



AFRICA MEDICAL COLLEGE

PROCEEDINGS OF FIRST ANNUAL RESEARCH CONFERENCE

*“Evidence for Health–Related Sustainable
Development Goal”*

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PREFACE

Africa Medical College, as a privately owned organization, is mandated to promote and enhance research focusing on knowledge and technology transfer consistent with the country's priority needs. To discharge these duties, it has to undertake and encourage relevant study, research, and community services in national and local priority areas (Higher Education Proclamation No. 1152/2019, Articles 4.2 and 8.3).

Consequently, Africa Medical College organized and conducted a one-day Annual Research Conference on the theme "Evidence for Health-Related Sustainable Development Goal" on February 17, 2023. The Conference was aimed at encouraging relevant research works that contribute to improving research practices in national and local priority areas, disseminating findings of the research, and fostering awareness of academic and administrative staff about research and development activities on health and medical education.

Originally, the College had announced a Call for Papers inviting researchers from higher education and research institutions to contribute research works on three identified sub-themes- Health Promotion and Disease Prevention, Mental Health and Substance Abuse, and Non-Communicable Diseases. Despite the Conference Organizing Committee members' high hopes, most of the abstracts submitted before the deadline (December 23) focused on only the first subtheme. As a result, the Committee, having deliberated on the issue, agreed to push the deadline a little further to the end of December. Nevertheless, the extension

of the closing date did not bring any change in the type of articles submitted. Thus, the Committee decided to go ahead with what was received and select five articles using an Article Review Score Sheet developed by the Ethiopian Academy of Sciences. To thwart similar challenges from happening in the future, the Conference Organizing Committee and the Research and Community Service Office learned that it is extremely important to promote and create awareness on the themes of the Annual Research Conference among academic staff of the College and other higher education and research institutions way before the call for papers has been made.

Africa Medical College strongly believes the Conference has provided an effective forum for the College's academicians and researchers to advance their knowledge of research. The Keynote Speaker, who has a great deal of experience in higher education and quality assurance, in particular, was believed to have motivated and encouraged participants to engage in research and community services. He underlined research is one of the pillars of higher education institutions and the basis for academic staff promotion and, therefore, there is no way to go around it (the full keynote address has been presented here). Paper presenters also shared their views, insights, and research findings with the academic and administrative staff of the College.

Kassahun Kebede (PhD),
Research and Community Service Head

OPENING MESSAGE FROM DR. MEKONNEN BELAY, V/P AFRICA MEDICAL COLLEGE



- **Dear Dr. Tesfaye Teshome our Guest of Honor and Keynote Speaker, Dear colleagues, lecturers, researchers, ladies, and gentlemen**

On behalf of African Medical College, I would like to express my sincere gratitude and welcome you to this Annual National Conference to be held on the theme “Evidence for Health-related Sustainable Development Goal”.

Ladies and gentlemen,

Africa Medical College is a privately owned higher education institution, which gives due consideration to research and community services. In the current academic year, the College has designed and implemented research policy and guidelines. It has also set aside a budget to fund researches that may be undertaken by the academic and support staff. Some of the College’s academic staff have also undertaken many relevant studies and community services.

The College believes that more has to be done in the future to increase the number of staff who are currently engaged in research and community service activities. That is why this conference has been organized. The agenda of this Conference covers interesting topics related to theoretical and practical aspects of health and medical practices and encourages both experienced and novice researchers of the College to present their papers and share their insights and research findings with the internal and external research community. One of the presenters, for instance, is a student who received his degree from Africa Medical College in one of the programs a few years ago and still pursuing his education in another program in the current academic year. It is hoped, the methods of scientific analysis used by the presenters will help those who are and who will want to engage in research activities in the future. It is therefore my hope that this conference conducted on the theme “Evidence for Health-related Sustainable Development Goal” will achieve its objective of providing an effective forum for academicians, researchers, and health practitioners to advance knowledge.

I would like to end my speech by thanking and appreciating the President and owner of the College, Mr. Berhane W/Georgis, for his unreserved support for all planned activities. I want also to extend my thanks to the Research and Community Service Office and Research and Publication Committee who worked hard to make this conference a reality. I would also like to say thank you to the Guest of Honor and Keynote Speaker, Dr. Tesfaye Teshome, Senior

Vice President of Unity University who has come to the Conference despite his crowded work schedule.

Finally, I wish participants a very productive conference with exciting and encouraging discussions and exchange of knowledge.

Thank you.



KEYNOTE ADDRESS BY THE GUEST OF HONOR, DR. TESHFAYE TESHOME, SENIOR VICE PRESIDENT, UNITY UNIVERSITY



- **Honorable Ato Berhane W/Georgis, President of Africa Medical College, Honorable Dr. Mekonnen Belay, Vice President of Africa Medical College,**
- **Distinguished Guests, Participants, Ladies and Gentlemen,**

First and foremost, I would like to congratulate Africa Medical College (AMC) for organizing its 1st annual research conference. Organizing such a kind of forum creates a platform for academic and research staff to present their research findings timely and provides the gateway for publications.

