creases on his palms and wrist creases, as well as corneal arcus and corneal opacification detected on slit lamp examination. Examination of his retina was normal, as was his neurological examination. On physical examination, he had blood pressure of 120/80 mmHg, height of 1.80 m, weight of 94.0 kg, body mass index of 29.0 kg/m^2, and waist circumference of 105 cm. He had no evidence of hepatosplenomegaly or enlarged orange tonsils. On laboratory testing, he had normal liver, renal, and thyroid function and a normal complete blood count. His fasting glucose was 92 mg/dl. His most striking laboratory finding was an HDL cholesterol level of 4 mg/dl. He had no history of chest pain, heart disease, hypertension, diabetes, or cigarette smoking. He was asymptomatic, but on coronary stress testing he had evidence of ischemia. On coronary angiography, he had a complete obstruction of his right coronary artery and a 90% narrowing of his left anterior descending coronary artery; he underwent successful coronary artery bypass surgery.

A family tree is shown in Fig. 1. The proband had three children who were healthy at ages 3, 6, and 8 years, with HDL cholesterol levels of 14, 21, and 34 mg/dl, respectively. The proband's 41 year old brother had previously sustained a myocardial infarction followed by coronary artery bypass graft surgery at the age of 38 years. His brother was also noted to have tuberoeruptive xanthomas as well as corneal arcus, and his HDL cholesterol was found to be 2 mg/dl. His daughter was in good health at age 12 years and was presumably a heterozygote, but she did not consent to having her blood drawn. Two other siblings of the index case were examined and were found not to have xanthomas, with HDL cholesterol levels of 15 and 22 mg/dl, consistent with heterozygosity. They were in good health at ages 33 and 35 years. Their children were in good health at ages 3, 8, and 10 years, with HDL cholesterol levels of 21, 45, and 42 mg/dl, respectively. The parents of the index case were alive and well at ages 65 and 66 years, with HDL cholesterol levels that were both 24 mg/dl. They were first cousins, and their fathers were

nonidentical twin brothers. Therefore, the index case, his homozygous brother, and his two heterozygous siblings were products of a consanguineous marriage. Control subjects matched for the age and gender of affected family members were also selected for study. This investigation was approved by the local scientific committee and an informed consent was obtained by all participants or their parents in the case of the children.

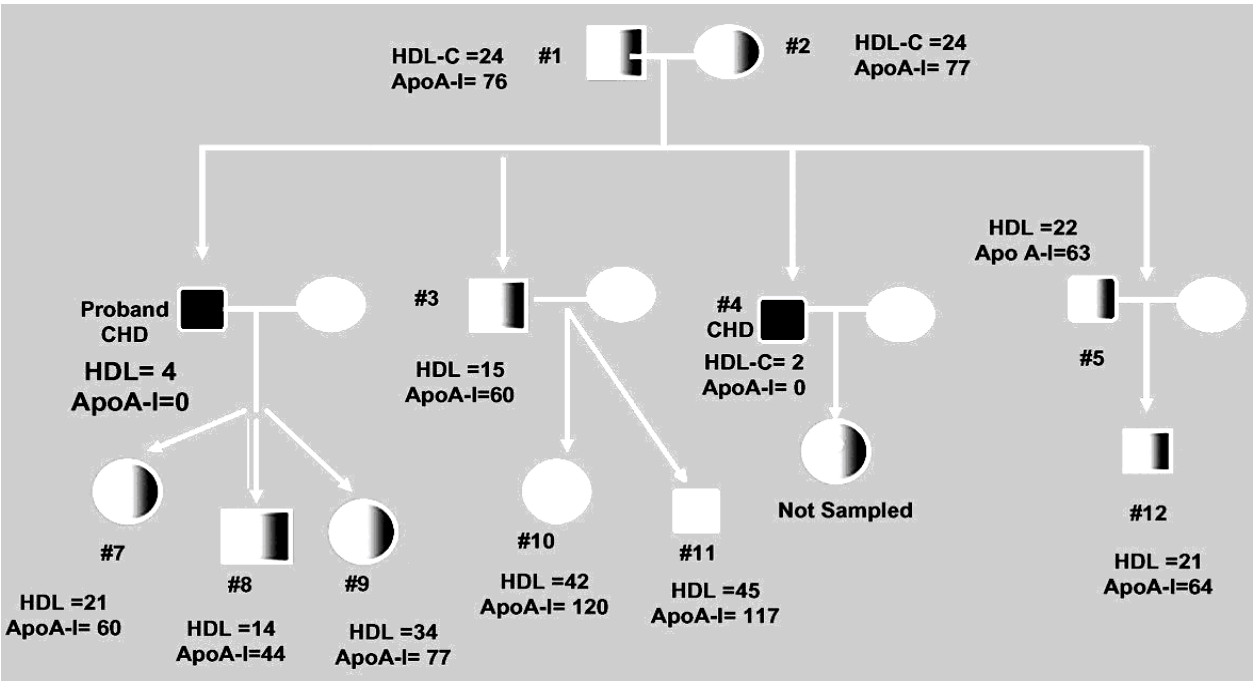

Fig1: A family tree

Biochemical measurements

Blood was collected from all subjects after an overnight fast and immediately placed on ice, and plasma was separated in a refrigerated centrifuge. Plasma cholesterol, triglyceride, HDL cholesterol, and calculated LDL cholesterol were assessed by standardized automated enzymatic methods in whole plasma and after precipitation of apoB-containing lipoproteins for HDL cholesterol in the clinical chemistry laboratory at El Shaab Hospital. All subjects had been off lipid-lowering medication for at least 2 months at the time of assessment. Plasma levels of the fat-soluble vitamins (A as retinol, D as 1,25-dihydroxyvitamin D, and E as α-tocopherol) were measured by high-performance liquid chromatography at the Alqasr Eleini Laboratory in Cairo.

Samples of plasma that had been stored at –80°C were shipped to the Biochemistry Laboratory at Khartoum University, and the results of the analyses from Cairo were confirmed. This laboratory also performed automated enzymatic lipid analyses, standardized by the Centers for Quality Control program in Laboratory

administration, Khartoum state. Free cholesterol was also assessed in whole plasma and in the HDL fraction [12, 16]. Plasma levels of apoA-I, apoA-II, apoB, apoC-III, and apoE were measured by immunoassay using kits obtained from Spinreact Inc., and apoA-I-, apoA-II-, apoA-IV-, apoC-III-, and apoE-containing HDL subpopulations were assessed by two-dimensional gel electrophoresis as described previously. Absolute plasma concentrations were calculated only for apoA-I-containing particles by multiplying the plasma total apoA-I concentration (mg/dl) by the percentile value of each subpopulation. HDL subpopulations were characterized by charge (preβ, α, preα) based on their relative mobility to albumin (first dimension), and size was determined from molecular weight standards [12, 13]. Each membrane was first probed for the apolipoprotein of primary interest, and percentage distributions of the particles were calculated. Subsequently, membranes were reprobed for apoA-I to colocalize each apolipoprotein with apoA-I and for human albumin as a reference point for the α-front. One representative subject of each group was selected for illustration of the various apolipoprotein-containing HDL profiles. All lipid and apolipoprotein data reported are from the Lipid Metabolism Laboratory at Tufts University and are shown in Tables 1 and 2 below. Coefficients of variation for all assays within and between runs were <5%.

Table 1: Characteristics of study participants

Variable (mg/dl)	Controls (n = 10)	Heterozygotes (n = 8)	Homozygotes (n = 2)
Male/female	7/3	5/3	2/0
Total cholesterol	200 ± 38	181 ± 37	157, 174
Triglyceride	117 ± 88	113 ± 45	56, 94
HDL cholesterol	54 ± 13	23 ± 7[a]	4.0, 3.2
LDL cholesterol	126 ± 33	140 ± 33	126, 146
ApoA-I	140 ± 25	65 ± 13[a]	0, 0
ApoA-II	38 ± 4	26 ± 5[a]	10, 12
ApoB	96 ± 16	97 ± 17	90, 106
ApoC-III	9.1 ± 4.2	8.7 ± 3.2	3.4, 4.0
ApoE	7.6 ± 3.6	4.1 ± 1.2	4.7, 3.8

ApoA-I, apolipoprotein A-I. Data are means ± SD.
[a] Significantly different from controls ($P < 0.05$).

ISSN: 1858-6147

Table 2: ApoA-I concentrations in HDL subpopulations

Variable	Controls (n = 10)	Heterozygotes (n = 8)
Preβ-1	12.2 ± 3.2 (9.9%)	6.3 ± 2.0[a] (9.8%)
Preβ-2	1.7 ± 0.9 (1.4%)	3.8 ± 1.5[a] (6.0%)
α-1	16.8 ± 8.9 (13.6%)	3.3 ± 2.4[a] (4.8%)
α-2	39.3 ± 9.7 (31.8%)	24.3 ± 6.2[a] (37.1%)
α-3	24.0 ± 5.7 (19.4%)	17.1 ± 3.5[a] (26.5%)
α-4	13.5 ± 3.6 (10.9%)	4.1 ± 1.2[a] (6.4%)
Preα-1	5.3 ± 3.4 (4.3%)	0.3 ± 0.4[a] (0.5%)
Preα-2	6.2 ± 2.4 (5.0%)	2.8 ± 0.6[a] (4.3%)
Preα-3	3.5 ± 1.4 (2.8%)	2.0 ± 0.7[a] (3.1%)
Preα-4	1.0 ± 0.4 (0.8%)	0.8 ± 0.3 (1.2%)

Data are means (mg/dl) ± SD, with the percentage of the total value in parentheses.

[a] Significantly different from controls ($P < 0.05$). No values are reported for homozygotes because of undetectable plasma apoA-I levels.

DNA sequencing

DNA was isolated from blood cells at InCor and shipped to Tufts University. An aliquot of DNA from the proband was then sent to Dr. Robert Hegele at the London Regional Genomics Center in Canada for sequencing of the APOA1 gene using genomic DNA. The proband was found to be homozygous for a mutation at APOA1 codon –2, namely Q[–2]X, identical to a mutation in a previously reported Canadian family with apoA-I deficiency [(11)]. This mutation results in the generation of a termination codon and the lack of any mature apoA-I being expressed in homozygotes. DNA from the proband, his homozygous brother, and all other available family members (n = 10) were then submitted for APOA1 gene sequencing to the core sequencing facility of Tufts University School of Medicine. Molecular analysis confirmed the prior results and revealed two homozygotes, eight heterozygotes, and two normal subjects, the latter of whom were the offspring of a heterozygote and had HDL cholesterol levels of 42 and 45 mg/dl, respectively. These two subjects were included in the control group in subsequent analysis. APOE genotyping and APOE gene sequencing were suggested by Dr. Jean Davignon of Montreal, because of the proband's striking planar xanthomas. APOE genotyping in all family members revealed either the E3/3 or E4/3 genotype; moreover, the proband's APOE gene was sequenced at the core facility at Tufts University and was found to be normal.

Data analysis

Mean values and standard deviations were calculated for all study groups. Data obtained from heterozygotes were compared with data from controls using ANOVA. A two-tailed *P* value of <0.05 was considered statistically significant.

Results

Table 1 shows data on plasma lipids, lipoprotein cholesterol, and apolipoproteins in controls (n = 10), heterozygotes (n = 8), and homozygotes (n = 2) for this kindred with familial apoA-I deficiency. Homozygotes (n = 2) had mean values of HDL-C, apoA-I, apoA-II, and apoE that were 6.7%, 0%, 28.9%, and 55.9% of normal, respectively; free cholesterol represented 30.2% of total cholesterol, and there was about the same percentage of HDL cholesterol as free cholesterol, ruling out LCAT deficiency. Heterozygotes had HDL cholesterol, apoA-I, and apoA-II values that were 42.6%, 46.4%, and 68.4% of control values (all $P < 0.05$), with relatively normal amounts of apoB, apoC-III, and apoE in plasma.

A schematic diagram from the two-dimensional electrophoresis of normal apoA-I-containing HDL particles is shown in Fig. 2, and control values are provided in Table 2. These data are expressed both in terms of the concentration of apoA-I in the various HDL subspecies and in terms of percentages of total plasma apoA-I. In normal subjects, ~12 mg/dl apoA-I (~10% of the total) is found in two small, discoidal preβ-1 HDL particles, and ~22 mg/dl (~20% of the total) is found in either large, spherical α-1 HDL or in the adjacent large, spherical preα-1 HDL (Figs. 2, 3). All of these HDL particles contain apoA-I without apoA-II. In contrast, intermediate-sized spherical α-2 and α-3 HDL contain both apoA-I and apoA-II and have a combined apoA-I concentration of ~60 mg/dl, or ~ 50% of the total plasma apoA-I and ~100% of apoA-II in normal plasma (see Figs. 2–4). Serum amyloid A protein can also be found in α-2 HDL. Adjacent to α-2 and α-3 HDL are the intermediate spherical preα-2 and preα-3 HDL, which contain apoA-I without apoA-II and together have an apoA-I concentration of ~10 mg/dl (~8% of total plasma apoA-I). The smallest αmigrating HDL particles are α-4 HDL and the adjacent preα-4 HDL, which both contain apoA-I without apoA-II and are discoidal particles, which together have an apoA-I concentration of ~2 mg/dl, representing ~ 1.5% of total plasma apoA-I. Finally, there are three large preβ-2 HDL particles that together have an apoA-I concentration of ~2 mg/dl, or ~1.5% of the total. These particles do not contain apoA-II (Figs. 2–4).

Table 2 also summarizes data on apoA-I-containing HDL subpopulations in heterozygous subjects. Homozygotes had undetectable levels of apoA-I-containing HDL, as shown in Fig. 3. They had HDL particles in the α-3 region containing apoA-II but no apoA-I (Figs. 3 , 4) as well as relatively normal amounts of the separate apoA-IV- and apoE-containing HDL particles, which have slow αmobility (Figs. 5 , 6). ApoC-III in the homozygotes was entirely in the free form and not associated with other apolipoproteins (data not shown). Heterozygotes had markedly decreased mean apoA-I concentration in large α-1 HDL

(19.6% of normal), some decrease in α-2 and α-3 HDL (61.8% and 71.3% of normal), and a more marked decrease in α-4 HDL (30.4% of normal). They also had preβ-1 HDL values that were 51.6% of normal, but their preβ-2 HDL levels were 2.24-fold higher than normal. Gel photographs of apoA-I-, apoA-II-, apoA-IV-, and apoE-containing HDL in heterozygotes are shown in Figs. 3–6. As mentioned previously, heterozygotes had marked decreases in α-1 and α-4 HDL and a marked increase in preβ-2 HDL, with relatively normal distributions and amounts of apoA-II-, apoA-IV-, and apoE-containing HDL. Both heterozygotes and homozygotes had reductions in the amounts of apoA-IV and apoE staining in HDL by ~50% compared with controls (Figs. 5, 6).

