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1- Analgesics and Anti-inflammatory Prescription for Pregnant Women in the Military and Khartoum Teaching Hospitals

2- Validity of Rapid Diagnostic Test compared to Light Microscopy for Malaria Diagnosis at Amana Clinic in Khartoum State

3- Testicular self-examination knowledge, Attitude and Practice of Alfajr College for Sciences and Technology Male Medical Students

4 Molecular Identification of Sudanese Females' Vaginal Microbiota and Microbiota Associated with Bacterial Vaginosis



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SUDAN JOURNAL OF HEALTH SCIENCES
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Editorial 1

The Sudan Journal of Health Sciences: This is how the Second Issue of the Journal was published during the Internecine Khartoum War.

**Hasan Abuaisha, FRCP.
Editor-in-Chief**

Praise be to Allah, Lord of the Worlds, and peace and blessings be upon the faithful Messenger of Allah, our Master Mohammed, and upon all his family and companions. Below are some thoughts and observations from the editor-in-chief of the *Sudan Journal of Health Sciences* (SJHS).

The *Journal* began its publication in January 2023, after preparation that took a full year before. SJHS has its main office at Alfajr College of Science and Technology (ACST) in Khartoum. It is a *Journal* open to partnerships from other Universities and Colleges in Sudan, as it bears the name of the Sudan, homeland of all Sudanese. The *Journal's* administration, in agreement with the senior management of ACST decided to publish the *Journal* three times a year initially, and then gradually increase the number of annual issues. The stated goal of the *Journal* is to encourage scientific research from all parts of Sudan and neighboring countries; especially prompting young researchers and faculty staff to conduct joint research for the purpose of answering scientific research questions and challenges in Sudan. The first issue of the *Journal* was published successfully during January to April 2023, and was well received by readers, and distinguished researchers from different specializations in health sciences. Then preparation started for the second issue, May to August 2023. However, the Khartoum war that suddenly broke out on April 15, 2023; Its aggressiveness was beyond imagination, as things were turned upside down throughout the Sudanese capital, and daily life in all institutions came to a halt with astonishing speed. In the following weeks, news came successively of the disruption and sabotage of all State institutions in the various aspects of the civil

service and the private sector. Educational institutions were not spared. At least 104 institutions of higher education, public and private, were partially or completely destroyed, as stated in the report of the Higher Education Committee to list the losses (see Professor Mohammed Elbagir's Editorial article). (1)

In this issue of the *Journal*, Professor Mohammed Elbagir describes how Al-Fajr College was able to withstand these challenges and pay special attention to graduate students in all its programs. The training of clinical medicine students was completed and the final examinations were held, in cooperation with Iqraa College in the Fadasi town near Wad Madani City, the capital of Gezira State in Sudan. With the help of number of professors from Khartoum and Gezira states. (1)

Alfajr College Research Center leads the scientific research and publishing in the College, and the SJHS represents the window through which the World sees the research activity of Alfajr College and the Universities that cooperate with it in this field. The big challenge was publishing the *Journal's* second issue, which coincided with the beginning of the war on April 15, 2023. The war resulted in the migration and scattering of the editors responsible for the magazine in the various states of Sudan and abroad. Thus, the offices of the ACST in Khartoum remained completely abandoned. We consulted with the *Journal's* management about overcoming this ordeal and we decided to communicate via electronic media and edit scientific articles using those media.

The challenge was great, and the editors worked on the various articles that the reader will find in this issue. There were many consultations with different scientists in the field of medical statistics and other specialties, as per the need to the published articles.

I am pleased to thank all those who contributed to editing and producing this issue, and I especially thank the Chairman of the Board of Trustees of ACST, Professor Mubarak Al-Majzoub, who has been in frequent contact with us to reassure and encourage the progress of the *Journal*. Likewise, the Dean of the College, Professor Mohammed Elbagir, has been following up on the timely publication of the *Journal*. All editorial board members made extraordinary

efforts at a time when the means of communication, the Internet and electrical current, were in a state of constant irregularity. I especially thank Professor Ishag Adam, a distinguished researcher at Qaseem University in the Kingdom of Saudi Arabia, who kindly wrote an editorial article on pain-relieving drugs during pregnancy, commenting on Dr. Azhari Al-Nour Haj Al-Amin and his colleagues entitled (**Analgesics and Anti-inflammatory Prescription for Pregnant Women in the Military and Khartoum Teaching Hospitals**). His response was timely and helpful, which the reader will find in this issue.

We are proud of publishing this issue on time despite the war that took place in the capital of Sudan, Khartoum, which became a deserted city to a large extent, as most of the inhabitants fled with their lives. Despite that, we were able to complete this work with the grace of Allah Almighty, and the diligence of all those involved in the *Journal*.

The production of one issue of a scientific *Journal* may seem reasonably doable in normal circumstances, but in this exceptional situation in which the war disrupted most of the administrative and scientific facilities in Khartoum, the issuance of the second issue of the SJHS in the midst of the war in Khartoum is a valuable achievement, and it indicates that ACST is keen to support scientific research and publishing, under all circumstances.

In the end, do we say "Praise be to Allah, the Lord of the Worlds"

Reference:

- Mohammed Elbagir Ali Elameen, Alfajr College for Science and Technology: Resilience in the face of the challenges of the Khartoum War, April 15, 2023, Editorial article in this issue.

Editorial 2

Alfajr College for Science and Technology: Resilience in the face of the challenges of the Khartoum War, April 15, 2023

**Professor Mohammed Elbagir Ali Elameen, FRCP Dean of
Alfajr College for Science and Technology**

The fifteenth of April, 2023, corresponded to the twenty fifth of Ramadan, 1444 Hijri, is a day carved into the memory of Sudan history with a lead chisel. On that day, the people of Khartoum woke up to the thundering sounds of varied gunfire. Now, as of writing this article, five months since its start, the war is still going on suspending every aspect of life in Khartoum. This war destroyed most of the infrastructure of the Triangular Capital. The destruction included all vital State utilities (water and electricity stations, State administrative offices and various information centres), private institutions (banks, companies and institutions), as well as citizens property (homes, cars and belongings); this included the destruction of public and private higher education institutions. The official committee formed by the Minister of Higher Education and Scientific Research, headed by the Director of the University of Gezira and published on the Ministry's website, provided a sufficient statement of what happened to higher education. Here is a summary of this report:

1. Higher education institutions lost a number of their scholars and employees.
2. All institutions of higher education and scientific research in the State of Khartoum and a number of other States were damaged (104 public and private higher education institutions and research centers were affected).
3. Many Universities were damaged in their infrastructure, including laboratories, workshops, and libraries.

4. In Khartoum State, all public Universities, ten private Universities, and twenty private Colleges were damaged.
5. In other States, six public Universities were damaged, in addition to private Colleges.
6. All means of transportation and movement were stolen from the affected Universities and Colleges.
7. Research Centres, laboratories and libraries were burned.
8. Faculty housing and property were also destroyed.

This is the end of the summary of the Ministry of Higher Education report.

The Ministry of Higher Education and Scientific Research addressed this emergency situation after shifting its offices to the University of Gezira, Gezira State, as follows:

The Minister of Higher Education and Scientific Research issued several directives to deal with the war situation, including:

1. Urge Universities and Colleges to adopt synchronous e-learning to continue studying and setting regulations for this type of study.
2. Urge public and private Universities in the unaffected States in Sudan to cooperate with Universities and Colleges from Khartoum State to help accommodating their students wherever possible. The Ministry laid down regulations and guidelines to regulate this interaction.
3. Universities and Colleges affected by the war were allowed to deal with Universities outside Sudan according to specific conditions and controls.
4. Start accepting applications and admission opportunities for the new academic year.
5. Resume documentation procedures at the Ministry of Higher Education.

In light of these directives, Alfajr College held its first Faculty Council virtual meeting on May 25, 2023, in which it was decided to resume education for all programs and levels in the College.

As a result, the program coordinators initiated several meetings with the aim of adapting study plans to the new situation. The Scientific Council continued to monitor the progress of preparations by holding another eight interactive meetings, the outcomes of which were as follows: -

1. Assess, through a questionnaire, the different locations of students and professors, the availability of the Internet and their ability to attend in person if necessary.
2. Direct the staff to take advantage of the available electronic infrastructure of the College to continue education electronically. This may be achieved through the College's educational platforms (Big Blue Bottom and Moodle), and applications like Telegram, and WhatsApp.
3. An agreement was concluded with Iqraa University College in Fadasi at the suburbs of Wad Medani (14 kilometers from the city) to host Alfajr College to complete the rest of the activities that need laboratories and other practical aspects, as well as to conduct examinations. This agreement was facilitated by the standing relationship between Iqraa College and Alfajr College, as they are both owned by Al-Majzoub Organization for the Service of the Holy Qur'an.
4. Postpone practical, laboratory, clinical and field activities until the end of the course, provided that there is a schedule for each program for these activities when students attend Iqraa College.
5. Contact the graduate students from the third batch of Medicine program to complete unfinished courses and attend various examinations in preparation for graduation.

This batch was supposed to sit for the Obstetrics and Gynecology examination immediately after the blessed Eid Al-Fitr in the first week of May.

The outcome of these efforts from May to mid-September was as follows:

- The College started the teaching process through electronic learning that continued effectively. This electronic process was needed to finish theoretical courses in all the programs which were already on the verge of ending.
- A memorandum of understanding was signed with Iqra University College. Iqra College at Soba Complex established administrative offices for Al Fajr College staff in their buildings.
- The College Information Technologies (IT) staff prepared a local network to accommodate 100 students for the purpose of electronic examinations in the Iqra Complex. The Internet was introduced in the Soba complex to access the cloud student system and to conduct examinations in the way used in the College. The electronic Tablets required for the examinations were provided by a specialized company in Wad Medani City.
- We contacted the graduate medical students to come to Fadasi to complete the courses on Obstetrics and Gynecology, Ophthalmology, Emergency medicine and rural residency. This would be followed by the final examinations for these courses and to start graduation research projects. Students were accommodated in boarding houses prepared for them in Fadasi. The attendance rate was 96.5% (83 out of 86 students), including foreign students from the Arab Republic of Egypt and the Kingdom of Saudi Arabia.

- The clinical Obstetrics and Gynecology examination was held in Fadasi. It was run by 23 professors who were recruited from the cities of Wad Medani, Al-Hasahisa and from Al-Butana.
- The theoretical and clinical training of the Ophthalmology course was successfully completed. This was followed by theoretical and clinical training for emergency medicine held at the Police hospital in Wad Medani City. The examinations for both courses were finalized successfully with the help of professors from the University of Gezira.
- We completed the rural residency course in Fadasi and Al-Muslimiya rural hospital under the supervision of Dr. Bashir Al-Tayeb, who came all the way from Khartoum to Fadasi in a two day trip that should have taken two hours in normal circumstances.
- The students continued to complete their graduation research under the guidance of supervisors who were scattered in the different States of Sudan and abroad. Regular meetings were held for the supervisors via Google Meetings platform to follow up on the progress of the students' research, discuss problems faced by students, and agree on unified solutions for them.

All the administrative and technical staff needed by the educational process reported to Fadasi. Rooms and amenities were prepared for them in the town. Teaching staff included professors of Obstetrics and Gynecology, Ophthalmology, Emergency Medicine, and Community Medicine. The program registrar, the staging officer, and the examination officer for the Medicine Program were also present, in addition to the financial and e-learning officers.

The conditions of the war and its psychological and social consequences did not hinder the College from its research activity and its participation in enriching the scientific arena.

It published the first issue of the *Sudan Journal of Health Sciences* (SJHS) in May 2023, and this second issue of the SJHS was also the result of an exceptional effort in the current context.