As we all are well aware, any higher learning institution, such as AMC, has responsibilities that rest on the three pillars of higher education; namely, teaching/learning, research, and outreach/community services. Even though all are important for the survival as well as the proper functioning of HLIs, institutions that do not embrace research undertakings as one of their mandates will be challenged by the community as they are expected to identify community problems, undertake relevant and demand-oriented health, business, and all rounded social-economic in nature and address the same.

Academic staff members who engage in research undertakings will have more comparative advantages than those who do not as they plow back their research findings and handle the teaching and learning process in a practically oriented way to ensure the quality of teaching. This encourages the academic staff to pick up community problems and create a fertile institutional research environment so that they can positively respond and contribute to solving community problems. This has several advantages, which include, among others, building knowledge and facilitating efficient learning and understanding of various health, business, and environmental and socio-economic development gaps, suggesting ICT and technology, and recommending solutions.

Moreover, research on timely and important topics attracts attention, which, in turn, leads to greater AMC visibility and reputation. As a college becomes known for its research endeavors in selected thematic fields, it becomes a magnet towards which students, grants, the media, and even reputation get attracted.

It is with these understandings that I felt very much grateful when I received the invitation to

appear here as a keynote speaker and pass my key messages to conference participants.

Ladies and Gentlemen,

I found today's conference a good start and remarkable in the sense that the five research papers selected addressing issues are pertinent to community problems.

The current trend in research undertakings has taken a different route and showed a shift as it has taken years back. The reasons are simple to understand as research is becoming a more complex, collaborative activity. Not only that the current research undertakings are emphasizing the growing needs of the community (business, urban and rural) as well as the real-world application, societal impact, and commercial return of academic research. The issues worth considering in this regard are the issue of technology which has growing importance as a tool to enable more complex and collaborative research across disciplines. To this effect, I suggest that AMC invest in subscriptions of reputable scientific journals, establishing a modern library, availing data analysis software, and making research an in-built culture of the College.

I, therefore, recommend that AMC management, particularly the Research Office consider such new trends of research undertakings and identify research thematic areas that have greater impact and relevance.

Ladies and Gentlemen,

As I can see from the program, I am convinced that the speakers of this conference and participants will benefit immensely from the deliberation and will have a fruitful stay at AMC.

As far as the topics selected for presentation and the profiles of the speakers are concerned, I can say that the organizing committee of the conference has diligently worked hard to make the conference interesting and informative.

I would like to congratulate AMC for realizing such an event. I would also like to take this opportunity to forward my earnest appeal to the leadership as well as the community of AMC to keep up their effort in similar future endeavors.

Ladies and Gentlemen,

As has been stated earlier one of the three mandates of HEIs is research undertaking; AMC is organizing this research conference to serve as a springboard for an in-depth discussion and deliberation among scholars. This being an effort that deserves appreciation, the most important issue in this regard, in my view, is the use of the research findings for the intended objectives so that they contribute towards the creation of vibrant socio-economic development of the nation. In so doing HEIs respond to the heart-felt needs of society and prove to be the hub of knowledge creation and application.

I am quite confident that you will enjoy the deliberation and will also learn and contribute a lot. Such kind of engagements will be more fruitful when the proceeding of this conference is ready and circulated to the wider user community as soon as possible. I hope AMC will do the same.

With this brief remark, I declare the conference open.



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SEROPREVALENCE AND ASSOCIATED RISK FACTORS FOR HEPATITIS B VIRUS INFECTIONS AMONG APPARENTLY HEALTHY PREGNANT MOTHERS ATTENDING ANC IN RUBKONA PRIMARY HEALTH CARE CENTER IN RUBKONA COUNTY, UNITY STATE, SOUTH SUDAN

By Michael Pou Machar, MSc in MI, MPH



Abstract

Background Hepatitis B is an infection caused by the hepatitis B virus (HBV), which is enveloped DNA virus that infects the liver, and the common complications are; cirrhosis, liver cancer and rest of organs failure.

Methods: A cross sectional study was conducted among 234 pregnant women attending antenatal Clinic in Rubkona primary Health care center in Rubkona County, South Sudan from March 1 to July 29. Data were collected using pretested interviewer administered questionnaire. Blood was withdrawn from each study participants and used to detect hepatitis B surface Antigen using an enzyme linked immunosorbent assay test kit. Bivariate logistic regression was carried out to identify the predictors associated with HBV infection. All variables with p-value of ≤ 0.25 in bivariate logistic regression were taken into multivariable model. Variables having p value ≤ 0.05 in the multivariate analysis were taken as significant predictors.

Objectives: To assess the sero-prevalence Hepatitis B surface antigen and its associated factors among pregnant women attending Rubkona Primary Health Care Centre (South Sudan).