Levels of fat-soluble vitamins were generally normal in this kindred in both homozygotes and heterozygotes, with mean values (standard deviation) being in the normal range for retinol at 41.3 (15.3) μg/ml (normal, 30–80 μg/ml), for 1,25-hydroxyvitamin D at 36.7 (46.4) pg/ml (normal, 16–60 pg/ml), and for α-tocopherol at 0.7 (0.4) mg/dl (normal, 0.5–1.8 mg/dl). In the two homozygotes, retinol was 33.9 and 37.6 μg/ml, 1,25-dihydroxyvitamin D was 19.4 and 30.2 pg/ml, and α-tocopherol was 0.4 and 1.1 mg/dl. Only α-tocopherol was somewhat below the normal range in one homozygote at 0.4 mg/dl.

Discussion

Three forms of familial apoA-I deficiency have been recognized: lack of apoA-I, lack of apoA-I and apoC-III, and lack of apoA-I, apoC-III, and apoA-IV. Schaefer and colleagues (2) in January 1982 described one homozygote and multiple heterozygotes in a kindred of English origin residing in northern Alabama. The index case had no xanthomas, marked HDL deficiency, low triglyceride, normal LDL cholesterol levels, and severe premature coronary artery disease. She had no history of diabetes, smoking, or hypertension and was premenopausal. She died at the time of bypass surgery at age 43 years (2–6). At autopsy, severe diffuse coronary atherosclerosis was documented (2–4). The defect in this kindred was subsequently found be a large deletion of the entire APOA1/C3/A4 gene cluster (5). Decreased plasma levels of the fat-soluble vitamins A, D, and E (<50% of normal) and a moderately prolonged prothrombin time, consistent with malabsorption of fat and fat-soluble vitamins in the homozygote, were noted (5). Heterozygotes were found to have plasma HDL cholesterol, apoA-I, apoC-III, and apoA-IV levels that were ~50% of normal (5). ApoA-I gene transfection studies indicated that apoA-I was essential for HDL formation, similar to what was noted in this initial kindred (6). With molecular characterization, the family was denoted as having familial APOA1/C3/A4 deficiency (5).

In June of 1982, a second kindred with apoA-I deficiency was described by Norum and colleagues (7) in two sisters with marked HDL deficiency and planar xanthomas. They had premature CHD and underwent coronary artery bypass grafting surgery at ages 29 and 30 years. They had no history of smoking, hypertension, or diabetes, and no fat malabsorption was reported. Their triglyceride levels were reduced, and their LDL cholesterol levels were normal. The genetic defect was subsequently found to be a DNA rearrangement affecting the adjacent APOA1 and APOC3 genes, resulting in a lack of production of these two apolipoproteins and their absence from plasma (8, 9). It was also subsequently reported that these homozygotes had small amounts of apoA-II-containing HDL and enhanced clearance of very low density lipoprotein apoB, presumably because there was no apoC-III present in plasma to inhibit lipolysis (10–14). This kindred was described as having familial APOA1/C3 deficiency. A second kindred with premature CHD, marked HDL deficiency, and absence of apoA-I and apoC-III in plasma has also been described (15).

Since 1991, 10 kindreds with isolated apoA-I deficiency have been described (16–20). However, only the homozygous probands in the two kindreds described by Matsunaga et al. (16) in 1991 (codon 84 nonsense mutation) and by Ng et al. (7) in 1994 (nonsense mutation at codon –2) had undetectable plasma apoA-I levels, marked HDL deficiency, and premature CHD. In the Matsunaga kindred from Japan (16), the female proband had normal triglyceride and LDL cholesterol levels and yellow-orange planar xanthomas. The APOA1 Q[–2]X mutation described in this report was first reported by Ng et al. (7, 12) in a Canadian kindred of mixed European ancestry, including Portuguese ancestry, living in Toronto. The index case was a 34 year old female who presented with marked HDL deficiency, mildly thickened Achilles tendons, xanthelasmas, mild midline cerebellar ataxia, and asymmetric bilateral neurosensory hearing loss. She also had bilateral cataracts and bilateral subretinal lipid deposition with exudative proliferative retinopathy, with resultant bilateral retinal detachments requiring surgical repair. Her apoA-I levels were undetectable, and her HDL cholesterol was 2 mg/dl; her triglycerides and LDL cholesterol levels were increased. Four other homozygotes from this pedigree also had marked HDL deficiency (mean, 4 mg/dl), normal triglycerides (mean, 123 mg/dl), and increased LDL cholesterol (mean, 175 mg/dl). One homozygous sister, at age 38 years, had xanthelasma, Achilles tendon xanthomas, and planar xanthomas in the web spaces of the hands and the cubital and popliteal fossae. She had sustained a myocardial infarction at age 34 years and had coronary artery bypass grafting surgery at age 37 years. A second homozygous sister had angina and documented reversible myocardial

ischemia on stress testing as well as cerebellar ataxia. The two other homozygotes, at ages 26 and 28 years, as well as four heterozygotes (ages 14–39 years) were asymptomatic and had no evidence of CHD, neuropathy, or visual impairment. In their discussion, the authors concluded that the combined hyperlipidemia in that kindred was probably not related to the APOA1 gene mutation. The findings in the present kindred would tend to support this speculation, because we observed neither combined hyperlipidemia nor Achilles tendon xanthomas. Although the Canadian kindred with APOA1 Q[–2]X did not have independently segregating classical familial hypercholesterolemia, the authors speculated that some other unmeasured defect of cholesterol metabolism resulted in the increased total and LDL cholesterol.

Other apoA-I-deficient patients reported had apoA-I present in their plasma, evidence of LCAT deficiency, no evidence of premature CHD or xanthomas, and corneal opacification (17-19). Therefore, LCAT deficiency can occur when there are mutations in the LCAT gene or in the apoA-I gene causing the formation of abnormal apoA-I, which does not allow for the normal activation of LCAT and interferes with the activation of LCAT by other apolipoproteins.

In our APOA1 Q[–2]X kindred, in contrast to the Canadian kindred, there was no evidence of Achilles tendon xanthomas. Moreover, we did not note the combined hyperlipidemia, ataxia, cataracts, or proliferative retinopathy in our kindred, in contrast to the kindred reported by Ng and colleagues (17, 18). That pedigree, as well as our own, had consanguinity documented, raising the possibility of other homozygous mutations being present, contributing to retinal disease, ataxia, combined hyperlipidemia, and tendinous xanthomas. The common features of the two kindreds are the APOA1 Q[–2]X mutation itself, marked HDL deficiency, planar xanthomas, and premature CHD. Members of our kindred had striking tuboeruptive xanthomas that were not observed in the kindred described by Ng and colleagues. In addition, we searched for APOE deficiency, APOE mutations, and E2/2 homozygosity but did not find these features.

These data indicate that apoA-I is essential for normal HDL formation and that its absence results in severe HDL deficiency, xanthomas, and premature CHD. The additional presence of apoC-III deficiency as observed in APOA1/C3 deficiency results in the same phenotype, except for the presence of very low triglyceride levels, consistent with the concept that apoC-III can impair lipolysis; thus, its absence is associated with lower triglyceride concentrations. The more complex and truly polygenic APOA1/C3/A4 deficiency results in the same phenotype, except that there are no xanthomas, whereas fat malabsorption is present, consistent with the concept that apoA-IV plays a

role in the intestinal absorption of fat and fat-soluble vitamins.

Research on HDL particle metabolism and function indicates that CHD patients have increases in preβ-1 and small α-4 and α-3 HDL and decreases in larger α-2 and α-1 HDL (13–15). Moreover, it is mainly the preβ-1 HDL that picks up free cholesterol from cells via ABCA1, and it is mainly the large α-2 and α-1 HDL that interacts with SRB1 in hepatocytes and other cells to promote bidirectional cholesterol flux (3). Studies in this kindred with apoA-I deficiency and in kindreds with Tangier disease indicate that the production of apoA-I is critical for HDL formation and the existence of preβ-1 HDL, whereas the presence of normal ABCA1 function and the addition of free cholesterol and phospholipids are critical for the conversion of this particle to small discoidal α-4 HDL (17–19). Studies in patients with familial LCAT deficiency indicate that cholesterol esterification via LCAT is critical for the maturation of α-4 HDL to larger spherical HDL particles, and the presence of cholesteryl ester transfer protein (CETP) is critical for the formation of normal spherical α-1 HDL that contain apoA-I but not apoA-II (6, 17). Surprising human subjects lacking plasma apoA-II have normal HDL levels and no evidence of premature CHD (36). In our studies in patients with homozygous CETP deficiency and lack of CHD, we noted the presence of very large spherical αmigrating HDL, which contain apoA-I, apoA-II, and apoE (16). It is precisely these particles that we have observed in C57BL6 mice, which naturally lack CETP (B. Asztalos, unpublished observations). Therefore, mice may not be an ideal model for human lipoprotein metabolism, and apoA-I knockout mice do not have increased atherosclerosis unless they also lack the LDL receptor (17, 18).

A critical element of our data is the clear documentation of slow αmigrating HDL particles containing only apoE and only apoA-IV, which exist in normal plasma as well as in the plasma of patients lacking apoA-I. These particles have a distinct metabolism and probably distinct functions separate from apoA-I-containing HDL, which require further elucidation. ApoA-I is known to activate LCAT, but other apolipoproteins can also do this; therefore, in this kindred, there is no evidence of LCAT deficiency. In contrast, kindreds with apoA-I mutations affecting LCAT activity are well described, and although they have HDL deficiency, apoA-I is present in their plasma usually in excess of 10 mg/dl, and they do not appear to have premature CHD (19, 21–23, 25). ApoA-IV in HDL is clearly on its own HDL particle, and its residence time in plasma is ~3 days, considerably different from that of HDL apoA-I, which is ~5 days, and HDL apoA-II, which is ~5.5 days (20, 21).

Moreover, apoA-IV, like apoA-I, is a significant protein component of intestinal apoB-48-containing

triglyceride-rich lipoproteins [21]. ApoA-IV appears to play a role in fat absorption; hence, its absence there indicates a modest degree of malabsorption of fat and fat-soluble vitamins, not observed in this kindred with isolated apoA-I deficiency. Both apoA-I and apoA-IV proteins are transferred from triglyceride-rich lipoprotein (TRL) to HDL during TRL lipolysis, then reassociate with newly formed TRL in the extraplasma space, and they can recycle multiple times in this manner, similar to the C and E apolipoproteins (20–22). ApoE also has its own HDL particle, and its residence time within HDL is substantially less than that of other apolipoproteins in HDL at ~1 day (23, 24). Only apoB-100 and apoA-I have production rates in the plasma space in humans in excess of 10 mg/kg/day, whereas apoE has a plasma production rate in humans of ~3.5 mg/kg/day and a somewhat lower HDL apoE production or transport rate. ApoE in TRL clearly plays a critical role in the fractional clearance of apoB-containing particles of both liver and intestinal origin, because in is absence there is a marked increase in remnant apoB-100 and apoB-48 particles with markedly delayed clearance, but HDL levels are normal (25). ApoE HDL could clearly play an important role in reverse cholesterol transport, because it can bind to hepatic LDL receptors. In humans, familial apoA-I deficiency results in much more severe and premature atherosclerosis than familial apoE deficiency, whereas the converse is true in mice, underscoring the concept that the best model for human lipoprotein metabolism remains the human, not the mouse.

The overall data in the present kindred indicate that isolated familial apoA-I deficiency results in marked HDL deficiency, xanthomas, premature CHD, and no evidence of fat or fat-soluble vitamin malabsorption. Moreover, the evidence indicates that in the absence of apoA-I, small amounts of A-II particles can be found in the αmigrating region of HDL at the α-3 position, and levels of apoA-IV- and apoE-containing HDL are only moderately reduced and have normal electrophoretic mobility and particle size. The data also indicate that there are at least three separate and distinct types of HDL that exist in normal plasma: *1*) multiple α, preα, and preβ-1 particles containing apoA-I (the predominant HDL type); *2*) two small- and intermediate-sized slow αmigrating particles containing mainly apoA-IV (a relatively minor HDL type); and *3*) large slow αmigrating HDL containing mainly apoE (also a relatively minor HDL type). These latter two types of HDL are only moderately reduced in homozygous familial apoA-I deficiency, and their role in health and disease requires further exploration.

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Original Article

Calcium permeation and blocking effect in human leukaemia cells

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Abstract

Cell-attached and inside-out patch-clamp methods were employed to identify and characterize mechanosensitive (MS) ionic channels in the plasma membrane of human myeloid leukaemia K562 cells. A reversible activation of gadolinium-blockable mechanogated currents in response to negative pressure application was found in 58 % of stable patches (n = 317). *I-V* relationships measured with a sodium-containing pipette solution showed slight inward rectification. Data analysis revealed the presence of two different populations of channels that were distinguishable by their conductance properties (17.2 ± 0.3 pS and 24.5 ± 0.5 pS), but were indistinguishable with regard to their selective and pharmacological properties. Ion-substitution experiments indicated that MS channels in leukaemia cells were permeable to cations but not to anions and do not discriminate between Na^+ and K^+. The channels were fully impermeable to large organic cations such as $Tris^+$ and *N*-methyl-D-glucamine ions ($NMDG^+$). Ca^{2+} permeation and blockade of MS channels were examined using pipettes containing different concentrations of Ca^{2+}. In the presence of 2 mM $CaCl_2$, when other cations were impermeant, both outward and inward single-channel currents were observed; the *I-V* relationship showed a unitary conductance of 7.7 ± 1.0 pS. The relative permeability value, P_{Ca}/P_K, was equal to 0.75, as estimated at physiological Ca^{2+} concentrations. Partial or full inhibition of inward Ca^{2+} currents through MS channels was observed at higher concentrations of external Ca^{2+} (10 or 20 mM). No MS channels were activated when using a pipette containing 90 mM $CaCl_2$. Monovalent mechanogated currents were not significantly affected by extracellular Ca^{2+} at concentrations within the physiological range (0-2 mM), and at some higher Ca^{2+} concentrations.

Introduction

It is generally accepted that temporal changes in the concentration of free ionized cytosolic Ca^{2+} constitute an important intracellular signal. Whereas Ca^{2+} channels in electrically excitable cells are well described, there is a dearth of information about Ca^{2+} transporters in non-excitable cells. In cells of blood origin, Ca^{2+} regulation and cation-transporting membrane systems may play a pivotal role in the control of cell growth and differentiation (1). In particular, it has been suggested that cytosolic Ca^{2+} is involved in the mechanism of action of DIF-1, a potent anti-leukaemic agent that induces erythroid differentiation in K562 cells (2). The mechanisms responsible for the fast Ca^{2+} entry through the leukaemic cell membrane remain poorly understood. In a variety of blood cells, plasma membrane channels operated by receptors, store

ISSN*: 1858-6147*

depletion or some other mechanisms are assumed to be involved [1].