As we are on the verge of completing the graduation of the third batch of students and are preparing to receive the dental students according to the schedule approved by the Scientific Council, Al-Fajr College and other immigrant Colleges And Universities inside and outside Sudan were surprised by the decision of the Minister of Higher Education and Scientific Research to stop University studies of all kinds in higher education institutions on August 14, 2023. This decision will abort all efforts made by the College to overcome the obstacles imposed on us by this war. We hope that the Ministry of Higher Education will review this decision.

It was an exciting, new and wonderful experience, especially since the weather was autumn, and the nature of the countryside in Sudan is beautiful. It was a nice opportunity for the professors and students to be in one place. This created an atmosphere of familiarity and ease of contact in the educational process, helped in its success by the full cooperation of the Iqra College administration. Students and professors celebrated the end of this successful period, and at the end of it, they honored Iqra College's management, employees, and workers.

I take this opportunity to extend my sincere thanks to everyone who contributed to the resilience of Alfajr College of Science and Technology to stand in the face of these difficult circumstances. I especially thank the College professors and those who participated in teaching from other Universities and Colleges, the administrative staff and workers. I pay tribute to the graduate students who came to the town of Fadasi and lived in the boarding houses of Iqraa College throughout studying these courses; their attendance rate was remarkable (96.5%).

I also particularly thank Iqraa College, which provided us with opportunities for success, including accommodation, hosting, and using all its available academic aids.

Reference:

Sudanese Ministry of Higher Education and Scientific Research
website

Editorial 3

Analgesics and Anti-inflammatory Drugs Prescription during Pregnancy

**Professor Ishag Adam, Department of Obstetrics and
Gynecology, Unaizah College of Medicine and Medical
Sciences, Qassim University, Saudi Arabia**

Pregnant women encounter several challenges during pregnancy when managing health conditions including pain and fever compared to the general population.(1) Poorly managed pain, fever, and conditions necessitating the use of analgesics and anti-inflammatory drugs during pregnancy can have several adverse effects on the mother and her unborn child. (2, 3) Generally, the use of most medications during pregnancy is not without risks to the mothers and their unborn children. (1, 4) This editorial aims to address analgesics and anti-inflammatory drugs prescription during pregnancy and to suggest suitable recommendations to improve pregnancy outcomes.

During pregnancy analgesics and anti-inflammatory drugs are used solely or with other drugs in treating different kinds of health conditions such as pain, rheumatic diseases, urinary tract infection, malaria, tuberculosis, dengue, chikungunya, etc. (3, 5, 6) Moreover, some anti-inflammatory drugs like low-dose acetylsalicylic acid (aspirin) are used to reduce the risk for preeclampsia, and its related morbidity and mortality (preterm birth and intrauterine growth restriction) .[2] Fever during pregnancy including pyrexia of unknown origin (PUO) is associated with several poor maternal and perinatal outcomes including maternal morbidity and mortality, preterm birth, premature rupture of membranes (PROM), fetal anomalies, intra uterine fetal demise (IUFD), development attention deficit hyperactivity disorder (ADHD) and autism.(5) Such poor pregnancy outcomes associated with fever necessitate urgent treatment. However, the use of analgesics and anti-

inflammatory drugs is not without risks and there is limited research about their safety during pregnancy. (2, 4) The challenges exist when pregnancy occurs with preexisting health conditions such as chronic rheumatic diseases, where drugs are needed during pregnancy. Some anti-rheumatic drugs have potential maternal or fetal toxicity, for example, nonsteroidal anti-inflammatory drugs (NSAIDs) which are known to be effective anti-inflammatory, antipyretic and analgesic drugs; but should be avoided in the third trimester due to the risks of premature closure of the ductus arteriosus. (3)

Although pain in pregnancy exists, there is limited research addressing the impacts of pain on maternal and perinatal outcomes. (6) Ray-Griffith, et al. reviewed 144 articles that studied the evaluation and management of pre-existing chronic pain in pregnancy, chronic pain associated with pregnancy, and chronic pain in relation to the mode of delivery. They recommended seven guidelines for chronic pain management during and after pregnancy: a) complete history and physical examination; b) monitor patients for alcohol, nicotine, and substance use; c) collaborate with the patient to set treatment goals; d) develop a management plan; e) for opioids, use lowest effective dose; f) formulate a pain management plan for labor and delivery and g) discuss reproductive health with women with chronic pain. (6)

Even for long-used drugs during pregnancy, which are considered to be safe in pregnancy at the recommended dose, some side effects were reported recently. (4) For example, there have been recent concerns about the safety of the use of acetaminophen (paracetamol); which is the preferred antipyretic/analgesic for pregnant women worldwide; when taken during early pregnancy. These include negative impacts on offspring reproductive development (cryptorchidism and hypospadias). (4)

This indicates the need for continued monitoring of drug safety on unborn children exposed to drugs during pregnancy until puberty and adulthood. In other words, first approval of drug safety does not guarantee safety forever.

The need for proper management of pain, fever or any pre-existing health condition that require treatment during pregnancy and the absence of conclusive data regarding the usage of analgesic/anti-inflammatory drugs during pregnancy; put more pressure on researchers to come up with a conclusive solution without jeopardizing women and unborn children health. Based on the evidence literature, the current situation of analgesics and anti-inflammatory drugs during pregnancy remains a real challenge that needs to be overcome. We came up with the following recommendations to overcome these challenges; aiming to improve maternal health and pregnancy outcomes:

1. Analgesics and anti-inflammatory drugs should only be prescribed by qualified health professionals, after a thorough diagnosis and determination of the needs for prescription. However, at present, this is not the case, as taking over-the-counter drugs is common during pregnancy. The successful approach of any patient should start with a complete history and physical examination and with the lowest effective dose if needed. (6)
2. Whenever possible, health professionals should avoid using drugs during pregnancy. The majority of pregnant women's conditions (aches and pains) can be treated without the use of analgesic/anti-inflammatory drugs. For example, some pregnant women may experience headaches often, so taking enough rest may be the best treatment approach. In case drugs are needed, using the lowest dose of the prescribed analgesic and/or anti-inflammatory drugs for the minimum period to relieve the discomfort during periods of ill health during pregnancy should be the strategic approach. (4. 6)

3. Establishing effective collaboration between health professionals and women even before pregnancy. (6)
Encouraging preconception counseling to implement appropriate preventive measures, and to manage any existing health conditions that may require treatment including analgesics and anti-inflammatory drugs during pregnancy, such as chronic pain, rheumatic diseases, PUO, malaria, visceral leishmaniasis (Kala-azar) and tuberculosis. For example, unplanned pregnancy, especially among women with rheumatic diseases can pose a threat to the health of the woman and the fetus. Therefore, appropriate management of rheumatic diseases during pregnancy (consisting of a multidisciplinary care team) can prevent maternal end-organ damage and minimize the adverse effects of the disease on pregnancy outcomes. (3)
4. Honest and accurate sharing of information with pregnant women is essential to support them in making choices that are in the best interests of their unborn babies. These information include thorough counseling of pregnant women about using medications during pregnancy and the probable risks based on the current evidence. (1) In case a pregnant woman encounters drug side effects such as teratogenic or fetotoxic effects, the risk must be assessed on an individual base and a risk management strategy must be determined. (1)
5. More research is needed regarding drug safety in pregnancy including analgesics and anti-inflammatory drugs. Women should not be excluded from drug trials, especially when there are preliminary benefits to mother and her fetus, and the benefits outweigh the risks of the potential tested drug. Currently, pregnant women depend on drugs that are usually studied on the general population and the results may not be suitable for pregnant women due to physiological changes during pregnancy.

In conclusion, prescription of analgesics and anti-inflammatory drugs during pregnancy is often challenging. Not prescribing such drugs, may lead to increased maternal and fetal morbidity and mortality including abortion, PROM, preterm birth, anomalies, IUFD, etc. Preconception counseling, timely investigations, and early appropriate drug doses and duration may help prevent the aforementioned poor outcomes. Further research trials are needed to ensure safety of analgesics and anti-inflammatory drugs during pregnancy; and whenever possible, pregnant women should participate in such researches. This is in the hope that drug safety during pregnancy should not be extrapolated from the results of observational studies on the general population.

In this issue of The Journal, there is a cross-sectional study entitled (Analgesics and Anti-inflammatory Prescription for Pregnant Women in the Military and Khartoum Teaching Hospitals, Khartoum, Sudan, 2016) authored by Azhari Elamin and his colleagues. The study showed that various analgesics and anti-inflammatory drugs were used to treat minor to moderate pain, headaches and/or fever during pregnancy. Acetylsalicylic acid (aspirin) was mostly used in low doses for its prophylactic/therapeutic effects of reducing fetal and maternal ill health during pregnancy. The researchers reported that occasional analgesic/anti-inflammatory drugs can be safely taken during pregnancy. However, in the light of possible long term side effects on the offspring such as cryptorchidism and hypospadias, children born to mothers who used analgesics/anti-inflammatory drugs should be followed up until puberty and adulthood.

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Analgesics and Anti-inflammatory Prescription for Pregnant Women, Military and Khartoum Teaching Hospitals, Khartoum, Sudan, 2016

Azhari E. H. Elamin^{1*}; Rasha. M.A.Rifat²; Omer.E.H.Elamin³;
Iman. A. M.Mohamed⁴

¹Department of Clinical Pharmacy and Pharmacy Practice, Alfajr College for Science and Technology and Khartoum University, Khartoum, Sudan.

² Omdurman Islamic University, Khartoum, Sudan.

³ Sudan Medical Specialization Board, Khartoum Teaching Hospital, Khartoum, Sudan.

⁴ Omdurman Islamic University, Khartoum, Sudan.

*Corresponding Author:

Dr. Azhari Elnour Haj Elamin

E mail: azhari_elamin@yahoo.com; azhari_elamin@hotmail.com

Abstract:

Background: Pregnancy loss, preterm delivery and maternal illness are all made more likely by active rheumatic, musculoskeletal, fever, and pain conditions. Pregnant women endure a range of aches and pains, and the majority of them can be treated without the use of analgesics/anti-inflammatory drugs. Many of these drugs are accessible over the counter. Some analgesic treatments may be teratogenic or fetotoxic in pregnancy.

Aim: This study aims to provide information on the analgesics and anti-inflammatory prescription to pregnant women and to correlate the prescriptions to women's characteristics including their medical history, in Khartoum, Sudan.

Methods: The study was a descriptive cross-sectional hospital-based study conducted at the Military and Khartoum Teaching Hospitals in Khartoum State. A data collection form designed by the researcher was used to obtain information on pregnant women from records during the period from January 2015 to January 2016. Women's characteristics, their medical history and the prescriptions for different types of analgesics and anti-inflammatory drugs were extracted. The Chi-square test was used and a p -value < 0.05 was judged statistically significant when analyzing the data using SPSS version 16.

Results:

The records of 650 expectant mothers were reviewed. Analgesic and anti-inflammatory medications were prescribed to 63 (9.7%) of the women. Of those women, 53 (84%) were in their third trimester; $p=0.0001$. Therapeutic agents used were: acetylsalicylic acid (aspirin) used in 39 (61.9%) of this group; followed by dexamethasone in 15 (23.8%); diclofenac sodium in 5 (7.9%), mefenamic acid in 2 (3.2%), and acetaminophen (paracetamol) in 2 (3.2%). The rate of prescription of Analgesic/anti-inflammatory drugs during pregnancy in this study is comparable to international literature. Acetylsalicylic acid (aspirin) was used in low doses (81 – 100 mg). Indications for using low dose aspirin (LDA) in this group were, Systemic hypertension (n=24; 61.5%); venous thromboembolism (n=7; 17.9%); hypertension-during-pregnancy (n=4; 10.2%); diabetic mellitus (n=2; 5.1%) and congenital hyperadrenalism (n=2; 5.1%).

Conclusions: In this study, various analgesics and anti-inflammatory drugs were used to treat minor to moderate pain, headaches and/or fever during pregnancy at a prescription rate comparable to the international literature. Although studies have shown that analgesic and anti-inflammatory medications can be safely taken during pregnancy, they should be under the supervision of qualified physicians and pharmacists for the appropriateness of the treatment and to avoid drug-related problems.