The data available to date indicate that in a variety of non-excitable cells, cation-transporting mechanosensitive (MS) channels may provide an effective pathway for Ca^{2+} influx from the extracellular medium to the cytosol. It has been shown that mechanically gated channels are present ubiquitously in various types of mammalian cell (3). Using the patch-clamp technique, it has been demonstrated that non-selective cation channels that are activated by stretching of the plasma membrane exist in a number of tissues including skeletal muscle, smooth muscle cells, neurones, various epithelial and endothelial cells, and osteoblasts. In recent years, the existence of physiologically active stretch-activated channels has been demonstrated, using both single-channel and whole-cell current measurements (4). Very little is known about the expression of calcium-permeable MS channels in cells of blood origin. Volume-regulated Cl^- currents have been described in lymphocytes and myeloma cells (5; 6), and it has been reported that macrophages contain stretch-sensitive K^+ channels (7). The presence of non-selective cation channels was demonstrated in a patch-clamp investigation of the permeability properties of trout red blood cells (8). In previous studies, we found sodium-selective channels activated by F-actin disruption in human leukaemia K562 cells (9, 10). These channels were shown to be independent of membrane stretch. In addition, we have observed in cell-attached patches on leukaemia cells, a reversible activation of single currents that differ in their conductive properties in response to suction (9; 11). In the study presented here, we have examined the selective characteristics and Ca^{2+} permeation of MS channels in K562 cells that have the properties of multipotent precursors of blood cells. The data obtained show that cation-selective stretch-activated channels permeable to Ca^{2+} in the physiological concentration range are expressed in the plasma membrane of leukaemia cells. The effects of extracellular Ca^{2+} that are of great importance for channel functioning and cellular responses have been also studied.

Methods

Cells

Human myeloid leukaemia K562 cells obtained from a cell culture collection (Institute of Cytology, Doha) were maintained in glass flasks in RPMI-1640 containing 10 % fetal bovine serum and (in some experiments) antibiotics (100 µg/ ml streptomycin and 100 units/ ml penicillin) at 37 °C. Cells were plated on cover slips (0.4 × 0.4 cm) 2-4 days before an experiment.

Electrophysiology

Single channel currents were recorded using standard cell-attached and inside-out configurations of the patch-clamp technique (12). The membrane voltage was calculated as the potential on the intracellular membrane side minus the potential on the extracellular membrane side. Pipettes were pulled from soft glass capillaries to a resistance of 7-15 MΩ when filled with normal external

solution. Membrane currents were recorded essentially as described by (10). Unless otherwise stated, data were filtered at 200 Hz and sampled at a rate of 1 kHz by a 12-bit A-D converter for analysis and display. The recordings were performed at room temperature (22-23 °C) on the stage of an inverted microscope that possessed Nomarsky optics (magnification 256 ×). Pieces of cover slips with adhered cells were transferred into a recording chamber filled with a normal Na^+ external solution, and a giga-seal was formed between the pipette and one of the cells. For cell-attached measurements, this bath solution was replaced with the potassium-containing solution to nullify the resting membrane potential. The pipette interior was connected to a manometer with a valve to allow either application of positive and negative pressure or equilibration to atmospheric pressure. The channel open probability (P_o) was determined using the following equation: $P_o = I/i \times N$, where I is the mean current determined from the amplitude histograms, i is the unitary current amplitude and N is the number of functional channels in the patch. The Goldman-Hodgkin-Katz modified constant-field equation was used to estimate the relative permeability of channels from current reversal potential (E_{rev}) values (13). Averaged data are given as the mean ± S.E.M. (number of experiments).

Solutions

The normal external solution in the bath and in the pipette typically contained (mM) 145 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Hepes/ TrisOH. In some of the experiments, the solution contained no divalent cations. The bath solution for cell-attached measurements contained (mM) 145 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Hepes/KOH. The cytosol-like bathing solution for inside-out measurements contained (mM) 145 KCl, 1 $MgCl_2$, 20 Hepes/KOH, 2 EGTA and an appropriate quantity of $CaCl_2$ (0.175 mM) to establish the final free ionized Ca^{2+} concentration at the desired level of 0.01 μM. In experiments with anion substitutions, KCl was replaced with 145 mM potassium glutamate, 145 mM potassium aspartate or 70 mM K_2SO_4 145 mM. Tris-Cl or NMDGCl were used to substitute monovalent alkali cations. To decrease the concentration of permeable electrolytes in the course of inside-out recordings, 75 mM KCl was replaced by 150 mM sucrose. To measure Ca^{2+} currents we used pipette solutions with different concentrations of Ca^{2+}. The solution of high Ca^{2+} concentration contained (mM) 90 $CaCl_2$, 10 Hepes/TrisOH. Solutions of lower Ca^{2+} concentrations contained (mM) 20 $CaCl_2$ + 110 NMDGCl, 10 $CaCl_2$ + 125 NMDGCl, or 2 $CaCl_2$ + 137 NMDGCl. Experiments involving Gd^{3+} were carried out using the highly dissolvable salt $GdCl_3$. The pH of all solutions was set at 7.3. All substances were purchased from Sigma.

Results

Activation of MS channels in cell-attached patches on K562 cells

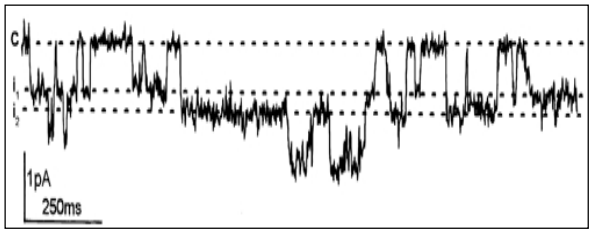
The first series of experiments was carried out to study the effect of mechanical stimulation on channel activity in the plasma membrane of cultured leukaemia cells. The cell-attached patch

configuration is the most suitable for single-current measurements under quasi-physiological conditions. Without stimulation, the major part of cell-attached patches on K562 cells displayed no channel events. In other patches, control cell-attached recordings revealed outward currents through potassium-selective channels. Rare inward currents representing the background activity of sodium-selective channels (9, 14) were also observed in a few experiments. To examine the effect of membrane stretch on single-channel activity in human leukaemia K562 cells, negative or positive pressure was applied to the patch via the suction pipette. Positive pressure application (10-20 mmHg) induced no change in membrane current while the patches remained stable. We found that mechanically gated ion channels were activated in response to application of negative pressure (10-30 mmHg). An increase in the pressure level resulted in a concomitant increase in P_o. It should be emphasized that the background activity of sodium-selective and potassium-selective channels in K562 cells was not affected by suction. In cell-attached recordings, mechanically gated currents did not inactivate during prolonged (up to 300 s) stimulation. Moreover, after removal of suction the activity could be evoked repeatedly. After excising the membrane patch from the cell, the activity of MS channels was not so stable as in cell-attached recording, exhibiting a rundown in a number of experiments.

The MS channels were identified in 58 % of stable patches (n = 317) on K562 cells; the activity of two to four, or more channels was recorded with increasing pressure level. According to their gating properties, MS channels in K562 cells could be identified as typical stretch-activated channels. This was confirmed by the fact that addition of 20 mM Gd to the pipette fully prevented the activation of all types of MS channels. We also found in cell-attached experiments that a high concentration of the diuretic amiloride (1 mM) caused a full inhibition of stretch-activated MS channels in K562 cells.

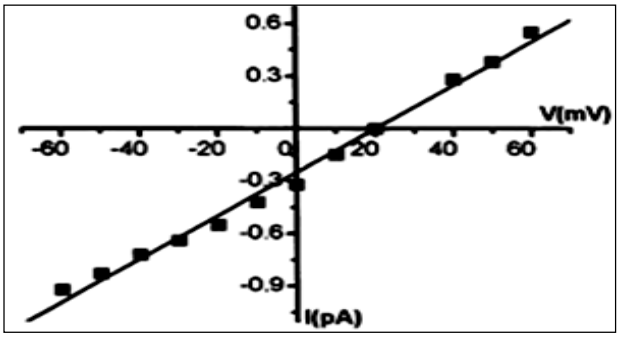
The other data revealed that MS channels were active throughout the range of holding potentials tested and P_o was not affected by membrane voltage. The unitary *I-V* curves measured for MS channels with Na^+ in the pipette showed slight inward rectification; the E_{rev} of currents was close to zero. In the range of negative membrane potentials, the *I-V* curve could be approximated as a linear regression to estimate single-channel conductance. *I-V* relationships measured with Na^+ in the pipette solution showed unitary conductance values in the range 10-30 pS. Importantly, MS channels characterized by different conductance values displayed very similar gating properties and selective characteristics. Two conductance levels of about 17 and 25 pS were predominant. Different conductances only rarely coexisted in the same patch.

Figure1: shows one of these examples



Channel events corresponding to conductance levels of 17 and 25 pS were observed. At a holding potential of −40 mV, the amplitudes of these single-channel openings were i_1 = 0.68 pA and i_2 = 0.98 pA. There were no direct transitions between levels i_1 and i_2, representing possible conductance states. On the contrary, successive openings of two independent channels of different conductance levels occurred. At the same time, there was no direct transition from the closed state (zero current) to the level equal to the sum of the current amplitude (i_1 + i_2). Thus, according to the classical scheme (15), examination of single currents provided no evidence that the different conductances represent different sub-states of the same MS channel. Consequently, we had to consider that there were different populations of MS channel in the K562 cell plasma membrane. Data analysis revealed two major channel populations having the following conductance values: 17.2 ± 0.3 pS (n = 37) and 24.5 ± 0.5 pS (n = 34). In a few experiments, MS channels characterized by lower unitary conductance values (10.9 ± 0.4 pS; n = 20) were observed.

Figure 2: activity of several channels of the same conductance

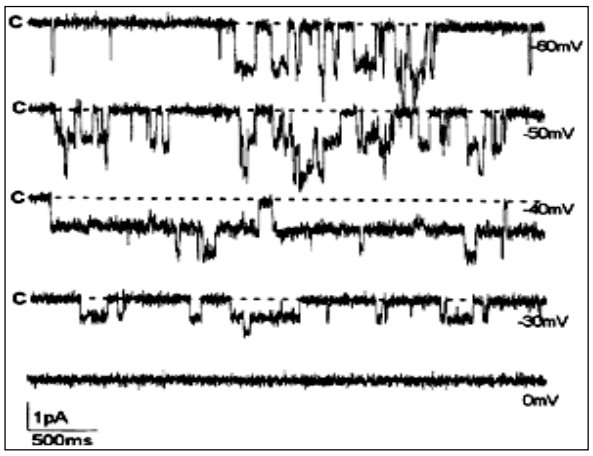


It should be emphasized that activity of several channels of the same conductance was observed frequently, whereas coexistence of difference channel types was only rarely encountered. This imbalance compelled us to assume that the populations of MS channels are characterized by cluster organization. Alternatively, it seems reasonable to suggest that the MS channel properties are controlled by some intracellular mechanisms that provide rather stable conductance levels. As a result, different conductance states may be realized in the same channel protein, although we did not record any direct transitions in the course of our single-current measurements.

Permeation properties

In some of the experiments, channel activity could also be evoked by negative pressure application after patch excision. Inside-out recordings showed similar kinetic properties of mechanogated currents.

Figure 3: Na^+ as the major cation in the pipette



The above curves measured with Na^+ as the major cation in the pipette and K^+ in the cytosol-like bath solution were similar to those obtained in cell-attached recordings (Figs 1 and 3). To identify reliably MS channels in the plasma membrane of K562 cells we examined their selective properties via ion substitution.

Figure 4: substitution of anions in the cytosol-like bathing solution

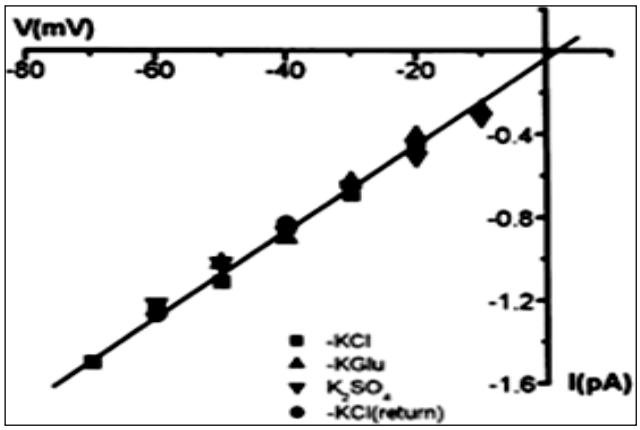
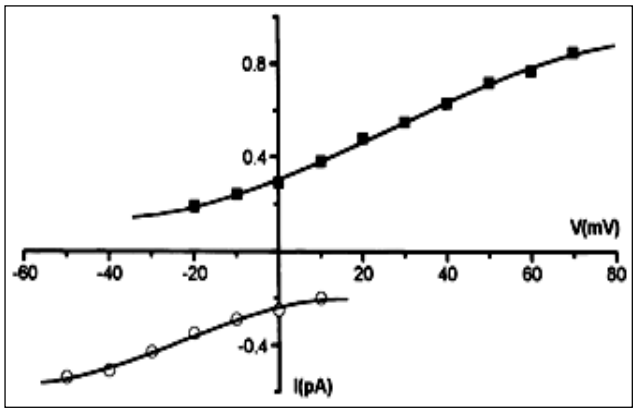


Figure 4 demonstrates that substitution of anions in the cytosol-like bathing solution caused no changes in the amplitude of single-channel openings; in different experiments where Cl^-, SO_4^{2-}, glutamate and aspartate anions were tested. This suggests that the channels are likely to be impermeable to anions, or else they do not discriminate between anions at all. The latter possibility seems to be very unlikely. However, to confirm directly the cation specificity of MS channels, single currents induced by suction in inside-out patches were measured after a non-electrolyte solution replaced half of the saline solution (Fig. 2). Thus, both the anion and cation concentration were diminished, whereas the tonicity of the cytosol-like bathing solution was not altered. This is a simple and unequivocal test that allows the determination of channel cation or anion specificity. In our experiments, this substitution led to a shift in the *I-V* curve to more positive potentials (i.e. the value of E_{rev} shifted from zero to 20.1 ± 0.9 mV). This indicates that MS channels are highly cation/anion selective.

Figure 5: relationship of MS channels with the substitution of cations in the extra- and intracellular solutions



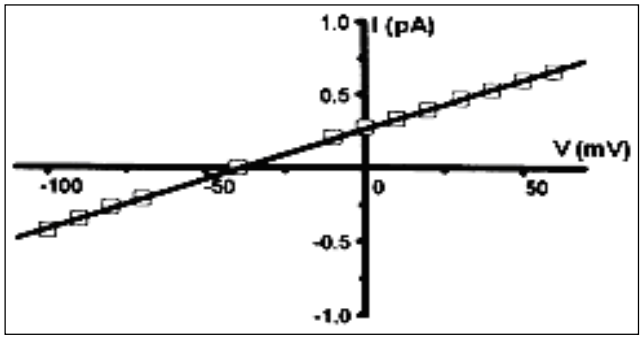
In the next series of experiments, relationships of MS channels were measured with the substitution of cations in the extra- and intracellular solutions (Fig. 5). When the pipette solution contained large organic cations ($Tris^+$ or $NMDG^+$) instead of Na^+, only outward currents through MS channels were recorded; no currents of the opposite (inward) direction were observed in the range of potentials up to −150 mV. Figure 4 also demonstrates the analogous results obtained with inside-out patches using the control Na^+ solution in the pipette and 145 mM NMDGCl in the cytosol-like bathing solution. In this case, we observed only inward mechanogated currents; no reversal of currents took place at potentials up to +60 mV. Thus, current recordings made over a wide range of membrane

potentials revealed unequivocally that the MS channels in these K562 cells were not permeable to organic cations such as $Tris^+$ and $NMDG^+$. These results also confirm that MS channels in leukaemic cells are permeable to cations but not to anions, and do not discriminate between Na^+ and K^+. Moreover, the data obtained allowed us to examine the cation permeability of MS channels in further experiments using these impermeant cations for partial cation substitution.

Ca^{2+} permeation and blocking effects

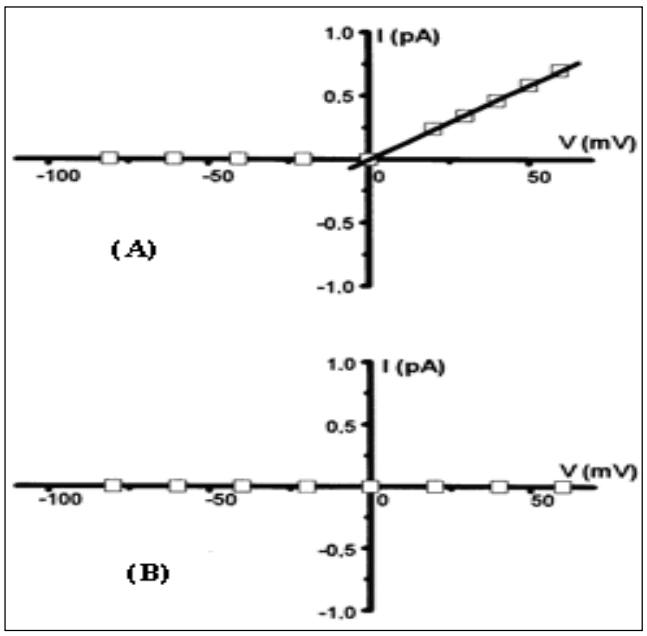
It was found in our experiments on K562 cells that physiological concentrations (1-2 mM) of Ca^{2+} and Mg^{2+} did not prevent the activation of MS channels. In some of the experiments, mechanogated currents were recorded with no bivalent cations present (Ca^{2+} and Mg^{2+}) in the sodium-containing pipette solution. The data obtained revealed channel populations with two major conductance levels (17 and 25 pS) similar to the values measured in the normal solution.

Figure 6: determination the Ca^+ permeability of MS channels



To determine the Ca^+ permeability of MS channels, cell-attached measurements with different concentrations of Ca^{2+} in the pipette solution were performed; other cations in the pipette were impermeant NMDG and Tris (Fig. 6). In the presence of 2 mM $CaCl_2$, outward and inward single-channel currents could be observed; the *I-V* relationship showed a unitary conductance of 7.7 ± 1.0 pS (n = 4) and E_{rev} = −39 ± 2.2 mV (Fig. 5*A*). The relative permeability value (P_{Ca}/P_K) estimated with 2 mM Ca^{2+} in the pipette was equal to 0.75.

Figure 7: affect of $CaCl_2$ onMS channels



We found that when the pipette solution contained 90 mM $CaCl_2$, no activation of MS channels occurred throughout the voltage range tested (Fig. 7B). At a pipette $CaCl_2$ concentration of 20 mM, the outward mechanogated currents activated in response to pressure application were observed at positive potentials, whereas the inward currents were fully inhibited (Fig. 7A). When the pipette solution contained 10 mM $CaCl_2$, rare inward openings of MS channels could be measured at potentials more negative than −20 mV.

Presumably, the inhibition of mechanogated Ca^{2+} currents at increasing extracellular concentrations of Ca^{2+} may be due, at least partially, to the modification of channel kinetics, resulting in an apparent decrease in P_o. *I-V* curves obtained at 10 and 20 mM $CaCl_2$ displayed typical outward rectification; for outward currents, the single-channel conductance measured at positive potentials was about 12-13 pS. Inward channel openings recorded with 10 mM Ca^{2+} in the pipette solution and in the voltage range −100 to −30 mV corresponded to a conductance value that did not exceed 10 pS. These data imply a blocking effect or saturation of open channel currents at higher concentration of Ca^{2+}. Thus, an increased level of extracellular Ca^{2+} resulted in a full or partial inhibition of Ca^{2+} currents through MS channels in K562 cells.

A question arises as to whether monovalent currents through MS channels could be blocked at higher Ca^{2+} concentrations. Figure 6 shows inward and outward currents recorded in a representative cell-attached patch after the addition of 20 mM $CaCl_2$ to the sodium-containing (115 mM NaCl) pipette solution. MS channel activity was observed as at both negative and positive membrane potentials. Single-channel conductance was about 13 pS in these patches. Taking into account that the Na^+ concentration was diminished to maintain ionic strength in these experiments, it is reasonable to consider that this value may correspond to one of the main conductance levels found for MS channels under normal conditions (i.e. 16 pS at 145 mM NaCl). However, we have not made a detailed, quantitative estimation of the possible effect of external Ca^{2+} on mechanogated Na^+ currents for two reasons: (1) we have no data on the possible saturation of Na^+ currents and conductance for these channels and (2) at least two conductance levels were measured in normal Na^+ solution. If we assume that currents and conductances are linearly dependent upon the Na^+ concentration (activity), we can conclude no significant effect of increasing Ca^{2+} in the range 0-20 mM on monovalent currents through MS channels.

Discussion

Very little is known about MS calcium-permeable channels in cells of blood origin. In the present study, MS cationic channels in the plasma membrane of cultured human myeloid leukaemia K562 cells were first identified and then examined. The data obtained confirmed our preliminary results on K562 cells showing stretch-sensitive activation of single-channel currents in cell-attached patches. Moreover, MS channels identified in leukaemia cells displayed some similar features with non-selective cation channels that could be activated by suction in fish erythrocytes [(8)]. Previous studies on blood cells revealed other channel types involved in cell mechanosensitivity and volume regulation: K^+ and Cl^- channels activated during osmotic swelling in frog erythrocytes [(16)], stretch-sensitive K^+ channels in human macrophages [(7)] and volume-regulated anion channels in lymphocytes and myeloma cells [(6)]. In

our experiments on K562 leukaemia cells, the background activity of potassium- and sodium-selective channels was not directly affected by pressure application. We found that MS channels were permeable to Ca^{2+} in the physiological range of concentration. Therefore, the indirect effects of mechanical stimulation on the non-voltage-gated Na^{+} channels may be assumed due to the calcium-dependent channel regulation via actin cytoskeleton rearrangement previously shown in K562 cells [17]. These data suggest that the actin-severing protein gelsolin, which is activated by even a local increase of cytosolic Ca^{2+}, provides an important physiological mechanism controlling ion-channel activity and signal transduction in a variety of non-excitable tissues including blood cells.

It is known that mechanogated channels function as physiological transducers in mechanosensory cells; more general and basic roles such as cell volume and growth regulation in animal cells have been also considered [3, 18]. These functions of MS channels may be realized in blood cells and their precursors. Numerous haemopoietic growth factors were reported to evoke Ca^{2+} mobilization; thus, an essential role of Ca^{2+} signalling in cell proliferation and differentiation processes was suggested [1]. In particular, putative anti-tumour agents were shown to suppress cell growth and to induce erythroid differentiation in human leukaemia K562 cells that had properties of multipotent precursors of blood cells [19, 20]. Investigations of the signalling pathway have indicated that an increase in cytosolic Ca^{2+} is involved in the mechanisms of action of DIF-1 and its analogues, and is required for their anti-leukaemic activity. Importantly, the authors suggested that similar mechanisms could exist in different mammalian cells, emphasizing their therapeutic potential in the treatment of cancer [18]. The MS channels described here may provide one of the physiological pathways for Ca^{2+} influx in different blood cells that are stimulated mechanically during their normal and pathological functioning.

Ca^{2+} influx through MS channels is considered to play an important role in Ca^{2+} signalling in a variety of non-excitable cells [21; 22; 23]. Our results represent the first identification of calcium-permeable MS channels in leukaemia cells. These channels were activated in both cell-attached and inside-out patches by negative pressure application. Without stimulation, the P_o value for MS channels was close to zero. In terms of gating, the channels described here could be assigned to the stretch-activated type. We have demonstrated that MS channels in K562 cells were highly selective for cations over anions and they were non-selective with regard to Na^{+} and K^{+}. The conductance values of MS channels in leukaemia cells for monovalent cations resemble those reported for stretch-activated cationic channels in endocardial endothelium [20] and in Reissner's membrane [24], and for channels reconstituted by expression of the α-subunit of the epithelial Na^{+} channel cloned from osteoblasts [25]. Thus, it may be concluded that leukaemia MS channels are rather similar to the stretch-activated cation channels described in cells of various types [26, 27]. Typically for this type, the channels were shown to be blocked by Gd and to be permeable to Ca^{2+}. However, in numerous

studies, conductive characteristics for bivalent cations were obtained at rather high extracellular concentrations of Ca^{2+} or Ba^{2+} [1, 28, 29]. The advantage of our study is the measurement of mechanogated Ca^{2+} currents and Ca^{2+} permeability in the physiological concentration range.

Channel conductance calculated from the *I-V* relationship with 2 mM $CaCl_2$ in the pipette was about 8 pS, which is comparable with the values measured for much higher Ca^{2+} concentrations. This may be due to the inhibition of channel currents at the higher bivalent ion concentrations used. The blocking action of Ca^{2+} on stretch-activated channels in *Xenopus* oocytes has been described previously [30]. An inhibitory effect of Ca^{2+} and Ba^{2+} on mechanogated monovalent currents was analysed when the concentration of bivalent cations varied within 0-2 mM [31]. Analogous variations of external Ca^{2+} did not alter the amplitude or kinetics of MS monovalent currents in leukaemia cells noted a reduction in channel open time and in the amplitude of the current caused by addition of Ca^{2+} to a sodium-containing bath solution. Unlike our data on K562 cells, an increase in external Ca^{2+} (Ba^{2+}) up to 70 mM [5, 32] or even 160 mM [33] did not result in a full inhibition of inward mechanogated Ca^{2+} currents in frog oocytes [34] also reported that pressure-activated currents in the endothelium were reduced by extracellular Ca^{2+}. The slope conductance for inward currents was diminished from 33 pS in calcium-free solution to 6 pS in isotonic $CaCl_2$ solution. Recently, [35] examined the activation of mechanogated channels initiated by the removal of external Ca^{2+} and other known stimuli. The blocking effects of extracellular Ca^{2+} that are of great interest for MS channels were examined in our experiments on leukaemia cells. Importantly, we found that an increase of external Ca^{2+} resulted in a partial or full inhibition of mechanogated Ca^{2+} currents; at a high concentration (90 mM $CaCl_2$ in the pipette solution), Ca^{2+} completely inhibited channel MS activity. It should be emphasized that monovalent mechanogated currents were not significantly affected by the variations of external Ca^{2+} in the physiological concentration range and at some higher levels. The data allow us to suppose that the pore-forming region of the channel includes an anion site that is characterized by an apparent binding constant for Ca^{2+} that is higher than 2 mM and possibly equal to 10 mM; a site position near the extracellular membrane surface is more probable. Binding of Ca^{2+} could result in an inhibition of Ca^{2+} currents and did not alter monovalent ion fluxes. The complete inhibition of channel activity at high Ca^{2+} concentrations may be due to the existence of one more binding site.

Over the last decade, patch-clamp studies together with fluorescence imaging data have revealed an apparent ubiquity of MS (or mechanogated) channels [36]. These data challenged two different views with regard to the functional significance of these channels in living cells. On the one hand, MS channels, including stretch-activated cation channels, are thought to mediate a variety of functions in both excitable and non-excitable tissues. On the other hand, specific reports have questioned

the reality of mechanogated channels as biological transducers and proposed that such channels are artefacts of patch-clamp recording [37]. It has been suggested that the effect varies with ion channel and cell type and presumably arises because of the disruption of membrane-cytoskeleton interactions. It is worth noting that functional coupling between Na^+ channels and the actin cytoskeleton has been clarified in our previous studies on leukaemia K562 cells [3]. Patch-clamp data obtained in different configurations and using cytochalasin D, gelsolin and different forms of G-actin support the idea that microfilament organization remains essentially undamaged during the course of single-current recordings on K562 cells. Since the cortical cytoskeleton is predominant in erythroleukaemia cells and erythrocytes, it may be that K562 cells present a suitable model with which to examine stretch-activated cation channels and mechanotransduction.

Cation-permeable MS channels are thought to be closely related to the epithelial Na^+ channel (ENaC)/degenerin family of channel proteins that are characterized by the presence of two hydrophobic domains [38]. Studying cation-transporting pathways in non-excitable cells, we found non-voltage-gated sodium-selective channels in macrophages [12], carcinoma cells [36] and leukaemia cells [23]. Amiloride-sensitive Na^+ channels in other cells of blood origin have also been reported [32]. Several lines of evidence show that the characteristics of these channels are similar to those of the well-known ENaC [33]. It is reasonable to assume that the MS channels identified in the present study and the Na^+ channels described previously in leukaemia cells [39] belong to the same superfamily of channel proteins that mediate cation membrane permeability, and are characterized principally by the similarity of their molecular organization and functional properties. The blocking effect of amiloride found in our experiments confirms the similarity of stretch-activated cationic channels and sodium-selective channels in leukaemia cells. Sodium-selective channels were reported to be impermeable to bivalent cations (Ca^{2+} and Ba^{2+} at a concentration of 100 mM). The data obtained here show that high extracellular concentrations of Ca^{2+} may block cationic channels, whereas at lower concentrations the channels may be permeable to this cation.

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Original Article

Sudan Medical Laboratory Journal

Protein/carotine – enriched flaky snack food (Rugag)

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Abstract:

Attempts were made to fortify a Sudanese traditional cereal-based snack food "Rugag". Addition of 27% chickpea or pigeon pea increased the protein contents of the snack significant ($p \leq 0.5$) from 10.1mto 18.3 and 15.3%, respectively. Incorporation of a dry organ sweet potato at 10% level gave a vitamin A content of 540 RE/100g snack material almost three folds increase over the conventional market product (190 RE/100g). Rugag material containing chickpea recorded highest reference ($p \leq 0.05\%$) from those containing pigeon pea or conventional market ones.

The protein quality of snack was assessed by both in vitro and in vivo means. Snack containing chickpea were found to be more susceptible to proteases. The protein efficiency ratio and the net protein ratio of snack containing chickpea were (2.33-2.46; 3.8-3.96, respectively), and they were significantly ($p < 0.05$) higher than respective values obtained from market Rugag (1.41, 2.68 respectively). The relative net protein ratio of one fortified snack food was closed to that of casein (81.3% of casien). The newly developed high protein/carotein rich Rugag flakes can successfully replace poor quality street food unhygienically sold to school boys; yet fortified flakes can be used as emergency foods.