Keywords: *Pregnancy, Analgesic/Anti-inflammatory Drugs, NSAIDs, Acetyl salicylic acid, Low-Dose Aspirin, Acetaminophen, Paracetamol.*

Introduction:

Pregnancy loss, preterm delivery and maternal illness are all more likely made by active rheumatic, musculoskeletal, fever and pain conditions.(1) Many of the aches, pains, and headaches that pregnant women feel don't need analgesic treatment. During pregnancy, headaches and soreness from dental operations are frequent. (2,3) With just a few exceptions, the majority of commercially available non-narcotic analgesics (many of which are over-the-counter medicines) can be used safely to relieve minor pregnancy-related pain. (4,5) Some pregnant women may experience headaches often. The best approach is to strike a balance between activity and rest. Although occasionally taking acetaminophen (paracetamol) is safe, it is typically best to avoid medications while pregnant. (2,3,6)

Depression and anxiety can develop from continuous pain that is not properly handled. These could have a negative effect on a woman's physical and psychological health as well as her pregnancy. Pain should not be endured by women during pregnancy or nursing. Common analgesics like paracetamol, aspirin, non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are relatively safe when used as directed and appropriately (7, 8,9). Due to lack of information regarding possible risks and benefits, choosing and taking these medicines can be difficult for doctors, pharmacists, and patients.(1) Fortunately, guidelines for the use of anti-rheumatic drugs like NSAIDs in pregnancy and lactation have been published by the American College of Rheumatology, American College of Obstetrics and Gynecology, British Rheumatology Society, and the European League against Rheumatism.

When counseling women about using medications during pregnancy, the physician should always emphasize that all couples have a background risk of about 3% of having a child with a serious birth defect and that 15% of all known pregnancies end in miscarriage, regardless of any drug exposures. (10,11)

Over 85% of women in their third trimesters of pregnancy take some form of medication, with analgesics being the most frequently used medicines second only to vitamins. Overall, 50% of women use analgesics during pregnancy. (10, 11)

Honest and accurate discussions about pregnancy planning and management are essential to support women in making choices that are in the best interests of their unborn children because many patients are reluctant to use analgesics during pregnancy.(1)

This study aims to provide information on the analgesic and anti-inflammatory prescription rates for expecting mothers who visited the outpatient clinics of the Military and Khartoum Teaching Hospitals between January 2015 and January 2016. This study also aims to find the correlation between the characteristics of the pregnant women and the frequency/rate of prescription of analgesic/anti-inflammatory drugs on participating subjects.

Materials and Methods:

This was a descriptive cross-sectional hospital-based study carried out in the Military and Khartoum Teaching Hospitals in Khartoum State, Sudan, from Jan 2015- Jan 2016. The two hospitals provided Obstetric Care in separate Departments where services were run by house officers, medical officers, registrars, and consultants. The study population included the records of pregnant women attending the two hospitals during the study period. Data collection forms were used to extract the information from pregnant women's files. The information extracted included characteristics of subjects (age, trimester and parity), medical history, maternal disorders and analgesic/anti-inflammatory drugs prescription by type. The sample size was a full coverage to all 650 records of pregnant women attending the outpatient clinics of the two hospitals in the study period.

Data was analyzed using SPSS version 16. Chi square test was the statistical method used. *p value* of <0.05 was considered statically significant.

Results:

The study covered 650 files of pregnant women who attended the outpatient clinics of the two hospitals. The age range of the study population was 15 to 45 years, the majority (67.7%) was multigravidas and 86.6% were in their third trimester (**Table1**)

Table (1): Characteristics of 650 Pregnant Women who attended the Outpatient Clinics of the Military and Khartoum Teaching Hospitals, Khartoum, Sudan

Characteristic	n (%)	
Age		
15-25 years	256	(39.4%)
26-35 years	310	(47.7%)
36-45 years	84	(12.9%)
Trimester		
First (1-3 months)	9	(1.4%)
Second (4-6 months)	78	(12.0%)
Third (7-9 months)	563	(86.6%)
Parity		
Primigravida	210	(32.3%)
Multigravida	440	(67.7%)

Maternal Medical Disorders:

One hundred and fifty-four (23.7%) of the pregnant women had maternal medical disorders. In this group, hypertension-during-pregnancy was the most common disorder affecting 14.3%, followed by primary hypertension and urinary tract infections (UTI), in 9.74% each. (**Table 2**) shows details of these medical disorders during pregnancy.

Table (2): Types of Maternal Medical Disorders among 154 Pregnant Women who attended the Outpatient Clinics of the Military and Khartoum Teaching Hospitals, Khartoum, Sudan

Maternal Medical Disorders	n (%)	
Hypertension during pregnancy	22	(14.3%)
Systemic Hypertension	15	(9.74%)
UTI*	15	(9.74%)
Pneumonia	11	(7.14%)
Diabetes during pregnancy	11	(7.14%)
Anemia	11	(7.14%)
Others**	69	(44.8%)
Total	154	(100%)
*UTI = urinary tract infections		
** Others = sporadic medical problems.		

Prescription of Analgesic and Anti-inflammatory Drugs:

Sixty-three (9.7%) of the 650 pregnant women were prescribed analgesic and anti-inflammatory drugs. In this group, acetylsalicylic acid (aspirin) was the most prescribed drug for 61.9% of patients, followed by dexamethasone for 23.8%. (Table 3) shows details of all the analgesic and anti-inflammatory drugs prescribed.

Table (3): Prescription of Analgesic/Anti-inflammatory Drugs to 63 Pregnant Women attended the Outpatient Clinics of the Military and Khartoum Teaching Hospitals, Khartoum, Sudan

Analgesic/ Anti-inflammatory Drugs	n (%)	
Acetylsalicylic acid (Asprin)	39	(61.9%)
Dexamethasone	15	(23.8%)
Diclofenac sodium	5	(7.9%)
Mefnamic acid	2	(3.2%)
Paracetamol	2	(3.2%)
Total	63	(100 %)

In the third trimester of pregnancy, aspirin was the most frequently prescribed analgesic/anti-inflammatory drug. It was used for various stages of systemic hypertension; followed by venous thromboembolism and hypertension-during-pregnancy (Table 4). Aspirin was used in low doses (81-100 mg/day) as a platelet inhibitor for this group of medical conditions.

Table 4: Use of Acetylsalicylic acid (Aspirin) in low doses for medical conditions during the third trimester in 39 pregnant women

Conditions	n (%)
Systemic Hypertension	24 (61.5%)
Venous Thromboembolism	7 (17.9%)
Hypertension-During-Pregnancy	4 (10.2%)
Diabetic Mellitus	2 (5.1%)
Congenital Hyperadrenalism	2 (5.1%)
Total	39 (100%)

Correlations between the Frequency of Prescription of Analgesic/Anti-inflammatory Drugs; parity and medical history are shown in (Table 5). In the 63 pregnant women who were prescribed analgesic/anti-inflammatory drugs during pregnancy, this was highest during the third trimester (84.1%).

Table (5): Correlation between the Characteristics of Pregnant Women and Frequency of Prescription of Analgesic/Anti-inflammatory Drugs among Women attended the Outpatient Clinics of the Military and Khartoum Teaching Hospitals, Khartoum, Sudan.

Item	Analgesic and Anti-inflammatory prescription frequency	p- Value
Trimester		0.0001
First (1-3 months)	4	
Second (4-6 months)	6	
Third (7-9 months)	53	
Medical History		0.0001
Hypertension	13	
Asthma	2	
Diabetic	2	
Migraine	2	

Discussion:

In this study, prescription of analgesic/anti-inflammatory drugs increased with pregnancy timing, so that 84.1% of prescriptions were noted during the third trimester.

Asprin was the most frequently prescribed analgesic/**anti-inflammatory** drug during the third trimester of pregnancy. It was used for various stages of systemic hypertension, hypertension-during-pregnancy and venous thromboembolism. The most likely indication was prophylaxes or therapy for various maternal and fetal effects.

Numerous studies have examined the impact of low dose aspirin (LDA) therapy on various maternal and fetal effects. The most recent research findings, published in the year 2021, are included in a summary of the present LDA usage recommendations, along with potential LDA treatment implications for the future.(12)

LDA use appears to be effective in reducing the risk of preeclampsia, preterm birth, and intrauterine growth restriction. However, current research advises raising the recommended dose to >100 mg to maximize these risk reductions. (12) The recent recommendations of reevaluation of using LDA emphasize on dosing and treatment initiation options. When treatment is started before 16 weeks of pregnancy, there is evidence that a decreased incidence of preeclampsia is observed in using aspirin at doses >100 mg.(12) Generally, aspirin is one of the drugs most used by pregnant women. In one prospective study of 1529 pregnant women, almost 50% of the women reported taking aspirin, and about 3% took it daily. High or

normal doses (>325 mg) block the production of prostacyclin and thromboxane, and low-dose aspirin (60–83 mg) results in a selective block of thromboxane production and favors the prostacyclin (vasodilation) pathway (4,5).

A meta-analysis study included a total of 35 placebo-controlled randomized studies with 46,568 pregnant women. Pre-eclampsia, premature delivery, perinatal mortality, and intrauterine growth retardation were all significantly reduced with aspirin prophylaxis without increased bleeding risks. (13) Although LDA did not reduce the chance of gestational hypertension, it significantly increased neonatal birth weight. The subset analysis showed that women who started taking aspirin before 20 weeks of pregnancy had significantly lower pre-eclampsia risk and higher birth weight and gestational age at delivery (RR=0.76, 95% CI=0.64, 0.90, p=0.001). (13)

Among 144 pregnant women who were 'heavy aspirin' users, the frequency of major birth defects in offspring was 4.2% ; not significantly different from that in the general population (3.5–5%).(4) Similarly, among 62 women who used aspirin in the first trimester, the rate was not significantly higher than that expected in the general population.(5) High-dose aspirin taken late in the third trimester may be associated with (a) closure of the ductus arteriosus, (b) persistent pulmonary hypertension, (c) decreased fetal renal function and (d) oligohydramnios. (4,5).

Care should be taken of aspirin adverse drug reactions if used by pregnant women; such as gastric irritation and bleeding; hypersensitivity reactions; provoking asthma attacks; urticaria and angioedema. Aspirin is also associated with Reye's syndrome, a rare but potentially fatal encephalopathy of infants and children (6, 8, 14). Maternal use of high-dose aspirin is associated with increased frequency of post-term pregnancies, low birth weight, neonatal bleeding disorders, intracranial hemorrhage in premature infants, and premature closure of the ductus arteriosus in the fetus. However, these complications were not associated with aspirin use when pharmacologically controlled doses of salicylates were used. (4,5,8,15,16) As long as aspirin daily doses don't surpass 100mg, its use during pregnancy is regarded as safe. In high-risk patients, aspirin has been shown to be effective in preventing pre-eclampsia and fetal development restriction. Neither the prevention of vascular placental disease in low risk individuals nor the benefits of prescribing aspirin in instances of unexplained recurrent miscarriages have been proven.(17)

In this study paracetamol was used during pregnancy, second only to aspirin. In a large longitudinal study, 41% of pregnant women reported paracetamol use during pregnancy.(4) The frequency of congenital birth defects was not increased among more than 1200 offspring of mothers who used paracetamol during the first trimester compared to non-users.