Introduction:

Snack foods are used universally for different dietary purpose (Gudith and Clara, 1971). Rugag is an analogue of flakey snacks prepared by Sudanese for their fasting month of Ramadan (Gorafi and Ahmed, 1995). Rugag is based on wheat flour with other ingredients that constitute ordinary meal having no dietetic claims (Ali and Ahmed, 1996). Fortification of such traditional snackshas been recommended earlier (WHO, 1988) to help combat nutritional disorders among population of developing countries. The aim of this work was to attempt fortification of Rugag with a protein rich source (legumes) and vitamin A source (orange sweet potatoes) to extend its food uses among needy groups.

Materials and methods:

Legumes (chickpea and pigeonpea) were purchased from the local market (Khartoum North). Orange sweet potatoes (var. Rimaila) were obtained from Shambat Research station. Other food ingredients were obtained from local markets.

Preparation of raw materials:

Orange sweet potato tubers were sliced, sulphited (1.5g metabisulphite/Kg), sun dried, powdered (Falling Number A. B. 3100) to pass mesh size 500µg and stored for different uses. Chickpea and pigeonpea were decorticated (TADD –Model

4E 200 Serial number 0122) and powdered (Falling Number A. B. 71849) to pass a 60 mesh screen.

Preparation of the snack products:

Table 1 show recipes used in preparation of snack foods. Ingredients were mixed with water (3:1 water to material ratio) to form a thin batter which was drum dried (c/o A. Musa – Industrial Area , Khartoum North) at 175± 25°C for 25 seconds as contact time. Dried flakes were then packed in plastic containers and kept or different experimental purposes.

Table (1): Recipies used in preparing snack food

Recipe code*	Wheat flour%	Legume flour%	Skimmed milk powder%	Sugar (sucrose powder)%	Dry sweet potato powder%	Vanillin%
C_1	38	27	12	10	10	3
P_1	38	27	12	10	10	3
C_2	40	27	10	10	10	3
P_2	40	27	10	10	10	3
C_3	53	20	5	10	10	2
P_3	53	20	5	10	10	2
Control (lab)	85	-	5	10	-	2

*C- denotes chickpea legume; P- denotes pigeonpea legume

Proximate composition of snacks:

Levels of nutrients (protein, fat, carbohydrates, etc) in Rugag sample were measured according to official method of analysis (AOAC 1975). Vitamin A content was determined according to an official method described in the AOAC (1990).

Organoleptic quality:

Different sessions were conducted to determine acceptability of newly developed Rugag flakes. In two consecutive sessions, semi-trained staffs from the FRC (15-19 persons) were asked to assess quality attributes (appearance, flavor, taste, texture, etc) of end products according to the ranking procedure of Ihekoronye and Ngoddy (1985).

Protein assays:

In vitro protein digestibility:

Total protein digestibility was measured according to the method of Saunders et al (1973) using gastric pepsin (activity 3200-380 units /mg protein) pancreatin (activity equivalent to USP specification).

Protein efficiency ratio (PER), net protein ratio (NPR) relative net protein ratio (RNPR):

PER, NPR and RNPR were determined as describe by Pellett and Young (1980). Wistar strain weanling male rats ((35-45 g) were housed individually and fed at libitum for 4 weeks on diets containing about 10% protein. One group of rats was fed a basal diet (nitrogen free) to allow assessment of NPR. The RNPR was computed from NPR of samples against NPR of casein diet.

Results

Table (2): Composition of snack food

Type of snack*	Moisture%	Fat%	Protein** (NX6.25) %	Total Ash%	Total*** CHO%	Vit. A (retinol equivalent/100g)%
C_1	6.4	2.9	18.3	2.5	69.9	540
P_1	6.5	1.7	15.3	2.3	74.2	475
C_2	4.6	2.2	16.1	2.3	74.8	540
P_2	5.8	1.4	14.9	2.3	75.6	475
C_3	5.8	3.5	12.2	2	77	540
P_3	5.1	1.7	11.6	2	79.6	475
Control (lab)	6.2	0.9	10.1	1.3	81.5	196
Control market	2.1	1.9	10.7	1.6	83.7	190

*C- denotes chickpea legume; P- denotes pigeon pea legume
**Any two mean values having different superscript letters differ significantly ($p<0.05$)
***Total carbohydrate by difference (Pearson 1976).

Table 3: Organoleptic quality* of snack products

Type of snack	Sum of Ranks**					
	Appearance	Taste	After taste	Flavor	Texture	Preference
C1	32	21	22	23	32	22
C2	51	38	38	42	48	43
P3	70	64	60	57	65	61
Control (market)	37	67	70	68	45	64

* For best snack products selected from previous session
** Any two sums of ranks having different superscript letter differs significant ($p\leq0.05$)

Table 4: In vitro protein digestibility (IPD), protein efficiency ratio (PER), net protein ration (NPR) and relative net protein ratio (RNPR) of snack foods

Type of snack	IPD%	PER*	NPR	RNPR%
C1	95.1	2.46	3.96	81.3
C2	90.8	2.33	3.80	79
Control (Market)	60.4	1.41	2.68	66.6
Casein**	-	2.68	4.86	100

* Any two values having different superscript letters differ significantly ($p\leq0.05$)
**Casein standard diet.

Discussion:

Table 2 shows proximate composition of snacks. The protein contents of the fortified snacks increased significantly ($p<0.05$) compared to the control samples.

Carbohydrate- based snacks with legume sources was known to improve their protein level (Green et al 1978, Walter 1978). All th snacks were characterized by low fat contents (1.7-3.5%), an advantageous property expected to appreciably extent their shelf life under Sudanese climate. The rest of the parameters may reflect more or less expected variations in energy value and mineral matter contents of the snacks. Vitamin A level in snacks improved significantly ($p\leq0.05$), naturally through inclusion of rich β-carotene source (sweet potatoes).

Table 3 concludes two successive sessions carried out to determine organoleptic quality of the snack products. It was obvious that C1 formula was superior and significantly ($p \leq 0.05$) better than the rest of the snack in all attributes tested. Inclusion of pigeon-pea in conventional Rugag resulted in significantly poor taste and poor texture, two attributes considered most critical for human acceptability of these types of snacks.The quality of the snacks protein as measured by in vitro and in vivo means is presented in table 4. Snack containing chickpea (C1 & C2) recorded digestibility of 95.1% and 90.8% respectively, which are significantly higher than both pigeon-pea containing snacks (83.3%) and market control (60.4%). The efficiency ratio o C1 proteins (2.46) were almost similar to that casein (2.68) suggesting suitability of such snack formula weaning (growth) purpose. The absence of significant differences between NPR of C1 and casein reflects the body maintenance role which can be played by fortified Rugag.

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Original Article

Sudan Medical Laboratory Journal

Prevalence of group A rotavirus among children in Khartoum state

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Abstract

This study was carried out to identify rotavirus group A in children suffering from diarrhoea in Khartoum State (Khartoum and Khartoum North) . Samples of stool were collected during the period from May to June 2006 from 60 infants aged between 4 months to 5 years suffering from diarrhoea.
Using direct enzyme linked immuno sorbant assay (ELISA). Forty five samples were detected in Khartoum hospitals , the positive samples for rotavirus group A was 4 (6.7%) and 15 samples in Khartoum north , the positive samples was one (1.7%). The total of positive rotavirus group A was five samples in khartoum state (8.3%).

Introduction

It is estimated that over one billion episodes of acute diarrhoea occur annually in children under 5 years of age in Asia, Africa, and Latin America, resulting in about 5 million deaths. Moreover, the dehydration caused by diarrhoeal diseases accounts for one-third to one-half of infant mortality in many developing countries [(1)].

The word diarrhoea is derived from the Greek words for (flowing through) . For most persons, diarrhoea means the frequent passage of loose stools [(2)]. Diarrhoea is a universal human experience and persons have had acute infectious diarrhoea at some time during their lives [(3)]

Most forms of acute diarrhoea (i.e., those lasting less than 4 weeks) are caused by infections and are self-limiting; the majority are caused by viruses (e.g. rotavirus, adenovirus, Norwalk agent,), but some are caused by bacteria (e.g., Salmonella, Shigella, and *Escherichia coli*) and others by protozoa (e.g., giardia and amoebas) [(3)]

Acute diarrhoea is frequent among travelers in whom enterotoxigenic *E. coli* is particularly common (4). In practice, most episodes of acute diarrhoea that are assumed to be caused by an infectious agent are treated without the causative agent being identified. [(5)].

Study in Atlanta and Georgia between 1986 and 1999 indicated that rotavirus causes approximately 22% of childhood diarrhea hospitalizations. From 2000 to 2004, this proportion increased to 39%. Application of this proportion to the recent World Health Organization estimates of diarrhea-related childhood deaths gave an estimated 611 thousands rotavirus-related deaths [(6)].

The identification of different infectious agents found in infantile diarrheas was undertaken in 237 children hospitalized in pediatric institutions in Libreville. In neonates between one day to 18 months of age, the most common pathogen was Rotavirus (20.6%), followed by Shigella (10.8%), Escherichia coli (9.24%), Salmonella (3.46%), Entamoeba histolytica (0.8%) and Yersinia enterocolitica (0.4%). The highest percentage of diarrheas caused by rotaviruses was found among children between 6 and 11 months of age. The frequency of Salmonella among children 2 to 4 years of age was almost the same as that in children under 2 years of age (3.44%). Isolation of Shigella in this group reached 12.06% [(7)].

Diarrhoeal diseases are noted to be a major cause of morbidity in children bellow 5 years of age in Sudan. Viruses such as rotaviruses and coronaviruses are reported as primary causes of diarrhoea in infants [(8)].

The epidemiology of gastroenteritis (diarrhoea) in children less than 5 years of age in Sudan was studied during 2002-2004 by, (personal communication). They found that out of 134 stool samples from diarrhoeic children in different localities in Sudan (Khartoum, River Nile and White Nile States) tested for group A rotavirus using ELISA, 35 gave positive results. Twenty nine of these were examined by page. Twenty four of them were positive with long electropherotype and five had different profiles. In the positive samples, the characteristic appearance of rotavirus was seen under the electron microscope.

This study is intended to investigate the prevalence of rotavirus in children with diarrhoea in Khartoum state.

Materials and methods

Specimens collection:

Faecal specimens were collected from 60 hospitalized children aged 4 months to 5 years. Specimens were collected in sterile containers and transported directly to the laboratory and were frozen at -20°C until used.

Samples were collected from both sexes during the period from May to June 2006 from Ibraheem Malik hospital (Khartoum), Khartoum Teaching Hospital and Ahmed Qasim Hospital for children (Khartoum North).

Detection of group A rotavirus antigen:

All stool samples were examined by enzyme immunoassay) rotavirus IDEIA, DAKO diagnostics, United Kingdom). The test was performed as specified by manufacturer instructions.

Results:

It was found that out of the 60 faecal specimens, 5 contained rotavirus group A (8.3 %). The details are presented in table 1:

Table 1:

Area	**No. tests**	**+ve**	**%+ve**
Khartoum	45	4	6.7 %
Khartoum North	15	1	1.7 %
Total	60	5	8.3%

Discussion:

In developing countries like Sudan, infections are often caused via faecal-oral transmission and are

thus more common in situations where sanitation is poor and water supply is contaminated and malnutrition occurs. Motarjemi et al (10) noted that diarrhoea among infants and children under 5 years of age was associated with malnutrition, water supply and sanitation.

The most common causes of diarrhoea in children are mainly viral, followed by bacterial then parasitic etiologies (11, 12 and 13). Children 6 months to 2 years of age are more often presented with diarrhoea due to viral infections mainly rotaviruses **(14)**. In Sudan Sixl *et al.*, **(15)** during a study of diseases associated with diarrhea in Melit District in South Sudan in 1981 – 1982 showed that the major detected pathogens were amoebie dysentery and rotavirus. In this study rotavirus type A was found in five (8.3%) of the diarrhoeal specimens. Unlike result previously obtained in Sudan Ali et al (2001) (personal communication).This study was conducted during summer between May and June, but in winter months virus would have detected in more cases. Cann AJ (2001) isolated rotavirus more frequently during the winter months.

Huilan et al., (14) stated that enteropathogens are found during all seasons of year except infection with rotavirus which showed a seasonal variation. A study documenting rotavirus *(*RV*)* diarrhoea in Asian infants in South Africa, described the virological and epidemiological aspects of this disease in this population. Fifty five per cent of 1142 hospitalized cases investigated over a 31 – month period showed positive stools for RV using ELISA. Most of these children stopped shedding RV by days 4-6 of hospital admission, though prolonged excretion was recorded in some acute cases for up to 13 days. Mixed RV-bacterial infection occurred in 7% of the total gastro-enteritis (GE) patients. The age-groups mainly affected were between 3 and 14 months, with a peak at 9-11 months; 3% of the rotavirus gastroenteritis (RVGE*)* patients were neonates. Both the RVGE and the total gastroenteritis (GE) admission showed well-marked winter peak, with an inverse relationship between RV prevalence and both temperature and humidity. It was concluded that RV was the most important cause of infantile GE in this population, whereas pure bacterial infections played a relatively minor role (16). Due to the major role play by rotavirus in diarrheal episode there is a global trend to introduce vaccine, this needs of country surveillance to explore the prevalence of this viral infection. Further study to cover this area and to characterize the virus in Sudan is highly recommended.

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Original Article

Sudan Medical Laboratory Journal

The in vitro effect of the snake venom in Sudan on haemostatic mechanism

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Abstract:

The present study was carried out on the snakes captured from Khartoum North, Algazzera and Nuba mountains, where venoms were collected from 4 types of snakes belonging to the family Viperidae, genera: Echis and Bitis, species: ocellatus, pyarimidum, leucagaster and arietans. The study was concerned with the detection of the in-vitro effect of snake venoms on haemostatic mechanism with emphasis on the coagulative effect, fibrinolytic effect and specific action on the on coagulation and fibrinolytic factors. The result showed that venom of the genus Echis has an effect on the fibrinogen solution. There was a variation of the venoms effects on the plasma deficient factor, which varied according to the species of the snakes. The study revealed that there is a variation in the results of the species ocellatus and pyarimidum when added to plasma deficient factor II. It was found that the venoms of the snakes of the genus Bitis has an effect on dissolving fibrin while with the 3 species of the genus Echis, this dissolving effect occurred after the coagulative effect previously occurred. Electrophoresis results revealed the separation of different proteins, which varied according to the species of snakes involved

Introduction:

Venoms of viperid and crotalid snakes are rich sources of components that can affect haemostasis by causing changes in blood coagulation and platelet function. Venom components affecting the clotting system include thrombin-like enzymes (TLE) which convert fibrinogen to fibrin, and activators of prothrombin, factor V and factor X. Platelet function can be affected by venom components such as hemorrhagic metalloprotein-ases, phospholipases. As a result, it is common to find consumption of clotting factors and blood incoagulability accompanied by hemorrhage in victims of snake bite. Various reviews on this subject have been published [1, 2, 3 & 4].