The Intelligence Quotient (IQ) of offspring at 4 years of age whose mothers had ingested paracetamol during pregnancy was no different than controls. (4,5)

A summary of available data on the negative effects of long-term paracetamol use, with particular emphasis on cardiovascular disease, asthma, renal injury, and in utero exposure was published by McCrae *et al* in 2018.(18)

In our study, paracetamol was used only to relieve pain and/or fever. Paracetamol is generally safe, its only major drawback being hepatotoxic in overdose. The toxic level of paracetamol need not be greatly above the therapeutic level (4 g daily for adults and children over 12 years) and symptoms of overdose may not appear for two days or more. (8,13,14)

Dexamethasone is commonly used in the late second and early third trimesters to promote fetal lung maturity and prevent respiratory distress syndrome.(19) In this study dexamethasone was used in 15 pregnant women (23.8%) which is comparable to other studies in the literature. (20, 21) Glucocorticoids, which include dexamethasone and betamethasone are frequently used to encourage fetal lung maturity and avoid respiratory distress syndrome in the late second and early third trimesters. (19, 20, 21) However, glucocorticoids are reported to be associated with an increased frequency of cleft palate in the offspring of pregnant animals when used during embryogenesis. Nevertheless, this risk has been estimated to be small in human pregnancy being less than 1%. (22,23).

The use of non-steroidal anti-inflammatory drugs (NSAIDs) was small in this study (diclofenac sodium 7.9% and mefenamic acid 3.2%). This was probably reasonable due to their limited use during the second and third trimesters for symptomatic relief,

For low to moderate pain and fever, paracetamol and NSAIDs are both suitable options, but due to known risks, NSAIDs should be avoided during the third trimester. Although neonatal abstinence syndrome must be watched for after exposure in the third trimester, short doses of weaker opioids are typically harmless during pregnancy (24, 25)

Conclusion:

In this study, the use of analgesic and anti-inflammatory drugs in pregnant women was limited to only 9.7% of the study population. Acetylsalicylic acid (aspirin) in low doses was the most frequently prescribed; and mostly during the third trimester (23.8%). The indications for such use were appropriate. The rate of analgesic/anti-inflammatory prescriptions during pregnancy in Sudan is comparable to other relevant literature.

Pregnant and nursing women and their doctors should be reassured that there are safe ways to manage pain, both acute and chronic. Occasional

analgesic/anti-inflammatory drugs can be taken during pregnancy; but it should be prescribed carefully by physicians and checked by pharmacists for the appropriateness of the treatment and to avoid drug-related problems.

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Authors own resources

Conflict of interest

None

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Validity of Rapid Diagnostic Test compared to Light Microscopy for Malaria Diagnosis at Amana Clinic in Khartoum State, 2020

Tasabeeh M. I. Mohamed¹, Aya A. A. Fadul², Mohamed A. Alsharef^{3*}

¹ Department of community medicine, Program of medicine, Alfajr College for Science and Technology,

^{2,3} Department of Parasitology, Medical Laboratory Science Program, Alfajr College for Science and Technology.

***Corresponding author**

Mohamed Awn Alsharef, Department of Parasitology, Medical Laboratory Science Program, Alfajr College for Science and Technology.

Email: mohawn2018@gmail.com

Abstract

Background: Early diagnosis and proper management are important issues in malaria control. Since malaria is a serious disease and highly endemic in Sudan, there is a need to identify the best diagnostic tools to help in the proper management and control of the disease. Rapid diagnostic tests (RDTs) and microscopy are routinely used for the diagnosis of malaria in Sudan. This study was conducted to assess the validity of RDTs of malaria compared to microscopy, using sensitivity and specificity of the RDTs against the standard method (Light Microscopy).

Materials and Methods: One hundred patients with a clinical diagnosis of malaria attending Amanah Clinic Laboratories were tested by two methods: (a) Peripheral thick and thin blood films for the light microscopy diagnosis and (b) immunochromatographic test (ICT) for Malaria as a RDT. The results were compared using the sensitivity and specificity of the ICT against light microscopy.

Results: The ICT detected Plasmodium species infection with a sensitivity of 89%, specificity of 86%, positive predictive value was 99% and negative predictive value was 31.7%.

Conclusion: Our study shows that diagnostic accuracy of RDT was suboptimal compared to the gold standard method of microscopy.

However; RDT is a valid diagnostic test for the majority of malaria cases, because it's quick and easy to use. In difficult cases, microscopy in expert hands remains the gold standard method and the final court of appeal

Keywords: *Malaria Diagnosis, Microscopy, ICT, Validity, Sensitivity, Specificity, Predictive values.*

Introduction:

Malaria is a serious and sometimes fatal disease caused by a parasite that commonly infects a mosquito species that feeds on humans. Malaria is considered the most severe public health problem worldwide. (1) Nearly half of the World's population lives in areas at risk of malaria transmission in about 87 countries as per the 2021 World Malaria Report. In that report, 241 million clinical episodes of malaria were estimated in the year 2020. The World Health Organization (WHO) African Region report estimated the African continent malaria prevalence as 95% and the death toll to be 627,000 deaths. (1)

The African Region continues to carry a disproportionately high share of the global malaria burden. In 2019, six countries accounted for approximately half of all malaria deaths worldwide: Nigeria (23%), the Democratic Republic of the Congo (11%), the United Republic of Tanzania (5%), and (4%) for each of Burkina Faso, Mozambique and Niger. (2) In Sudan, over one million malaria cases were reported across the country in the first half of 2022. The most vulnerable groups were travelers and migrants from areas with low or no malaria infections, , pregnant women, especially during the first two trimesters of pregnancy and children under five.(3)

People who get malaria are typically very sick with high fever, shaking chills, and flu-like illness. Although malaria can be a deadly disease, illness and death from malaria can usually be prevented. (4) A rapid and accurate diagnosis is the first step in the malaria effective management. (5) Early diagnosis and treatment of malaria reduces disease and prevents death. It also contributes to reducing malaria transmission. The best available treatment, particularly for *Plasmodium falciparum* malaria, is artemisinin-based combination therapy (ACT). (6) Light microscopy and Rapid Diagnostic Tests (RDTs) are currently considered the two diagnostic procedures with the highest impact on controlling malaria. Microscopy is a valuable diagnostic tool, and in expert hands, it can detect up to 50 parasites/ μ l of blood. (7) Immunochromatographic test (ICT) is a commercially available malaria Plasmodium/pan rapid test, which allows the detection of malaria antigens. It is a qualitative membrane-bound assay for *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* detection. The test membrane is pre-coated with anti-Histidin Rich Protein-2 (anti-HRP-2) specific antibodies for *Plasmodium falciparum* and Plasmodium specific anti-aldolase antibodies for the detection of the other three species of Plasmodia. (7) In Sudan, like many other African countries, malaria is highly prevalent in areas where health recourses are often poor and expert health personnel are scarce. Malaria diagnosis by quality microscopy is often not available in such areas. ICT is a RDT that is not operator dependent and is a welcome addition to the diagnostic armaments of malaria. This study aims to test the diagnostic accuracy of ICT compared to the standard microscopy operated by expert personnel in malaria diagnosis.

Materials and Methods:

A descriptive cross-sectional study was conducted at Yathrib area in Khartoum State from August 2019 to August 2020.

The study was carried out in the Amanah Clinic. This center was established in the year 2018 and is equipped with a modern laboratory, run by qualified personnel.

Study population:

Patients referred to the Amana Clinic laboratory during the study period requesting laboratory diagnosis of malaria were considered for the study. Critically ill patients were excluded. Patients who agreed to participate in the study after informed consent were included.

Sample Size:

The sample size was determined according to the following formula:

$$n = N / [1 + N(E^2)]$$

Where **n** is the sample size; **N** is the population of the study and **E** is the margin of error (0.05) indicated by the 95% C.I.

The population of the study was N=134 patients. Using the above formula, **n** was computed to be 100 patients. They were selected by simple random sampling technique. Peripheral thick and thin blood films for the microscopy diagnosis and a rapid ICT for Malaria were performed on the study group.

Procedures for thin and thick blood films and RDTs preparation:

The skin of the index finger was sterilized using a 70% alcohol swab and pricked with a sterilized lancet. Capillary blood sample was obtained for the thin and thick blood films as well as the ICT for malaria diagnosis.

- (a) Thin blood film preparation: A small drop of blood was placed on a pre-cleaned labeled slide. Another slide (spreader) was held at a 30-45° angle and used to spread the blood along the contact line up to the end of the lower slide. The thickness of the blood smear decreased progressively towards the feathered edge of the slide, thus forming cells in a monolayer.

The thin smear was allowed to dry, and then fixed by dipping in absolute methanol for two seconds.

(b) Thick blood film preparation:

A thick blood film consists of a thick layer of lysed red blood cells. To prepare such a film, three small drops of blood were placed in the center of a pre-cleaned labeled slide. The corner of another slide was used to spread the drops to form a circular pattern of 2-3cm in diameter. The slide was laid flat and the smear was allowed to dry thoroughly and protected from dust, fluid and insects. To accelerate the drying of a smear a fan was used. After that Geimsa stain was used to stain the smears. (8)

(c) Principles and preparation of RDTs, also known as ICT:

Malaria RDTs are qualitative immunochromatographic “lateral flow” tests. They are prepared in dipstick (strip), cassette or card forms to detect malaria antigens in the peripheral blood. The term “lateral flow” refers to the migration of liquid across the surface of a nitrocellulose membrane. Malaria antigen from a lysed blood sample is reacted with anti-malaria monoclonal antibody conjugated to colloidal gold (pink-mauve) particles. The antigen-antibody colloidal gold complex migrates along the nitrocellulose membrane, where it becomes bound (captured) by a line of specific monoclonal antibodies, producing a pink line in the test result area. This line can be seen after a washing buffer has removed the background hemoglobin. A further pink line (i.e. inbuilt positive control) is produced above the test line, indicating that the test reagents have migrated satisfactorily (it is not a malaria antigen control). (8) The Kits used in the study were manufactured by SD BIOSENSOR company from Republic of Korea.

Interpretation of malaria diagnostic tests:

- (a) Interpretation of microscopic examination: In this study, malaria diagnosis was considered positive if the parasite trophozoite stage was detected.

Simple criteria were used to differentiate between the two species of malaria. For the *P.falciparum*, the trophozoites were small to medium in size. Many of them had ring and comma shapes. *Chromatin dots* (often *two dots*) and regular cytoplasm were observed.

For *P.vivax* the trophozoites were large in size and had few to moderate numbers of broken rings. The most common shapes were irregular forms. The trophozoite contained a *single chromatin dot*.

(b) Interpretation of ICT: The test results were interpreted as follows;

- Test considered *P. falciparum* positive if one line appeared in the control region and one in the *P. falciparum* specific region.
- Test considered as non-falciparum Plasmodium-species-specific positive if one line appeared in the control region and one in the pan malarial region.
- Test considered to be mixed infection if one line appeared in the control region, one in the *P. falciparum* region and one in the pan malarial region.
- Test considered to be negative if one line appeared only in the control region. (8)

Data Analysis:

Data were analysed using statistical package for the social sciences (SPSS) version 20. Matthews Correlation Coefficient (MCC) was used to calculate the ICT sensitivity and specificity.

Validity of a test is defined as its ability to distinguish between who has a disease and who does not. (9, 10) Validity has two components: sensitivity and specificity. (9, 10) **Sensitivity** is defined as the ability of the test to identify correctly those who have the disease. **Specificity** is defined as the ability of the test to identify correctly those who do not have the disease. **Positive predictive value** is defined as the proportion of patients who test positive and actually have the disease.

Negative predictive value is defined as the proportion of patients who do not test positive and actually do not have the disease. (9, 10) All these values are calculated using the following formulae:

Sensitivity =	$\frac{\text{True positive (TP)}}{\text{TP} + \text{false negative (FN)}} \times 100$
Specificity =	$\frac{\text{True negative (TN)}}{\text{TN} + \text{false positive (FP)}} \times 100$
Positive predictive value =	$\frac{\text{TP}}{\text{TP} + \text{FP}} \times 100$
Negative predictive value =	$\frac{\text{TN}}{\text{TN} + \text{FN}} \times 100$

Results

A total of 100 blood samples were tested for malarial parasites by light microscopy. The same samples were tested by the ICT malaria Plasmodium falciparum/Pan test device (P.f/Pan). The results of light microscopy and ICT were compared.