Several venom enzymes lacking coagulant activity but possessing the ability to cause local hemorrhage have been characterized, as recently reviewed by Bjarnason and Fox (5). They are zinc-containing metalloproteinases varying in size from 20 to 100 kDa, capable of inducing rapid local bleeding. Metalloproteinases of different sizes are often present in the same venom. The high molecular weight enzymes are classified as metalloproteinase/ disintegrin-like/cysteine-rich (MDC) proteins, according to a structure containing an N-terminal catalytic site, a disintegrin-like domain followed by a cysteine-rich C-terminus. These enzymes have a potent proteolytic effect on the extracellular matrix proteins. Their effects on the blood vessel wall

components have been extensively studied (5, 6). The arrest of bleeding from the damaged blood vessels, however, depends on normal function of blood platelets and clotting factors, which are the principal blood components involved in haemostasis. The extent to which these latter components are directly affected by venom hemorrhagic metalloproteinases has not been explored in detail.

Another group of snake venom components that affect platelet function is comprised of small non-enzyme polypeptides (5-10 kD) called disintegrins. Disintegrins have also been recently investigated in many other cells besides platelets. Since disintegrin domains of venom MDC enzymes and disintegrins show pronounced structural homology, they will be reviewed together here, referring particularly to their effects on cell-cell interactions using platelets as a model.This study aimed to detect the in-vitro effect of snake venoms in Sudan on the haemostatic mechanism and also to separate the venoms conistituents by electrophoresis.

Materials and methods:

Study design:

This is an experimental type of study.

Study area:

This study was conducted in the snakes captured from rural areas in Khartoum north, Elgazeera and Nuba mountains.

Study population:

The study was conducted on snakes of the family viperidae, genera: Echis and Bitis, species, E. ocellatus, E. pyarimidum, E. leucagaster and B. arietans.

Study duration:

The Study commenced in 2004 and ended in 2006.

Sampling:

Capturing of snakes:

This was done by using a snake catcher and holding the snake in its neck by the catcher.

Collection of the venom:

Sterile glass beaker was prepared for the venom collection. The snake was caught from the end of the neck exactly at the area of venomous glands. Snake fangs once erected, were placed at the edge of the glass beaker. Smooth squeezing was applied for the venomous glands to help the withdrawal of the venom in the glass beaker.

Preparation of the venom:

The venom was prepared by dissolving 1 mg of desiccated snake venom in 1 ml PBS to give a stock solution of venom.

Calcium chloride (CaCl2 0.025mol/l), factor deficient plasma, Thromboplastin reagent and partial thromboplatin reagent were commercially obtained from DiaMed company.

Preparation of Platelet Poor plasma (p.p.p):

P.P.P was prepared according to the method described by Dacie 7.

Assessment of procoagulant activity without addition of 0.025mol/l CaCl2:

100 uL of plasma was transferred into coagulometer cuvette.

200 uL from each species of venom was then added.

Clotting formation was measured as endpoint of reading in the coagulometer.

ISSN*: 1858-6147*

Assessment of procoagulant activity with addition of 0.025mol/l CaCl2:

- 100 µL of plasma was transferred into coagulometer cuvette.
- 100 µL from each species of venom was mixed with 100 µL of $CaCl_2$ then added.
- Clotting formation was measured as endpoint of reading in the coagulometer.

Assessment of thrombin like enzyme activity:

- 100 µL of fibrinogen solution was transferred into coagulometer cuvette.
- 200 µL of Echis species venom was added to the solution.
- Thrombin like enzyme activity was measured as the end point of the clotting formation by reading in the coagulometer.

Assessment of the action of snake venom on the plasma deficient factor:

- 100 uL of each plasma deficient factor was transferred into coagulometer cuvette.
- 200 uLof Echis species venom was added to the tested plasma deficient factor.
- Clotting formation was measured as endpoint of reading in the coagulometer.

Detection of the fibrinolytic activity:

- Fibrinolytic activity of the Echis species was tested at the same cuvet where the clot was formed. For the Bitis species, which showed no clot during the study and to further, investigate the role of the Bitis venom on the haemostatic mechanism, CaCl2 was added to the normal P.P.P. The solution incubated till clotting occurred.
- 200 uL of Bitis arietans venom was added to the recalcified plasma.
- The mechanism of firinolysis was followed till dissolving of clot occurred.
- N.B: All the above-mentioned methods were compared against the controls
- (PBS and CaCl2) instead of the venom.

Method of polyacrylamide gel electrophoresis (PAGE):

5*uL* of each venom sample was added to 5 *uL* of loading buffer. The sample and protein marker was then layered on 5% stacking gel, which was prepared by adding the following contents (D.W, 30% polyacrylamide mix, 0.5 M Tris (PH 6.8), 10% SDS, 10% APS and TEMED). The electrical current was then adjusted to 80 volt.

The electrical current was increased to 100 volt when the samples attached the 5% resolving gel, which was prepared by adding the following (D.W, 30% polyacrylamide, 1.5 M Tris (PH 8.8), 10% SDS, 10% APS and TEMED) with different concentrations from stacking gel. The electrical current was stopped once the loading buffer reached the end of the resolving gel.

The gel was then taken out and splitted in staining solution and was then incubated for 24 hrs at room temperature.

The excess stain was removed by the destaining solution, which contains absolute methanol, glacial acetic acid, and D.W.

Data analysis:

Data was analyzed using Microsoft Excel program.

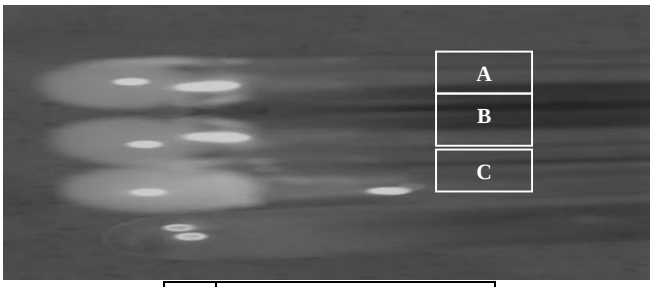
Results:

ISSN*: 1858-6147* S*ud Med Lab J, issue 1, vol 1 (2011)*

The results showed that when viperidae snakes venom were added to the plasma (p.p.p), its effects varied not only according to the genera but also to the snake species, whether it is the genus *Echis* or the genus *Bitis.* Venom derived from the 3 *Echis* species showed a pronounced coagulating effect on the plasma. This differences was statically significant at P= 0.05. The venom of *Bitis arietans* showed no coagulating effects after 10, 20, 30 minutes and 1hour.

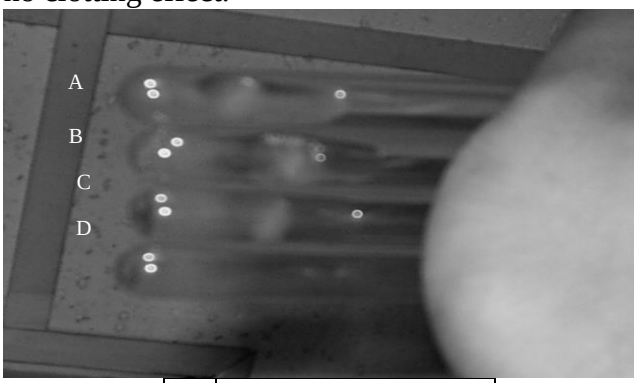
The results demonstrated showed also that venom of the genus *Echis* has an effect on the fibrinogen solution, which was statically significant at P= 0.05. When the venoms of the snakes of the genus *Echis* was added to the plasma deficient factor, their effects varied according to the species of the *Echis* snake and also according to the deficient (missing) factor. With the venoms of the three species of the genus *Echis*, coagulating effect occurred (figure 1) and was then followed after a while by the dissolving effect of the fibrin (figures 2, 3, 4, 5 and 6). the time observed for fibrinolysis was 10 to 2o minutes.

With regard to testing the snakes venoms on the recalcified plasma, it was found that the venoms of the species *Echis* have an effect on dissolving fibrin. *Bitis* venom as well showed an effect on dissolving fibrin (figures 7,8and 9). The electrophoresis results revealed that different bands were separated. This separation varied from species to another even within the same genus depending on the molecular weight of the bands. Protein marker was done with the test including (Lysozyme 14.3,Trypsinogen 24.0, Pepsin 34.7, Albumin 45.3 KD). The *ocellatus* species resulted in 2 bands with molecular weights 64.8 and 40.82 KD, while the *pyarimidum* resulted in 2 bands with molecular weights 72.83 and 71.52 KD. The *leucagaster* species resulted in 1band with molecular weight 68.49 KD. The last species (*arietans*) resulted in 7 bands with molecular weights 93.12, 79.95, 77.18, 66.77, 60.5, 60.58 and 48.86 KD (figure 10).



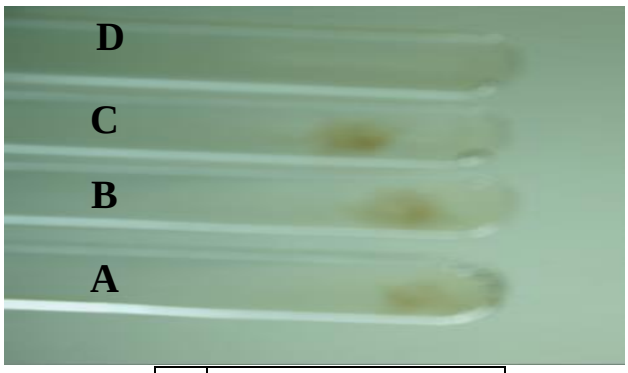
A	*Echis ocellatus*
B	*Echis pyuarimidum*
C	*Echis leucagaster*
D	*Control*

Figure 1: Clot of P.P.P with the venom of *Echis ocellatus, Echis pyarimidum* and *Echis leucagaster* in addition to control, which showed no clotting effect.



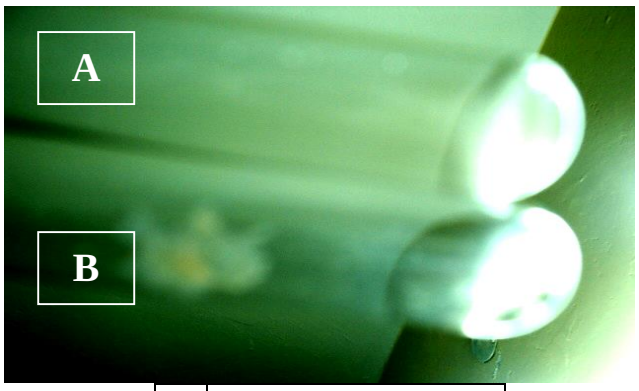
A	*Echis ocellatus*
B	*Echis pyuarimidum*
C	*Echis leucagaster*
D	*Control*

Figure 2: Starting of fibrinolysis and its effect.



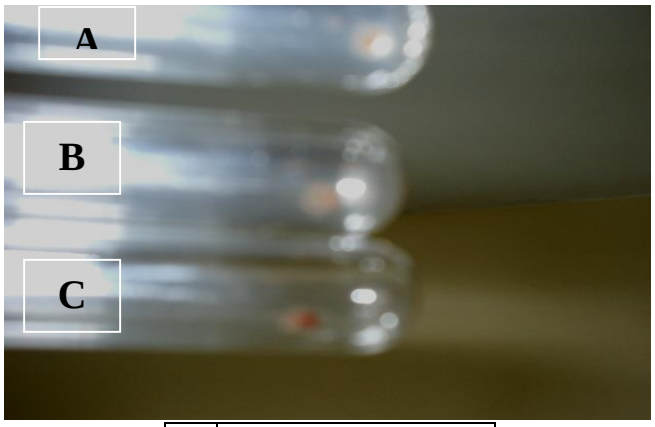
A	*Echis ocellatus*
B	*Echis pyuarimidum*
C	*Echis leucagaster*
D	*Control*

Figure 3: Starting of fibrinolysis and its effect.



A	*Control*
B	*Echis species Venom*

Figure 4: Dissolution of the clot.

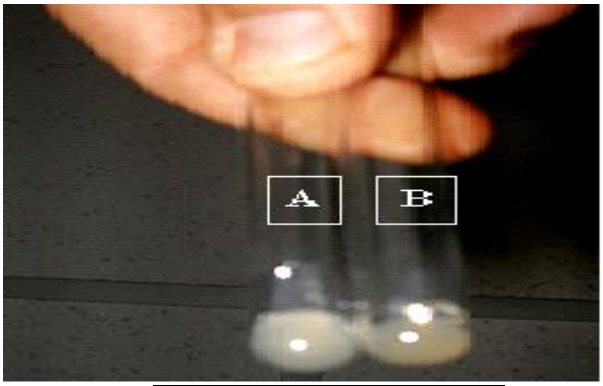


A	*Echis ocellatus*
B	*Echis pyuarimidum*
C	*Echis leucagaster*

Figure 5: The progressive diminution of clot**.**

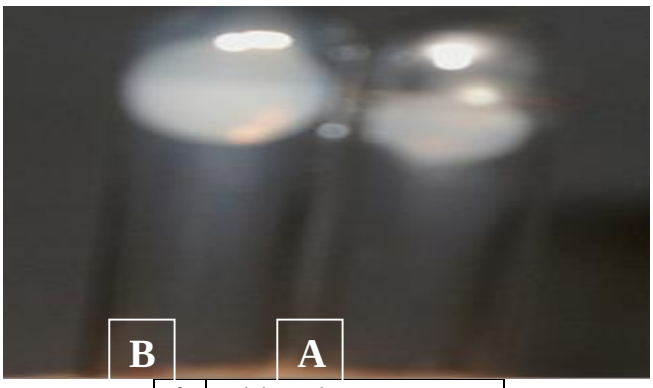
A	*Echis ocellatus*
B	*Echis pyuarimidum*
C	*Echis leucagaster*
D	*Control*

Figure 6: The final results of the fibrinolytic activity of the snake venoms.



A	*Bitis arietans Venom*
B	*Control*

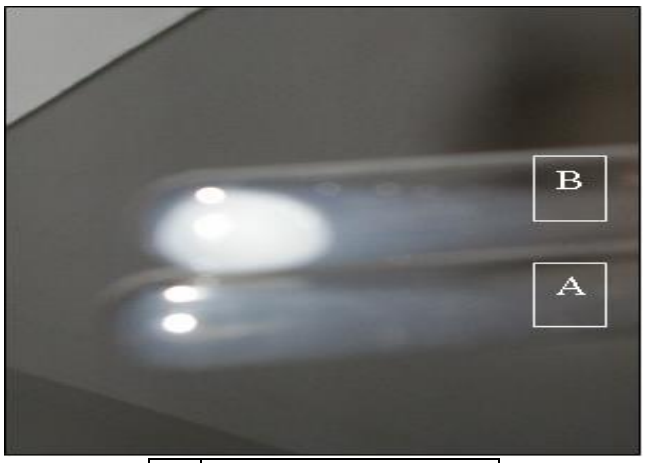
Figure 7: Recalcified plasma with tube of control.