Results of Microscopy of the blood films indicated that 60 (60%) patients were infected with malaria; and the rest 40 (40 %) were malaria negative. Among the positive patients *P. falciparum* was detected in 52 (87%) and non-falciparum Plasmodium species were found in the remaining 8 (13%). Mixed infection was found in none of them.

The ICT malaria P.f./Pan Test results showed that 54 (54%) of the patient samples were positive for malaria parasites and the rest 46 (46%) were negative for malaria parasites. Infection with *P. falciparum* accounted for 51 (94.4%) cases of the 54 positives; and non-falciparum Plasmodium species for the remaining 3 (5.5%). Mixed infections were not found. (Table1).

Table 1: Comparison between the results of blood film microscopy and immuno-chromatographic test (ICT) for detection of malaria in Amana clinic, 2020

Test	Result	
	Positive	Negative
Blood film microscopy (standard) n =100	60(60%)	40(40%)
ICT for malaria (test) n =100	54(54%)	46(46%)

The results of true positive (TP), true negative (TN), false positive (FP), false negative (FN) will obtained according to (table 2).

The results showed sensitivity of ICT was (89%), and the specificity was (86%) (Table 3), the positive predictive value was (99%) and the negative predictive value was (31.7%) (Figure1), and the validity of ICT was (89.2%). MCC of true positive, false positive, true negative and false negative was 0.78, which was significant with the gold standard method.

Table 2: comparison of results of Malaria ICT and Microscopy.

Test		Microscopy	
		Positive	Negative
ICT (Malaria RDT)	Positive	TP (Positive by both)	FP (Negative by microscopy but positive by ICT)
	Negative	FN (Positive by microscopy but negative by ICT)	TN (Negative by both)

Table 3: Sensitivity and Specificity of ICT for malaria in Amana clinic patients, 2020

Sensitivity and specificity of ICT for Malaria			
Sensitivity =	$\frac{(TP)}{(TP) + (FN)} \times 100$	$\frac{114}{114+14} \times 100$	89%
Specificity=	$\frac{(TN)}{(TN) + (FP)} \times 100$	$\frac{86}{86+14} \times 100$	86%

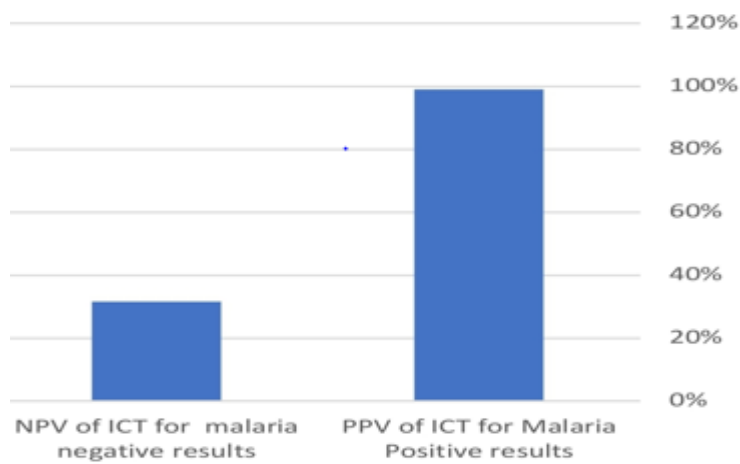


Figure 1: Positive predictive value and Negative predictive value of ICT

Discussion

Although microscopy remains the gold standard method for the diagnosis of malaria, yet there are several constraints such as the lack of trained microscopy technologists, time consumed for microscopy examination and scarcity of good devices. An easily performed, time-effective and accurate test for the detection of Plasmodial infections is needed especially in countries like Sudan with endemic malaria, large population and scarcity of trained laboratory staff. The current study was performed to compare ICT with microscopic malarial parasite detection and to determine the diagnostic validity (sensitivity and specificity) of the ICT method using microscopy as the gold standard diagnostic method. The results showed: the sensitivity of ICT was 89% and the specificity of ICT was 86%; the positive predictive value was 99% and the negative predictive value was 31.7%. These findings are consistent with many similar studies conducted within and outside Sudan. The findings were consistent with a similar study performed in Pakistan that compared the validity of ICT malaria with slide microscopy and showed that the sensitivity of ICT for malaria was 96.1% and specificity was 95.7%. (9) However our results are not in agreement with another study from Pakistan, where sensitivity and specificity results to evaluate the “Optimal Test” for the rapid diagnosis of Malaria in children in 2005 showed a sensitivity of 85% and specificity of 91%; positive predictive value of ICT was 68% and negative predictive value of ICT was 96%. (11) The disagreement may be due to the fact that the reagents of the two studies were directed to two different components of the test. In our study, the ICT was used to detect malaria surface antigens, while the “Optimal Test” was used to detect malaria enzymes. Our results are consistent with the findings of a similar study from Guinea, in which the sample size was 400, the sensitivity of ICT was 93% and the specificity was 95%. (12) Thus, the specificity was higher than our findings. Our findings were consistent with a study performed in Egypt to study the validity of rapid malaria test and microscopy in detecting malaria in a pre-elimination region in Egypt.

The sample size was 600 and the ICT sensitivity was 95%. (13) Another Egyptian study conducted in Mansoorah Hospital, using a sample size of 211 to evaluate sensitivity and specificity of rapid malaria test in reference to light microscopy, reported sensitivity of 98% and specificity of 96%. (14) These results were higher than those shown in our study; the discrepancy may be due to differences in burden of malaria disease between Sudanese and Egyptian population, the malaria burden being higher in Sudan than Egypt. Moreover, there is a likelihood of the Sudanese population having a more effective immune response against malaria. Unlike the above quoted studies, a study from Uganda showed that persistent antigenicity in patient's blood reduces the accuracy and showed low specificity of ICT and other RDTs (15), another study from Equatorial Guinea showed a low ICT sensitivity of 69.7% and a specificity of 73.7%. (16), in Pakistan study of ICT showed 97% Sensitivity and 98.3% Specificity for *P.falciparum*, and 89.7% sensitivity, and 97.9% Specificity for *P. vivax*, they concluded that RDTs shouldn't be used without reference method. (17)

Our study and most of the above quoted studies indicate that the sensitivity and specificity of ICT were suboptimal compared to the gold standard method of light microscopy in expert hands. This may be due to some biological factors, such as; the level of malaria parasitemia and cross reactivity with other microorganisms and antigens. Environmental factors may be operating such as: variability of temperature, humidity, transportation factors and storage environment of reagents. (18, 19, 20)

Conclusion:

Although the study shows that the diagnostic accuracy of ICT was suboptimal compared to the gold standard method of microscopy, yet RDTs are valid as a supporting diagnostic tool for malaria. In difficult cases where ICT was used to diagnose malaria, there may be need to use light microscopy in experts hand for the final verdict.

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Authors own resources

Conflict of interest

None

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Testicular Self-examination: Knowledge, Attitude and Practice of Male Medical Students in Alfajr College for Science and Technology, Khartoum, Sudan, 2022.

Ahmed S. A. Alawad¹, Rayan I. H. Mohamed², Egbal A.B. Abukaraig³

¹Graduate of Medicine Program, Alfajr College for Science and Technology.

²Research and Publication Coordinator, Research and Development Centre, Alfajr College for Science and Technology.

³Director of the Research and Development Centre, Alfajr College for Science and Technology.

*Corresponding author

Rayan Ibrahim Hamid Mohamed, Research and Development Centre, Alfajr College for Science and Technology, Khartoum, Sudan.

Email: rayan.ibrahim.hamid@gmail.com

Abstract

Background: Testicular cancer is one of the malignancies of young adult males, especially between the ages of 15 and 35. Although this type of cancer is relatively uncommon, being only 1% of all cancers globally, yet its incidence has doubled over the past 40 years. Testicular self-examination (TSE) involves examining and palpating the testes to detect potential changes that may indicate early signs of testicular cancer.

Aim: To determine Knowledge, Attitude and Practice about testicular self-examination among male medical students in Alfajr College for Science and Technology, 2022.

Materials and Methods: The study was a descriptive cross-sectional, institutional-based study conducted among male medical students, at all five levels in the College. The sample size was estimated at 149 distributed by stratified sampling technique over the five levels of medical students. Self-administered interview using structured questionnaire designed by the researcher and approved by the supervisor was used, then it was entered, revised, and analyzed using SPSS version 25.

Results: The study involved 148 participants with 99% response rate. Most of the students' age (79.7%) ranged between 15-26 years. Only 44 (29.7%) of the students had ever heard about TSE. The overall knowledge of students about TSE was 11.36%; and 20.45% had favorable attitude towards TSE.

However, practicing TSE regularly was rather poor being only performed by 2.27%.

These results are less than reports from other countries in the region like Nigeria, Ethiopia and Uganda, where studies from these countries showed more knowledge and practice among their male population about testicular cancer and TSE.

Conclusion: The low rate of knowledge and practice of TSE among medical students in Alfajr College is worrisome. This probably reflects the paucity of health educational programs about testicular cancer among males in Sudan compared to other countries in the region. This preliminary report draws attention to this important issue and probably more research and action are needed in this respect.

Keywords: *Testicular cancer, Testicular self-examination, knowledge, Attitude, Practice.*

Introduction:

Testicular cancer (TC), the malignant tumor of the male sex organ (testicle), is an uncommon form of cancer, being only 1% of all cancers globally; and ranking at the 20th mark in the world cancer record. Its incidence increased in most countries around the Globe over recent years. (1,2) Although Africa has been within the lowest incidence rates, the incidence in the sub-Saharan Africa is expected to increase by more than three folds by 2030.(3)

In Sudan, TC formed less than 1.5% of all male cancers treated during the period from August 2004 to September 2012 at the Radiation and Isotope Centre of Khartoum (RICK).(4)

TC risk factors include age, an un-descended testicle, abnormal testicle development, testicular torsion, family history of the disease, HIV infection, a history of testicular cancer, body size, painless tumors of the testicles, significant decrease or increase in the testis and loss of sexual activity. (5,6) Globally, incidence rates of TC are increasing among the 15 to 54-year-old males, especially between the ages of 15–35 years, with the majority of those cases affecting males under 40 years. (7)

In the United Kingdom (UK), around two thousand new cases are diagnosed each year, which led the Cancer Registries and support groups to recommend an awareness program on TC for young men. The program should include that medical advice has to be sought if any abnormalities are detected by self-examination. (8)

A useful tool for the early identification of TC is examination of the testicles by a male examiner to rule out any lumps. (3) Testicular self-examination (TSE) is

a screening method of examining and palpating the testes to detect potential changes that may be early signs of cancer. (9) TSE is advised to be performed when standing and immediately following a warm shower, when the scrotum is relaxed and the testes are lower.(10) Low income nations have a high rate of morbidity and mortality from TC because of late presentation, poverty, and lack of management resources. Sudan is not an exemption, where in a retrospective study conducted in RICK in 2019, 54.4% of the patients presented either with stage three or four TC. (4) Early detection can be facilitated if men are knowledgeable and encouraged to practice TSE. (6)

This study aims to explore knowledge, practice and attitude towards TC and TSE among medical students in Alfajr College for Science and Technology.

Materials and Methods

The study was institutional, descriptive cross-sectional study conducted during November 2022 in Alfajr College for science and technology (ACST).

The study population: All registered male medical students at ACST from the first to the fifth academic years were considered for the study. The students who agreed to participate after an informed consent were included.

Sample size and Sampling: Sample size was estimated by the formula

$$n=N/[1+N(E^2)]$$

Where n = the sample size, N = the total study population that included male medical students = 238, E is the margin of error at 95% C.I taken as 0.05.

$$n = 238 / (1 + 238(0.05)^2) = 149.21 = 149$$

The students were chosen by stratified sampling technique, proportionate to the number of students in each level. Thus, 21 students were taken from the fifth level, 25 from the fourth level, 29 from the third level and 37 from each of the second and the first levels. Students from each level were chosen by convenience, taking the first responding to the required sample size.

Data collection

The data was collected using self-administered interview using a pre tested, pre coded, standard Google form questionnaire which was quoted and modified from previous studies. (11) The questionnaire consisted of four sections; the first section was the socio-demographic data, the second section was assessing the knowledge of students regarding TSE and it contained eight questions, the third section was assessing the attitude of students regarding TSE and it contained six questions. The last section was measuring the practice of the students and it contained five questions. The questionnaire was reviewed and accepted by an expert supervisor. The questionnaire was distributed through social media to the target population.

Data analysis

Data was entered and analyzed using SPSS software version 25. Descriptive statistics were carried out and inputs summarized as frequencies and proportions at a 95% confidence level.

The knowledge score was valued by categorizing the outputs of correct answers as follows: (a) good knowledge if the participant answered correctly 75% or more of the questions; (b) moderate knowledge if correct answers were 50% to 74.9%, and (c) poor knowledge if correct answers were less than 50%. The same was used for the attitude and practice scores.

Ethical approval

Ethical and technical approval was obtained from the department of community medicine at ACST. Written consent was obtained from each student as part of the electronic form with clarification that their participation is voluntary and the information obtained will be treated with confidentiality.

Results:

The total number of participants involved in this study was 148 with 99% response rate.
The majority of participants (79.7%) were in the age group of 15-26 years. Personal and family histories of TC were positive in less than 4%. (Table 1).

Table 1: Socio-demographic Characteristics of Male Medicine Program Students at Alfajr College for Science and Technology; (n=148).

Variable	Frequency	Percent
Age group		
15-20	37	25%
21-26	81	54.7%
27 and more	30	20.3%
Personal history of TC		
Yes	4	2.7%
No	144	97.3%
Family history of TC		
Yes	5	3.4%
No	143	96.6%

Only 44 students of the sample (29.7%) heard of TSE before. The major source of information was social media in 61.4%. Of the participants who knew about TSE, only one fifth answered correctly when asked about the range of age that would be at risk of TC; and only 9% gave the correct answer about the appropriate time for doing TSE. More details about the participants’ knowledge are shown in (Table 2).

Table 2: Knowledge of the 44 Male Medicine Program Students (29.7% of the sample size) who heard of TSE before; Alfajr College for Science and Technology, 2022

Variable	Frequency	Percent
Source of information regarding TSE		
Social Media	27	61.4%
Friends	2	4.5%
Health profession	6	13.6%
Teachers	6	13.6%
Others	3	6.8%
What is the importance of doing TSE?		
For screening	18	40.9%
Others	26	59.1%
How often to do TSE?		
Once every month	8	18.2%
Other frequencies	36	81.8%
TC risk age group		
15-35 years	9	20.5%
Other age groups	35	79.5%
The best time to practice TSE		
Shortly after taking a shower	4	9.1%
Other times specified	40	90.9%
Probability that family history can increase TC		
Yes	18	40.9%
No	7	15.9%
Don't know	19	43.2%
TC can be more serious than other diseases		
Disagree	13	29.6%
Uncertain	6	13.6%
Agree	25	56.8%
TSE can help in finding lumps in the testes		
Disagree	13	29.5%
Uncertain	8	18.2%
Agree	23	52.3%
Routine TSE can help in finding changes in the testes before it is discovered by regular health checkup		
Disagree	15	34.1%
Uncertain	6	13.6%
Agree	23	52.3%

Of the 44 students who heard of TSE before, only 5 (11.36%) had overall good knowledge score of TC and the majority, 27 (61.36%), had poor knowledge score. **(Figure 1)**

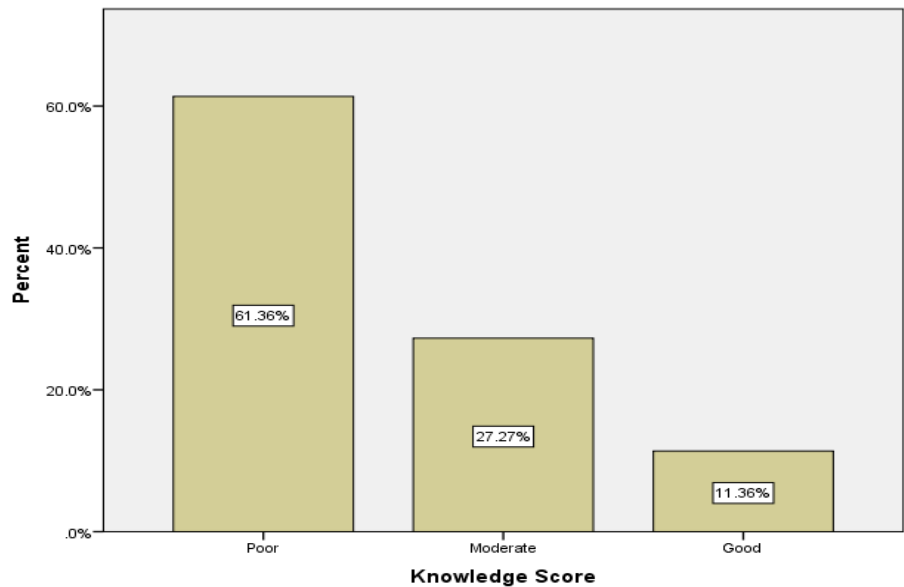


Figure 1: Overall Knowledge Score of Male Medicine Students in Alfajr College for Science and Technology, 2022 (n=44)

Questions probing for attitude regarding TSE showed that TSE was embarrassing for about 27% of the students; 18% thought that it could be painful and 20% thought that it could be time consuming. (Table 3)

Table 3: Attitude of the 44 Male Medicine Program Students (29.7% of the sample size) who heard of TSE before; Alfajr College for Science and Technology, 2022

Variables	Frequency	Percent
Routine TSE is embarrassing for me		
Disagree	15	34.1%
Uncertain	17	38.6%
Agree	12	27.3%
TSE can be painful		
Disagree	15	34.1%
Uncertain	21	47.7%
Agree	8	18.2%
TSE can be time consuming		
Disagree	16	36.4%
Uncertain	19	43.2%
Agree	9	20.5%

The majority of the participants (63.64%) had overall poor attitude score towards TSE, while only 20.45% had good attitude score. **(Figure 2)**

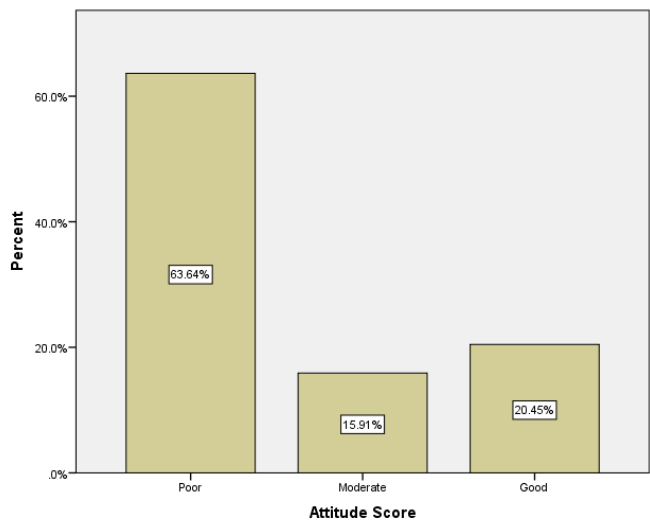


Figure 2: Overall Attitude score of Male Medicine Students at Alfajr College for Science and Technology, 2022 (N=44)

Only 23% of the 44 participants who knew of TSE actually practiced TSE and of them only 2.27% practice it as appropriate. **(Figure 3)**

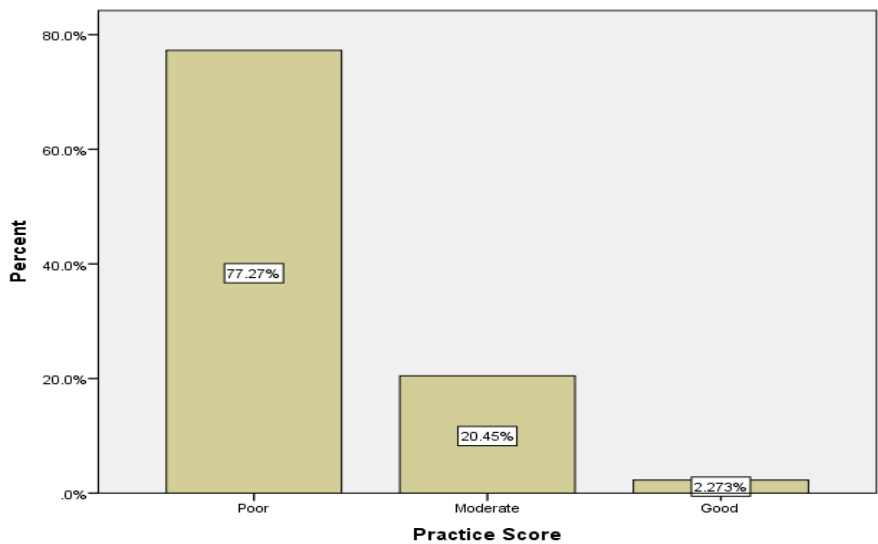


Figure 3: Overall practice score of Male Medicine Students at Alfajr College for Science and Technology, 2022 (N=10)

Discussion:

The most common cancers that can be detected by regular self-examination are breast cancer for women and TC for men. (11) While Breast Self-Examination is gaining much attention among women in the Sub-Saharan African region, knowledge of TSE in men in the same region remains poor. Although TC is far less prevalent than breast cancer, it is the most frequent cancer for men aged 18–50 who are considered a high-risk group. (11) In this study, the majority (79.7%) were in the age group of 15-26 years been within the targeted age of the most commonly affected by TC. (8)

The American Cancer Society (ACS) recommends that all men practice TSE every month and make the procedure a lifetime habit. The ACS based its recommendation on that nine out of every ten TC were detected by the patient self. (12). The ACS also advises that doctors should consider testicular examination as part of their routine cancer-related check-ups.(13) Besides TC assessment, males with knowledge about TSE can easily check themselves for male-specific urogenital health concerns, including scrotal abnormalities like varicoceles, hydroceles and unusual lumps and testicular torsion, which are all risk factors of TC. (5, 6) Every male at puberty should be taught and encouraged to perform TSE for detecting testicular tumors or other scrotal abnormalities. (14) It is reported that a better prognosis with a 5-year survival rate is likely in males who effectively carry out TSE and identify a lump in its early stages. (15) Although the ACS recommended young men between the ages of 15 - 35 years should practice TSE every month, a study done in the USA showed that only 46% of respondents reported performing TSE. (12)

In our study, a relatively good proportion, 44 (29.7%) out of the 148 respondents had heard about TSE. This figure is higher than studies done in Iran, Nigeria and Turkey, where those who heard about TSE were less than 5%, 5.9% and 1.3% respectively. (7,10,16) The discrepancy might be explained by the fact that the participants in our study were all medical students who should have better awareness of medical conditions than the general population. However, the overall knowledge level of respondents from our study was low, only 11.4% had good knowledge of TSE. This figure is lower than reports from Nigeria, Ethiopia and Uganda where TSE knowledge was of a good level in 52%, 30%, and 41.8% respectively. (10,11,17) This might be due to better health promotion programs for early detection of TC in those countries.

The sources for knowledge, attitude and practice of TSE include media information, health professionals, parents and teachers. (18) In our study, health profession was the source of information among 13.6% of the participants who had heard about TSE.