A	*Bitis arietans Venom*
B	*Control*

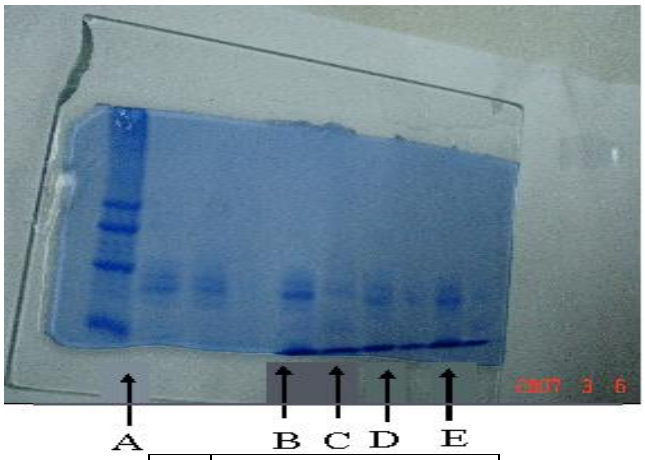
Figure 8: The diminishing size of the clot after the addition of Bitis arietans venom compared to control.

ISSN*: 1858-6147*



A	*Bitis arietans Venom*
B	*Control*

Figure 9: The complete dissolution of the clot.



A	*Protein marker*
B	*Echis ocellatus*
C	*Echis pyuarimidum*
D	*Echis leucagaster*
E	*Bitis arietans*

Figure 10: Venoms proteins in SDS-PAGE.

Discussion:

Might have the capability of converting fibrinogen to In this study four types of snake venoms belonging to the family viperidae were collected. The genera involved are: *Echis* and *Bitis*. There are 3 species belonging to the genus *Echis* (*ocellatus*, *pyarimidum* and *leucagaster*), and one species that belongs to the genus *Bitis* (*arietans*).The effects of all these venoms on plasma were tested.

The results revealed that all three *Echis* species are capable of coagulating plasma, with *Echis leucagaster* showing the least time for inducing coagulation.

On the other hand, *B. arietans* was shown to lack this capability. This coagulatory effect was previously reported by Dacie [(7)] with the *russels* species which is a snake belonging to the same viperidae family. The effect of the venom is similar to the factor X in promoting the coagulatory process.

From the study, it was obvious that the three *Echis* species have an effect on the fibrinogen factor, which might probably reflect the thrombin like action of the venoms. This finding was consistent with the finding of Dacie [(7)] who proved that the taipan gave the same action similar to the action of the venom of the three Echis species in converting fibrinogen to fibrin.

As the study revealed, the coagulatory mechanism induced by the venoms of the three *Echis* species when added to the plasma deficient factor may differ from species to another depending on the deficient factor as when the venom of the *E . ocellatus* was added to the plasma deficient in factors V, VII , X , XI and XII , the coagulatory effect occured in the same period of the coagulation of normal plasma . While, when the venom was added to plasma deficient in factor VIII and IX, the coagulatory effect occured in a period longer than the period of coagulation of normal plasma. This finding might probably ascertain the dependability of this venom on the presence of these two factors as they act on promoting the coagulatory activity of the venom.

When the venom of the *E. leucagaster* was added to the plasma deficient in factor VIII, the coagulation time was longer than the coagulation time of normal

plasma, which might probably indicate the importance of factor VIII to the venom in order to rapidly activate its enzymes.

From our findings, it was clear that when the venoms of *E. ocellatus* and *E. pyarimidum* were added to the plasma deficient in factor II, they failed to induce any coagulatory effect. This might probably be explained by that these two venoms might have an enzyme that directly acts on fibrinogen rather on prothrombin. When the fibrinogen is in the plasma, they cannot convert the fibrinogen into fibrin, which may be attributed to the presence of the some substances that suppress the action of the enzyme on the fibrinogen. In other types of plasma deficient factors, there must be other enzymes that are capable of accomplishing the coagulatory process by producing thrombin. This finding is in line with the results of Howes [8] who found that snake venom metalloproteinases SVMPs were capable of activating prothrombin to varying degrees and can therefore be described as procoagulants. He demonstrated that EoVMP1, EoVMP2 and EoVMP3 share sequence identity with other members of the reprolysin family, but differ greatly in their effects on some of the components that control haemostasis.

On the contrary, the venom of the *E. leucagaster*, when added to the plasma deficient in factor II, the coagulatory mechanism is accomplished which draws attention that the venom fibrin i.e it acts as a thrombin like enzyme. This finding was in agreement with Mitrakul [9] who reported that Malayan pit viper (MPV) has a strong thrombin-like coagulant action.

However, the three venoms involved in this study (*ocellatus* , *pyarimidum* and *leucagaster*) showed the ability to dissolve the fibrin following its formation after the coagulatory mechanism (figures, 3.6 ,3.7 ,3.8 ,3.9 and 3.12).

As from the results (figures 3.13, 3.14 and 3.15), the venom of *B. arietans* demonstrated an ability to dissolve recalcified plasma. This finding was previously confirmed by Mitrakul [9] who demonstrated the lytic activity on normal euglobulin clot.

As far as the electrophoresis is concerned, our finding revealed that there is variation in the results of the polyacrylamide gel electrophoresis, which by turn confirms the existing variation in the constituent of different venoms. These findings were in line with the findings of Huseyin (10) who used the same technique and indicated that the secretion of the colubrid snake had a total of 8 fractions or fraction groups (protein bands), while in the viperidae samples, he observed 10-14 protein bands. He concluded that the venom of viperidae snake is more complex than that of colubrid snake in Turkey and Cyprus.

It was revealed that the larger the number of proteins, the more complicated is the structure of the venom. Consequently, the variations in the structures in these venoms ascertain the variations in their effects on the different types of plasma and the fibrinogen solution.

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Original Article

Sudan Medical Laboratory Journal

Cultures of urine samples collected from catheters compared with those obtained by direct Supra-pubic aspiration

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Abstract

To examine the validity of cultures of fluid collected through drainage catheters, we reviewed retrospectively fluid specimens that had been collected through catheters in place for at least 2 days. These specimens were taken from patients at a large tertiary-care hospital. A total of 150 specimens representing 75 patients were collected as 2 samples from each patient, one sample is collected from drainage catheters and the other is collected directly as a supra-pubic aspiration. For 50 (67%) episodes there was no reliable imaging evidence for renal infection (UTI), making the results uninterpretable. The remaining 25 (33%) episodes were with infection. In 56% of cases, matched culture results were equivalent for therapeutic decisions. We conclude that direct aspiration of potentially infected fluid collections is the most reliable method of obtaining specimens for culture that should be used to guide therapy.

Introduction

One-step needle aspiration is not commonly used for diagnosis and collection of urine samples [(1)], percutaneous catheters often are inserted specially in case of renal obstructive disease (anuric patients). They are also placed manually during many surgical procedures to prevent accumulation of exudates and blood at the operative site. Over the days to weeks that these catheters remain in place, drainage urine may be submitted for culture, especially when symptoms and signs suggest infection. Culture of such urine is potentially misleading, however, when the fluid becomes contaminated within the catheter or collection apparatus. To further examine the validity of cultures of urine collected through drainage catheters, we initially looked at the clinical circumstances under which specimens from drainage catheters were submitted for culture, then compared the culture results for urine collected through a drainage catheter with those for direct supra-pubic aspiration, and finally assessed the potential therapeutic consequences of these comparisons.

Materials and methods

Study area:

The study was carried in Portsudan teaching hospital laboratory, Red Sea state in Sudan.

Types of study:

An observation case finding hospital-based study (comparative)

Study population:

The study was conducted on patient admitted to Portsudan teaching hospital, and treated with urinary catheter for any cause.

Sample size:

150 samples were taken from 75 patients.

Samples collection:

2 urine samples were collected in separate sterile containers from each patient, one sample is collected from catheter and the other is collected as aspiration, and then both samples were sent to the clinical microbiology laboratory for culture.

Data collection:

A questionnaire was designed for data collection. It includes questions on history of UTI, anuria and other renal diseases.

Techniques:

First, we examined the radiological records for each episode. Then, we compared the results of bacterial culture from matched specimens, reviewed the corresponding computed tomography (CT) or ultrasound (US) films, and examined the clinical records.

For each culture result of direct aspirate or catheter drainage fluid, an infectious-disease physician who had not been directly involved with the patient's care noted retrospectively one or a combination of antimicrobial agents that would have been reasonable therapy, in combination with drainage, for the microorganisms present. A combined beta-lactam and beta-lactamase-inhibiting agent with broad-spectrum aerobic and anaerobic activity frequently was selected. Individual samples were assessed according to the relative quantity of microorganisms on Gram stain microscopy and culture, the reputation of each isolate as a pathogen, and antimicrobial susceptibility results, when available. For example, yeasts were judged to require treatment only when detected by both Gram stain microscopy and culture. Actual antimicrobial use by the patients' physicians was not examined.

Assuming culture of directly aspirated urine to be the "gold standard," the potential consequences of selecting antimicrobial therapy based on the results of catheter drainage fluid culture were assessed: therapeutically equivalent drainage catheter results were defined as correct, and discrepant drainage catheter results were defined as excessive (if the result could have led to unnecessary antimicrobial therapy) or inadequate (if the result could have led to insufficient antimicrobial therapy).

Each image-guided direct aspiration was performed using an 18-gauge needle, aseptic technique, and single-use, sterile equipment. In the clinical microbiology laboratory, catheter drainage and aspirated urine samples were examined microscopically after Gram staining and were cultured on the following media: blood agar with 5% sheep blood.

Results

A total of 150 specimens representing 75 episodes were collected during the study period. For 38 (50.7%) episodes, no CT or US imaging of the corresponding body region was done within 2 days before or after submission of the specimen. For 50 (67%) episodes there was no reliable imaging evidence for renal infection (UTI), making the results uninterpretable. The remaining 25 (33%) episodes were with

infection. Thereby, allowing comparison of culture results of direct aspirates with those of prior catheter drainage.

For 25 (30%) of patients, the catheters were inserted to them during or after surgical operation for different purposes. On the day of initial collection of urine from the drainage catheter or the previous day, 10 patients (13.3%) had a temperature of ≥38.3°C, and 42 patients (56%) had more than 50 ml of urine draining from catheter present (not necessarily the catheter from which the index sample was collected). Catheter drainage fluid submitted for culture was described as brown, green, cloudy, purulent, or bloody for 53 (70.7%) patients.

Overall, culture of directly aspirated fluid was positive in 32 (42.7%) patients. Cultures of catheter drainage and aspirate fluids gave comparable results with regard to therapeutic decision making for 23 patients. Discrepant results between catheter drainage and aspirate fluid cultures that could have led to incorrect therapy were seen for 26 patients; in only 4 (5.3%) cases could these differences be attributed to changes in antibiotic therapy between sampling times or to the selection of the media and atmosphere for incubation. Potentially misleading results from catheter-collected specimens were equally frequent when the drainage catheter tip apparently lay in the same fluid collection that subsequently was sampled (9 [12%] of cases) as when the tip apparently lay in a site remote from the collection that subsequently was sampled (15 [20%] of cases). The median duration of drainage catheter placement before the initial specimen was collected was range 6 days to 2 weeks for patients with therapeutically equivalent culture results and range 3 to 8 days for patients with discrepant culture results; the median values for these two groups were not significantly different (P = 0.106).

Discussion

Although Kommar et al in 1980 that urine collected through a drainage catheter into sterile container could be "sent to the laboratory for bacteriological culture without fear of contamination from the external environment," no data were presented to support this conclusion [(2)]. Subsequently, several authors have examined prospectively the correlation between surveillance cultures of fluid collected through drainage catheters and cultures of samples from clinically infected sites [(2-4, 5)]. In each study, the predictive value of positive culture from the catheter drainage fluid for subsequent infection was poor; sensitivity varied but was as low as zero [(3)]. No study included more than 17 patients with documented infection. In contrast, we examined the accuracy of culture of catheter drainage fluid in actual clinical practice and included a larger number of patients with true infection (n = 46). We found that most (67%) catheter drainage specimens were submitted without accompanying reliable CT or US evidence for localized urinary infection, thereby rendering the culture result uninterpretable. Furthermore, when urine collection of potential

significance was radiologically confirmed, culture of the urine collected through preexisting drainage catheters yielded discrepant results in nearly half the cases, even in those cases in which the catheter tip apparently lay in the same collection that subsequently was sampled. Therefore, whether a significant collection is present or absent, the results of catheter drainage fluid cultures are potentially misleading for therapeutic decision making. There was no evidence that sampling drainage fluid from catheters in place for a shorter time (but more than 2 days) was more accurate than sampling from catheters in place for longer times.

Our findings support the recommendation that radiological imaging should be standard practice in the assessment of deep-tissue infections in patients with drainage catheters, and that direct aspiration of potentially infected urine collections is the most reliable method of obtaining specimens for culture that should be used to guide therapy, whether as part of a one-step aspiration procedure in the assessment of a new collection or as part of a reevaluation of a collection with a preexisting drainage catheter. In addition, the following interpretative comment was added to the results of all bacterial cultures of urine samples that were collected through a drainage catheter:

Bacterial cultures of fluid collected through drainage catheters in place for more than two days yield inaccurate results with potentially misleading therapeutic consequences in nearly half of cases compared with fluid collected by direct aspiration.

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Original Article **Sudan Medical Laboratory Journal**

Prolactin measurements in follicular fluid; a possible role in Sudanese women with polycystic ovary syndrome.

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Abstract
Increasing number of polycystic ovary syndrome (PCOS) among the Sudanese women, and its sequences in infertility and other complications. Objectives of this study are to determine the prolactin hormone changes occurring in the follicular fluid in Sudanese women with PCOS. One hundred eleven Sudanese women aged 20-43 years were enrolled in the study; 55 women with PCOS and 56 women with normal ovaries. The study was carried out in the Faculty of Medicine in the National Ribat University, during the period from July 2004- May 2008. The follicular prolactin measured in PCOS patients were compared with those measured in the control group, after detailed history and clinical examination. Follicular prolactin showed significant increase ($p = 0.00$); in the patients with PCOS, when compared with their controls. In conclusion the biochemical parameter (prolactin) measured in this study, was strongly correlated with the PCOS, and so the prolactin assay was suggested, as one of the means of its laboratory diagnosis.

Introduction

Prolactin (PRL) is a protein hormone produced by the lactotrogh (mammotroph) cells in the anterior pituitary; its molecular mass is about 22,800 daltons. It plays an essential role in the secretion of milk and has the ability to suppress gonadal function1. The circulating prolactin values are distinctly elevated at birth but decline to adult levels in less than three months [(1)]. Women are reported to have slightly higher levels than men, with slight rise at puberty – apparently estrogen related – and a corresponding fall at menopause [(2)]. Prolactin; in conjuntion with several other hormones, helps stimulate development of the breast tissue needed for lactation. In postpartum women, prolactin induces synthesis of milk in the

ISSN*: 1858-6147*

mammary gland. The prolactin is present in non-lactating women, men, and children, but the serum levels in these individuals are lower than those in pregnant women. Secretion of prolactin appears to be under control of a prolactin-inhibiting factor (PIF), which known as dopamine and comes from hypothalamus [(3)].