This is unlike the information from two studies from Nigeria in which none of the respondents got information from health professionals. (9,10)

Only 20.5% of our study sample knew the age group mostly affected by TC, This finding is slightly higher than data reported from Uganda where 13% of their study group were aware of the age group at risk. (17) This variation might be due to the higher awareness among medical students. When the participants in this study were asked about the appropriate time to perform TSE, 9.1% of the respondents knew the correct answer which is shortly after taking a shower. These findings are lower than a research done in Uganda, which was 29.0%. (17) This discrepancy might be due to lower information dissemination about TSE in our context.

About 41% of the respondents said TC probability would increase with family history. This result is lower than the research conducted in Nigeria which revealed that 67.4% of the respondents said “Men who have blood relatives with testicular cancer are more likely to get TC”. (10)

In our study about 20% of the students had an overall favorable attitude towards TSE. In Nigeria and Ethiopia the attitudes were more favorable towards TSE at 57.2% and 51.3% respectively. (10,11) Moreover, only 23% of our respondents had practiced TSE. This finding is much lower than the researches in Nigeria, Turkey and Uganda in which the practice of TSE among participants were 45.9%, 17.7% and 14% respectively (10,16, 17) This huge difference may reflect the lack of health promotion for early detection of TC in our context.

Regarding the regularity of performing the TSE in this study, only 4.2% of respondents examined their testis every month. This low figure is consistent with the report by Jemal et. al. that only 3.1% of respondents from low-and-middle-income countries examined their testis regularly. (19)

Conclusion:

Our results showed that TSE knowledge, attitude and practice among this sample of male medical students were inadequate. Only 20% of the students showed favorable attitude towards TSE. The major reason not to perform TSE was lack of knowledge about TSE and under-appreciation of the importance of early detection of TC. This reflects that there is shortage of promotion for early detection of TC in our institution among medical students. Students in non-medical programs may be even less informed. Therefore, the benefits of early detection of TC through TSE for reduction of mortality and health care expenditure associated with management of the disease should be promoted. A larger scale research is needed to verify the size of the problem in the country.

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Conflict of interest

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Molecular Identification of Sudanese Females' Vaginal Microbiota and Microbiota associated with Bacterial Vaginosis, Khartoum, Sudan, 2018

Deena A. A. Mohamed^{1*}, Sahar E. G. Osman², Hassan A. Musa³

¹Research and Development Center Alfajr Collage, Khartoum – Sudan

² Departments of Histopathology and Cytology; Medical Laboratory Science Program, Alfajr College for Science and Technology, Khartoum, Sudan.

³Faculty of Medical Laboratories, National Ribat University, Khartoum. Sudan.

***Corresponding Author:**

Deena Abdalfatah Abdelgadir Mohamed, Assistant professor, Research and Development Center, Alfajr Collage for Science and Technology, Khartoum, Sudan.

Email: deenamohammed@gmail.com

Abstract

Background: The microbiota of healthy women changes from birth to menopause. During their reproductive years, females' normal flora is dominated by Lactobacillus species. Females who are identified with vaginal infections are characterized by lacking Lactobacillus species and the vagina would be dominated by facultative and strictly anaerobic bacteria. Recently, a new detailed description of the vaginal microbiome has been attained using quantitative, real-time Polymerase Chain Reaction (PCR) targeting key bacterial genomes. In this study, we have used 16S Ribonucleic acid (RNA) sequencing to identify the Sudanese flora and flora associated with bacterial vaginosis within females in their reproductive age.

Materials and Methods: This is a descriptive cross-sectional and molecular laboratory-based study; carried out in one reproductive health Centre and two health care facilities in Khartoum State, Sudan. Hundred vaginal swab samples were collected from the participants who visited the Centres. Out of the 100 serial vaginal swabs, 11 samples were randomly selected. The DNA was then extracted from the swab samples and 16S RNA sequencing was performed. The samples were classified into four groups.

Results: *Lactobacillus* species was found to be the most dominant organism in all four groups except for the group with bacterial vaginosis (BV) which was found to be dominated by the genus *Gardnrellia* and *Lactobacillus inners*. Factors such as usage of contraceptives and some Sudanese traditions like circumcision and skin smoking (Dukhan) have significant effect on the flora (p-value 0.045 and 0.03 respectively)

Conclusions: Sudanese female flora is dominated mainly by *Lactobacillus* species among normal and asymptomatic females, while *Gardnrellia* sp. was dominant among females with BV. This is consistent with the global picture despite the differences in customs and traditions of Sudanese women, which was expected to affect the types of vaginal bacteria. Further studies and metagenomic sequencing are needed for better identification.

Key words: *Bacterial Vaginosis, Microbiota, 16S RNA, Lacbacillus*

Introduction:

The vagina has a complex ecosystem of microflora that changes throughout life, from birth to menopause (1). This microbiome consists of species and genera which typically do not cause symptoms or infections in women with normal immunity. The vaginal microbiome is dominated by *Lactobacillus* species (Döderlein bacilli) in the normally healthy vagina of women of reproductive age (2). Bacterial vaginosis (BV) is a clinical condition often referred to as an overgrowth of normal bacteria in the vagina. It is the most common vaginal infection among women of reproductive age. These species metabolize glycogen and break it down into glucose and lactic acid (2).

With the development of high throughput sequencing (HTS), 454 pyrosequencing (3), and Illuminasequencing (4), the tools available for studying the human microbiota, have taken a new major step forward.

For the analysis of bacterial communities, HTS is used in two separate ways; 16S rRNA sequencing uses PCR amplification, of one or more variable regions, in order to identify the microbiota present and their relative abundances. The advantage of sequencing the amplified 16S rRNA is the depth of the investigation, that even bacteria representing less than 0.01% of the bacterial abundance can be detected with a reasonable number of sequences from each sample at a low cost per sample (Fredricks, 2011(5).

In Sudan there is a huge variation among different tribes in their customs and diets, and hence different biota is expected to present. In this study we aim to isolate and identify the vaginal bacterial flora of Sudanese women and the flora associated with BV.

Materials and Methods:

Study design, area, and population:

This is a descriptive cross-sectional molecular laboratory-based study carried out in Khartoum State, Sudan. Women attending one major reproductive center (Sudan Fertility Center) and two health centers (Aylafon Health Center and Eid Babiker Health Center) were considered for this research. A self-administered questionnaire was filled by the participants. Different variables such as Skin-smoking (*Dukhan*), circumcision, contraceptives use and anti-biotic use, were included to determine the effect on the vaginal flora. Vaginal swab samples were collected from participants.

Sample Size: A hundred consecutive participants who fulfilled the inclusion criteria were included in the study. The vaginal swabs were collected from the mid-vagina using sterile swabs. All samples were preserved at -20°C and saved until processed. DNA was extracted using the Intron DNA extract Kit. All samples were tested for bacterial genomic presence using the PCR technique. The samples were grouped into according to their Nugent Score ranking (6): (Clinically and microscopically identified as normal, Symptomatic vaginosis, Asymptomatic vaginosis). Eleven samples were selected for sequencing from the identified groups using simple random selection. A simple random selection for the sample was made after the groups were, identified according to the microscopic identification.

Molecular Identification: For identification and phylogenetic classification of pathogens in diverse microbial populations, prokaryotic 16S rDNA was used. The protocol targets the variable V3 and V2 regions of the 16S rDNA for sequencing. Amplicon primers for amplifying a single amplicon of approximately ~460 bp were ordered from (Integrated DNA Technologies, USA). Amplicon library preparation and sequencing were performed at the National Institute for Communicable Disease Sequencing Core Facility in South Africa. (Table 1). Illumina adapter overhang nucleotide sequences were added to the gene-specific primers for compatibility with Illumina index and sequencing adapters. During library preparation using Nextera XT Library Preparation Kit (Illumina, San Diego, CA, USA), the V3 and V2 region of the 16S rRNA is amplified using a limited cycle PCR, followed by the addition of Illumina sequencing adapters and dual-index barcodes to the amplicon target.

Table (1): Amplicon Library Preparation and Sequencing performed at the National Institute for Communicable Disease Sequencing Core Facility, South Africa.

16S Amplicon PCR Forward Primer	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG
16S Amplicon PCR Reverse Primer	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC

16S data analysis:

Following the sequencing, the resulting paired-end reads were checked for quality, trimmed, and analyzed using the CLC Genomics Workbench version 11 (CLC, Bio-QIAGEN, Aarhus, Denmark). The tools from Amplicon-Based Analysis at the CLC Microbial Genomics Module are designed to cluster all reads within a certain percentage of similarity into Operational Taxonomic Units (OTUs) where they are then represented by a single sequence.

The tools clustered reads at some level of similarity into pseudo-species or OTUs, where all reads within for example 97% similarity are clustered together and represented by a single sequence.

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 20. A chi-square test was conducted to establish the relation between the variables.

Results:

Clinical and socio-demographic data: a total of 100 vaginal smears were collected from Sudanese females' mean age (30.7±7.05) from Khartoum and Khartoum suburbs. (Table 2) shows the age groups. Seventy five percent of the participants were in the younger age group of 16 – 36 years. The state of conception, contraceptive use, antibiotic use in the preceding 3 months and clinical symptoms are shown in (Table 3)

Table (2): Age groups of participants of the study population, Khartoum, 2018.

Age groups	n=100 No. (%)
16-26 years	31 (31.0%)
27-36 years	44 (44.0%)
37-46 years	20 (20.0%)
>46 years	5 (5.0%)

Table (3): Behavioral Characteristics of the Participants of the study, Khartoum, 2018

Variable	No (%)
Circumcision	
Yes	83 (83.0%)
No	17 (17.0%)
Skin smoke	
Yes	87 (87.0%)
No	13 (13.0%)

Table (4): Clinical Characteristics of the Participants of the study, Khartoum, 2018. n=100

Variable	No. (%)
Pregnancy	
Pregnant	34 (34.0%)
Non-pregnant	66 (66.0%)
Hormonal contraceptive use	
Yes	8 (8.0%)
No	92 (92.0%)
Antibiotics use (in the past three months)	
Yes	84 (84.0%)
No	16 (16.0%)
Symptoms	
Abnormal discharge	15 (15.0%)
Abnormal discharge with odor	6 (6.0%)
Irritation or discomfort	17 (17.0%)

Table (5): Vaginal Bacteriome Profiles Data Frequencies of Symptomatic / asymptomatic BV subjects, Khartoum, 2018.

Genus	15s	49h	10 h	11 h	21 s
	Asymptomatic BV		Symptomatic BV		
Frequency at genus level according metagenomic reads					
<i>Actinomycetaceae</i>	19	0	0	0	0
<i>Corynebacteriaceae,</i>	0	1	12	12	25
<i>Propionibacteriaceae</i>	11	0	1	3	4
<i>Gardnerella. Sp</i>	56	43	139	142	281
<i>Prevotellaceae</i>	15	0	1	3	9
<i>Veillonellaceae</i>	13	1	0	2	2
<i>A.vaginae</i>	0	3	0	0	0
<i>Lactobacillaceae,</i>	25	3	91	295	428
<i>L. iners</i>	53	41	1	636	1485
<i>L. reuteri</i>	1	0	1	15	17
<i>L. vaginalis</i>	0	0	28	8	36
<i>Ureaplasma.sp</i>	5	0	8	2	10
<i>Gemellaceae</i>	0	1	0	2	2
Other bacteria	115	13	63	138	258
Total	313	105	345	1258	2557

Table (6): The Effect of Different Variables against the Presence or Absence of Different Micro-organisms that might be found in Normal Females, Khartoum, 2018.

Variables	Percentage % of identified organisms findings					p-value
	G+ve Bacilli- Lactobacillus	Streptococcus G +ve Cocci- Staphylococcus	G +ve Rods- Bacteroides /Prevotella	G+ve Coccobacilli- Gardnerella	Coliform	
Contraceptive yes	1	25	0	0	0	0.000
no	99	75	100	100	100	
Skin smoking yes	93	92	67	50	100	0.003
No	7	8	34	50	0	
Circumcision yes	87	75	67	100	100	0.045
No	13	25	33	0	0	
Antibiotic usage yes	15	20	0	50	0	0.344
no	85	80	100	50	100	

PCR screening:

A total of 100 genomic DNA was extracted and amplified by 16S rRNA genes to detect the V1-V3 region bacteriome. Agarose gel electrophoresis showed the result of samples producing different band sizes <100bp for the V1 region, 341-534bp for the V3 region, and 338bp. The result of samples 8S, 9S, and 16S produced different band sizes Fig (1). 17 samples show bands for both *G.vaginalis* and *A.vaginae* however 100 samples all show different bands position.

16s rRNA sequencing: Eleven samples of mid-vaginal swabs were randomly selected and divided into 4 groups. The most prevalent phylotypes to genus level in 11 Subjects include for the 16S sequencing the results revealed the presence of: *Gardnerella* sp and *Prevotella* sp. *Lactobacillus* sp, *Streptococcus* sp, and *Staphylococcus* sp as the most predominant phylotypes (Fig 2). In contrast, less prevalent genotypes in 11 Subjects were detected including *Deinococcus* sp, *Meiothermus* sp, *Actinomyces* sp, *Arcanobacterium* sp, *Mobiluncus* sp, *Varibaculum* sp, *Corynebacterium* sp, *Probainobacterium* sp and *Prevotella* sp.. Normal vaginal biota profiles represented by Sample 9. Analysis showed the family *Lactobacillaceae* with the most dominant percentage was *Lactobacillus* sp by 95% (Fig 3), followed by 2.7% *L. iners* and 0.9% *L. reuteri*. The 9.5% lowest percentages of other bacteria phylum were *Corynebacterium*,
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Propionibacteriaceae, *Staphylococceae*, *Streptococcaceae* and *Mycoplasmataceae*. Moreover; other types of bacteria showed similar abundance profiles in both target sequence libraries. Samples with symptomatic BV were presented by 45.5% of the 11 samples selected. The most identified organisms were 48.4% *L. iners* and 14.4% *Gardenrella* sp, of the total genomic population. Presentation of other genera and species differs between samples such as *L. vaginalis* and *Gemella* sp, *Peptococcus* sp. *Sporosarcinapasteuri*, *Mycobacterium tuberculosis. bovis*, *Lactobacillus aviaris*, *Carnobacteriumgallinarum*, *Vagococcussalmoninarum* and *Halmonas*. Other genera identified such as *Salmonella* Sp. and *Streptococcus* Sp. were reported as well. (Table 5). Contraceptives, skin smoking, circumcision, antibiotic intake and age groups where analyzed against the presence and nascence of Lactobacilli and other organisms where some showed a significant effect on the type or vaginal flora as shown in (Table 6)

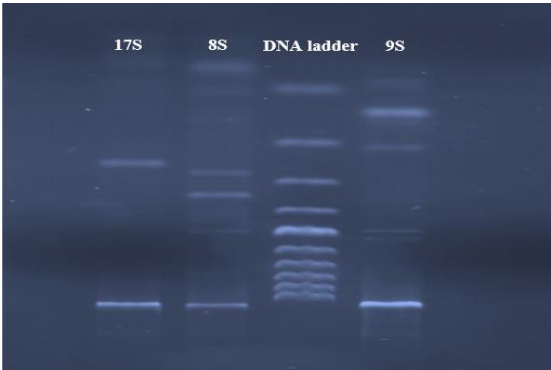


Fig 1: Agarose Gel Electrophoresis showing result of samples 8S, 9S and 17 S, Khartoum, 2018.

As shown in Fig 1, different band sizes were produced with the Pf/Pr_{1,2&3} primers pair multiplex PCR detection of B.V and vaginal microbial flora.

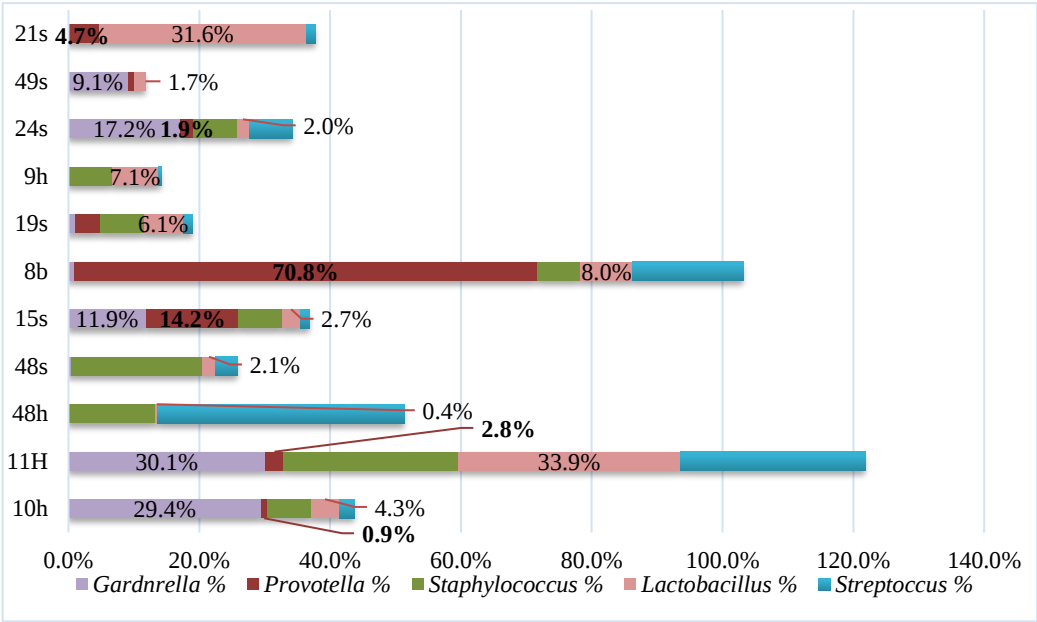


Fig. (2): Vaginal bacteriome profiles t genus level distribution

As shown in fig 2 the microflora of the 11 samples metagenomic reads for V1-V3 hypervariable region for most common genera read by the 16S sequencing.

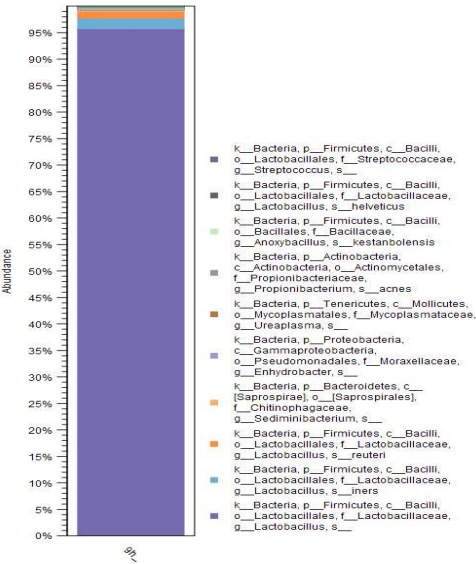


Fig 3: Normal bacterial flora Sample h9

Fig. 3 shows the normal flora and its metagenomic reads for V1-V3 in Sudanese female vaginal flora

Discussion:

According to metagenomic 16s rRNA sequencing; 11 samples of mid-vaginal swabs were randomly selected and grouped into 4 groups as more than 34 phyla of bacteriome were detected in Sudanese women mid-vagina either associated with BV or not. In each sample, Good's coverage was greater than 99% (range: 0.99 - 1.00). The metagenomic 16s rRNA sequencing; was highly robust resulting in a total of 14 phyla in samples 9h and based on a >75% cut-off identity to the V1-V3 gene target identified in our study. Revealing a predominance of 86.8% *Lactobacilli* sp of the normal vaginal biota and 2.7% *L. iners*. *Lactobacilli* sp promote a healthy ecosystem by producing lactic acid, hydrogen peroxide, and bacteriocins that have antimicrobial properties thereby excluding pathogens from this niche. Although both groups of those women were non-pregnant, the behavioral character of the vaginal microbiome gradually revert to baseline characteristics. Several studies have demonstrated that a significant proportion (7–33%) of healthy asymptomatic women lack appreciable numbers of *Lactobacillus* species in the vagina (7, 8), and instead have a vaginal microbiota that consists of other lactic acid-producing bacteria, i.e. species from the genera *Atopobium*, *Leptotrichia* and *Streptococcus* (Aldunate et al., 2015(9). The first report of isolation of *L. iners* in a woman with a normal Nugent score was in 2002 (10) and is one of the organisms most frequently isolated from the vagina of healthy women. (2,11,12,13)

In correlation to circumcision, skin smoke, and pregnancy with vaginal microbiome; 9h women were pregnant, not menopausal at the age of 29 years old, had not had a history of BV, were not circumcised, and were regular skin smoke users. However, microbial communities in the human vagina likely undergo shifts in the representation and abundance of key species over time that are influenced by factors that change vaginal microbiota. Studies of the normal bacterial flora of the female vaginal are primarily limited to the characterization of the types of bacteria present in women who do not have identifiable diseases. Studies by Chaban B *et al.* (14), MacIntyre DA *et al* (15), and Borgogna JL *et al* (16) stated that with the progress of pregnancy, the increased estrogen level has a positive effect on *Lactobacillus* activity and proliferation by increasing glycogen availability. Increased *Lactobacillus* rates are thought to inhibit pathogen growth through the secretion of antibacterial bacteriocins, as well as the production of metabolite, such as lactic acid which helps to maintain a low, hostile pH (17). Our study; showed that the abnormal bacterial colonization decreased significantly (p-value 0.034) (Table 6) as the women uncircumcised.

However, the studies do not address whether some proportion of “healthy” women were in transition to or from BV, or whether they have asymptomatic BV, ie. Abnormal flora but no symptoms due to genetic or other factors.

Our study found that 3/11 women had BV and a combination of eleven phyla is detected as the majority *Lactobacillaceae* and other bacteria including *Gardnerella* sp, *Gemella* sp. *Ureaplasma* sp and *A.vaginae* and *G. vaginalis* have been shown to be present in 78% to 96% of BV samples, in contrast to 5% to 10% of normal flora samples (18,19). Conversely, Menard et al. detected *A. vaginae* in 69% of samples from women without BV, suggesting that the mere detection of *A. vaginae* has a poor predictive value for BV (20). Nevertheless, their results showed that quantification of *A. vaginae* bacteria is a good predictor since higher levels were detected in BV-positive samples (18).

Hillier et al., 1993, reported that the prevalence of *U. urealyticum* was 78% in pregnant women with normal flora and was significantly higher, at 92%, in pregnant women with BV (21,22). Results reported by R Capoccia et al. were similar in that women with BV had a higher *U. urealyticum* carriage rate, at 65%, than did normal women, who had a carriage rate of 48%; however, the difference was not significant (23).

These studies found a strong association between BV and the isolation of *G vaginalis*, anaerobic gram-negative rods belonging to the genera *Prevotellaceae*, *Veillonellaceae*, *Ureaplasmaurealyticum*, and often *Mobiluncus* spp. A lower concentration of facultative species of *Lactobacillus* among women with BV in comparison to women with normal flora was noted in this study. *Lactobacilli* are reported to play an important role in the maintenance of normal vaginal flora (15). Furthermore, differences in the vaginal microbial composition between ethnic groups may potentiate their predisposition to BV and infections during pregnancy due to differences in community resilience, which describes the ability of a given community to resist stresses and perturbations and return to a stable equilibrium (24).

Conclusion:

Sudanese female flora is dominated mainly by *Lactobacillus* species among normal and asymptomatic females, while *Gardnerella* sp. was dominant among females with BV. This is consistent with the global picture despite the differences in customs and traditions of Sudanese women, which was expected to affect the types of vaginal bacteria. Further studies and metagenomic sequencing are needed for better identification.

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Authors own resources

Conflict of interest

None

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