Prolactin amino acid composition is similar to those of growth hormones (GH). In humans, PRL appears to function solely in the initiation and maintenance of lactation. In this regard, PRL acts in conjunction with estrogen; progesterone, corticosteroids and insulin to promote full development of breast tissue, these additional hormones; among the other functions increase the number of PRL receptors in mammal tissue. It is thought that the high levels of estrogen present during pregnancy inhibit PRL induced lactation [(4)]

Prolactin secretion is controlled primarily by prolactin-inhibitory factor (PIF), which is released from the hypothalamus. This factor has been shown to be dopamine. Other inhibitory factors have been identified but their physiologic significance is unclear, several prolactin-releasing factors have been identified inducing thyrotrophin-releasing hormone (TRH), vosacative intestinal peptide (VIP), and peptide histidine methionine [(5)].

Levels of PRL increase during sleep, and decline during waking hours, there also appears to be a gradual increase in PRL secretion in first half of the menstrual cycle with decline immediate after ovulation. The level then increases again until menstruation occurs. PRL secretion increases during pregnancy, reaching levels as high as 200ng/ml, in the third trimester [(6)]. Mild hyperprolactinemia has been reported in 5% to 30% of patients with PCOS, in which prolactin is generally; only 50% above the upper limit of normal. Furthermore, hyperprolactinemia is most often transient, with perhaps only 3% to 7% of hyperprolactinemic PCOS patients having persistently elevated prolactin levels. Thus, it is now felt that PCOS and hyperprolactinemia are independent disorders. Hyperprolactinemia may all cause secondary amenorrhea but do not cause hirsutism; however, they need to be ruled-out 7. PRL may be secreted from chromophobes cells of interior pituitary 8. Prolactin is secreted in response of to both stress and thyrotropin releasing hormone (TRH), and plasma concentrations also depend on esterogen status [(6)].

In postpartum period stimulation of the breast produces a rapid rise in PRL concentration, resulting in lactation. Mechanical stimulation of the breast also may produce increases in PRL concentrating in some normal non-pregnant [(5)].

During pregnancy the prolactin level climbs steady to ten or twenty times its former values. Then drops black down to normal after delivery, within three weeks in non-nursing mothers. Women taking oral contraceptives or under estrogen treatment may have prolactin levels higher than normal. Prolactin is stress hormones. Not only sugary, but events such as veinipuncture or clinical interview have been reported to transient rise. There is a sleep-related diurnal

variation, prolactin levels increase during sleep and reach their lowest a few hours after waking [2] Prolactin deficiency is usually seen as part of the general picture of panhypopituitarism, it's very rare [2].

Materials and methods:

This study was carried at the department of biochemistry, Faculty of Medicine in the National Ribat University, in collaboration with Reproductive Health Care Center (RHCC), Sudan Assisted Reproductive Center (SARC) and Asia Hospital in Omdurman. The study was performed during the period between: July 2004 and May 2008.

The subjects:

The study population included 111 women; all subjects were between 24 and 43 years of age, they were generally in good health. The women enrolled in the study, were Sudanese from the different Sudanese states.

All the patients underwent IVF process. They were examined by the obstetricians, especially abdominal and vaginal ultrasound using four dimensions Doppler machine. PCOS morphology was defined as at least one ovary with 10 or more follicles of 2-10 mm in a single plane or an ovarian volume greater than 10 ml in the absence of a dominant follicle greater than 10 mm, a corpus luteum, or a cyst.

The fertility of the husbands was proved with seminal analysis.

At study entry, all subjects underwent follicular fluid sampling for prolactin measurement.

The diagnosis of PCOS was made according to the European Society for Human Reproduction (ESHRE) and American Society of Reproductive Medicine (ASRM) criteria for the PCOS diagnosis. In particular, PCOS was defined when two of the following criteria were found: oligo-and/or anovulation; clinical and/or biochemical signs of hyperandrogenism; polycystic ovaries (PCOS); and exclusion of other etiologies [4].

Fifty five patients with PCOS were consecutively enrolled in this study, while fifty six healthy women matched for age, were enrolled and considered as the control group. The healthy state of the women in the control group was confirmed by medical history, physical and pelvic examination, and complete blood chemistry (hormonal profile). Their normal ovulatory status was confirmed by transvaginal ultrasonography (TV-USG). This procedure was performed during the luteal phase of the menstrual cycle (7 days before the expected menses). Exclusion criteria for healthy controls were PCO at TV-USG and/or clinical or biochemical hyperandrogenism. All subjects were non-diabetic, with primarily or secondary infertility, not suffering from any disease especially cardiac disease, nonsmokers and none drank alcoholic beverages.

Laboratory investigations:

Prolactin hormone in the follicular fluid was measured using automated chemical analyzer (Immulite 2000).

Results:

Follicular prolactin showed highly significant increase ($p = 0.00$); in the patients with PCOS,

when compared with their controls, as shown in (table 1).

Table 1: Descriptive statistics of follicular prolactin (PRL) in the study group of PCOS patients and their controls

Parameter	**Prolactin**
Controls (P :value)	N.S
PCOS (P: value)	0.02

The effect of the age was significant (P = 0.02) on the prolactin hormone in the patients with PCOS, when compared with their control group, (table 2) shows this correlation.

Table 2: The relationship of the age on follicular prolactin among the patients and their controls

	Mean	**SD**	**Number**	**P. value**
PCOS	49.9	22.8	55	0.00
Control	38.6	21.3	53	

The distribution of the age groups between the two PCOS patients and the women with normal ovaries was shown in the following table (3).

Table 3: Descriptive study of the age distribution among the PCOS patients and the control group

Parameters	**Age (years)**		
	<20	**20-35**	**35-43**
Controls	1.8%	35.7%	62.5%
PCOS	0.0%	77.8%	22.2%
Total	0.9%	56.8%	42.4%

Discussion

All the women included in the study, were in the reproductive age between 24 and 43 years of age, they were generally, in good health. The fifty five women with PCOS included in the study were younger (20-35y =77.8%), while the patients above 35years of age, represent (22%). In the fifty six control women with normal ovaries; only 35% were in the age group (20- 35 y), but the age group (> 35y) was the bulk, representing 62.5%. As prolactin is the hormone that stimulates development of the breast tissue needed for lactation. It induces synthesis of milk in the mammary gland. In this study highly significant follicular prolactin elevation (P value 0.00) was seen in the patients with PCOS, compared with their controls. These finding were in agreement with previous studies recorded by 6, 7, 9, 10, 11. While these results were contrary to what written by Minakami and his colloquies 12, who said that the true prevalence of this phenomenon (hyperprolactinemia), in PCOS patients, may be coincidental rather than a pathological condition. This study may be with value, remembering that the prolactin deficiency is very rare, which is usually seen as part of the general picture of panhypopituitrism 5.

This study revealed that, the follicular prolactin hormone was insignificantly increased with age, in the patients with PCOS, when compared with their controls.

The biochemical parameter (prolactin) measured in this study, was strongly correlated with the PCOS, and so the PCOS profile was suggested, as one of the means of its laboratory diagnosis. It is hoped that this study will add to the growing knowledge, in regard to the diagnosis of this challenging disease.

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Original Article **Sudan Medical Laboratory Journal**

Plasma zinc as biochemical marker on prostatic carcinoma

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Abstract:

Studies on the relation between zinc and malignant disease are numerous but, on the whole, desultory and inconclusive. The aim of this study was to investigate the concentration of zinc in the plasma of Sudanese patients with prostate carcinoma.

A case-control study of 150 Sudanese patients with prostate cancer was compared with 50 healthy subjects as control group their mean age was. A plasma samples was taken and zinc levels were analyzed using atomic absorption spectrophotometry.

The mean (±SD) plasma zinc level in normal case was 0.89±0.26 mg/dl. Among prostate cancer patients, the plasma zinc level was 0.28±0.11 mg/dl. There was statistically significant ($P<0.05$) difference in plasma zinc level between patients and normal subjects.

Conclusion: The determination of zinc levels can be used as a biochemical marker to evaluate prostate carcinoma development.

Introduction:

The prostate gland is one of the most intriguing organs of the human body and constitutes a very important part of the male genital apparatus. Diseases of the prostate are mentioned in Egyptian papyri dating back as far as 1500 BC [(1)].

The special functions associated with the high zinc level of the prostate have not been resolved. The ability of the prostate to accumulate high zinc levels is a function of the glandular secretary epithelial cells [(2)]. It was demonstrated that the epithelial cells of prostate contain high levels of gland intracellular zinc and most importantly, contain high levels of mitochondrial zinc. The accumulation of zinc results in the inhibition of mitochondrial aconitase activity which minimizes the ability of these cells to oxidize citrate [(3)].

ISSN*: 1858-6147*

In the United States, prostate cancer is the second leading cause of all male cancer deaths. Furthermore, incidence rates are higher in African Americans than in any other racial group [(4)]. It is hypothesized that African may have genetically down regulated their zinc absorption capacity; otherwise, they would absorb abnormally high levels of zinc resulting in various serious neuro degenerative and biochemical disorders [(5)]. It is therefore possible that people African origin may have a lower capacity to absorb zinc when compared with other racial groups because of their inherent down regulation of zinc transporter. Extensive research has shown that low serum levels of zinc are associated with the increased incidence of prostate cancer [(6)]. This study is aiming to investigate the plasma zinc level in Sudanese patients with prostate carcinoma.

Material and methods:

This case-control study included 150 Sudanese patients attending the Radiation and Isotope Centre in Khartoum; fifteen subjects were used as baseline control. Baseline values were formulated by considering those patients (age-matched) who presented with prostate symptoms but in whom, after investigation, no prostatic disease could be elicited (n=50). Patients were subsequently advised to have a digital rectal examination (DRE) and transrectal ultrasonography for PCa confirmation. Patients with evidence of carcinoma of the prostate at the time of presentation (n=150) were classified according to the present of metastasis. Information with the study objectives prior written consent was conducted to each subject. In this study, 150 blood samples were collected from prostate carcinoma, infected person with age between 55-70 years. The patients were attending in Khartoum and Ibn Sina hospital and other 50 healthy Sudanese people as a control group with age between 54-67 years.

5 ml heparinized blood was collected in polyethylene vial coated with an anticoagulant (EDTA) from each individual of study population. The blood was centrifuged at 5000 R.P.M for 10 minutes which was stored in small aliquots and kept in a deep freezer (-20 ˚C) until analyzed. The plasma sample obtained was subjected to atomic absorption spectrophotometry (Beckman System) analysis to determine plasma zinc levels.

Student's *t*-test was used to calculate a pooled estimate of the variance of the data. $P<0.05$ was considered significant.

Results:

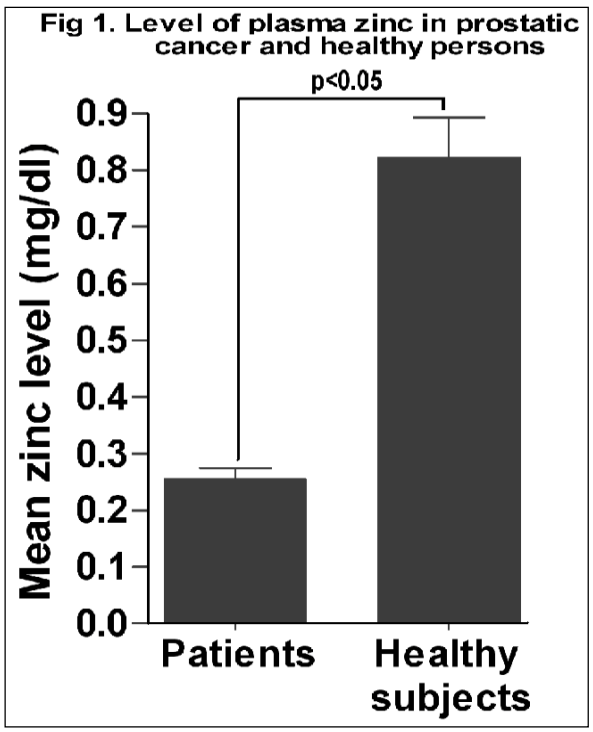
Demographic results for the study subjects were presented in (table 1). The mean (±SD) plasma zinc in the normal cases was 0.89±0.26 mg/dl. Among the prostate cancer patients, the plasma zinc level was 0.28±0.11 mg/dl (Table 1). There was a significant statistical difference ($p<0.05$) in the mean plasma zinc level between patients and normal cases (Figure 1).

Table (1): Demographic data of the patients (150) and the normal control in the study

Parameter	PCa patients (n=150)	Control (n=50)
Age (years)	55-70	54-67
Positive DRE	150/150	0/50
Biopsy procedure	140/150	0/50
Carcinoma of the prostate with metastasis	70/150	
Carcinoma of the prostate without metastasis	80/150	

Table (2): The mean of zinc levels for patients and control group

Parameter	Patients mean ± SD n = 150	Control mean ± SD n = 50
Plasma zinc	0.28±0.11	0.89±0.26



Discussion:

In this study, plasma zinc levels were dramatically reduced in patients with carcinoma of the prostate compared to normal subjects. Other authors showed similar results using samples of prostatic tissues [7-9].

Elizabeth [10] reported a marginal rise in plasma zinc levels in patients with a malignant prostate. Our findings, in contrast, revealed a significant fall in plasma zinc levels in such patients. Again, these variations may be due to the different techniques used to estimate zinc levels. In previous studies, chemical methods or the polarographic technique were used to determine

zinc levels. We used atomic absorption spectrophotometer (AAS), which is a superior and highly sensitive method for estimating the level of metallic ions.

Other recent studies [11-15] have all shown similar results to those obtained in the present study. The correlation between the plasma zinc values in our normal and diseased groups was significant ($p<0.05$), proving that the findings were in agreement with those of other studies.

Attention has shifted to the use of zinc as a benchmark for the determination of prostatic diseases and the possible mechanism for its development. Kristal et al. [16] suggested significant dose response effects for zinc and stated that zinc supplements may protect against the development of carcinoma of the prostate.

Costello and Franklin [17] showed that zinc inhibits mitochondrial aconitase and plays an important role in the citrate metabolism of prostate epithelial cells. The intra-mitochondrial accumulation of high zinc levels inhibits mitochondrial aconitase activity, which in turn inhibits citrate oxidation. This essentially truncates the Krebs cycle and markedly decreases production of adenosine triphosphate (ATP), which is normally coupled to citrate oxidation. These relationships form the basis for a new concept of the role of zinc and citrate related energy metabolism in prostate malignancy.

The inability of carcinoma cells to accumulate high zinc levels results in increased citrate oxidation and formation of the coupled ATP products essential for the progression of malignancy [18]. Liang et al. [19] showed the inhibitory effect of zinc on human prostate carcinoma cell growth, which was possibly due to induction of cell-cycle arrest and apoptosis. Feng et al. [20] showed a similar effect of high intracellular accumulation of zinc in prostate cells, which induced mitochondrial apoptogenesis.

More studies need to be carried out in this area, the result of which could be an evaluation tool for the diagnosis/screening of prostate carcinoma and as a biochemical marker to evaluate prostate carcinoma development.

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