Result: The overall Seroprevalence of HBV infection was, 16 (6.8 %), 95% CI; 3.8-10.3). Having history jaundice AOR= 10.91: 95%CI (2.6-45.2)], abortion history, [AOR= 5.5: 95%CI (1.5-23.5)] and history multiple sexual partner [(AOR=9.5:95%CI (2.3-39.7)]. Were found to be associated factors of sero-prevalence of HBV infection.

Conclusion: Hepatitis B is identified to be a major health problem in this community. According to WHO classification, the prevalence of HBV infection in this study area can be categorized as moderate prevalence (2–7%). Abortion and history multiple sexual partners found to be the risk factor associated with HBV infection.

Keywords: Hepatitis B virus; Seroprevalence; Rubkona Primary Health Care Centre

Introduction

1.1 Background

Hepatitis B is an infection caused by the hepatitis B virus (HBV), which is enveloped DNA virus that infects the liver, and the

common complications are; cirrhosis and hepatocellular carcinoma (HCC) (1, 2). Its incubation period ranges from 45 days to 6 months' days. About 10% of children and 30-50% of adults with acute infection are clinically diagnosed, with anorexia, vague abdominal discomfort, nausea and vomiting, sometimes arthralgia and rash, often progressing to jaundice (1).

HBV has been found in almost in all body secretions and excretions. Though, it is found in all body secretions and excretions, the risk of transmission is only blood, body fluids containing noticeable blood, semen and vaginal secretions. Major modes of HBV transmission include sexual contact with infected person or close household contact with an infected person, perinatal mother to infant transmission, injecting drug used and nosocomial exposure (1-5).

Parenteral exposures that have contributed to HBV transmission include transfusion of unscreened blood or blood products, sharing unsterilized injection needles for IV drug use, haemodialysis, acupuncture, tattooing and injuries from contaminated sharp instruments sustained by hospital personnel (2, 3).

The major complications of chronic hepatitis B are cirrhosis and hepatocellular carcinoma (HCC). Between 20% and 30% of those who become chronically infected will develop these complications, and an estimated 650, 000 people will die annually due to CHB (3). The majorities of people are unaware of their HBV infection, and therefore often present with advanced disease (1, 3, and 5). Universal hepatitis B immunization programs that target infants, with the first dose at birth, have been highly effective in reducing the incidence and prevalence of hepatitis B in many endemic countries, however these programs will not have an impact on HBV-related deaths until several decades after their introduction (1- 4)

1.2 Statement of the problem

Infection with hepatitis B virus (HBV) is a major global public health problem with significant morbidity and mortality (1-4)

Globally, in 2015, an estimated 257 million people were living with chronic HBV predominantly in low- and middle-income countries. The leading region infected for HBV are African and Western Pacific regions accounted for 68% of those globally infected (4).

There is no specific treatment for acute hepatitis B, and only general supportive care is used in symptomatic cases. Chronic HBV infection can be treated with drugs used for treating HIV infection such as tenofovir or entecavir. These drugs do not completely eradicate the virus from the patient's blood but can slow the progression of chronic disease and improve long-term survival, however may require life-long treatment (2).

Worldwide, 21 countries accounted for more than 80% of the total number of HBsAg-positive infections in the general population. More than half of all HBsAg-positive infections are from China, India, Nigeria, Indonesia, and the Philippines (6). Only 16 countries accounted for more than 80% of the estimated number of infections in children aged 5 years with HBsAg (6). Nigeria, India, Indonesia, and the Democratic Republic of the Congo accounting for almost 57% of all infections (6)

Among the 36.7 million persons living with HIV in 2015, an estimated 2.7 million had chronic HBV infection (4). Liver diseases are a major cause of morbidity and mortality among those living with HIV and co-infected with viral hepatitis (7). These people should be diagnosed and provided with appropriate and effective treatment for both HIV and hepatitis as a priority (3, 4 and 7).

In sub-Saharan Africa, vertical transmission HBV increases co-infection with HIV (8). A co-infected pregnant woman is twice as likely to test positive for HBeAg, three times more likely to have detectable HBV DNA (9). And have higher HBV DNA serum concentrations than

those who are not co-infected by HIV, hence the increased HBV DNA serum concentrations greatly increased the risk of vertical transmission. (8, 9)

South Sudan is also one of the countries with high prevalence HBV carrier, which is about 6.3 % of the pregnant women are Hepatitis B virus carriers (10). Perinatal transmission is the major route of HBV transmission in many parts of the world. It is the main mode of transmission in high-prevalence in Africa. Perinatal infection increases the risk of developing chronic infection of an infant about 90% of the cases, therefore; the risk of progression to chronic infection decreases to 20–60% between the ages of 6 months to 5 years (11). The ideal time of preventing HBV infection is through immunization at birth, which offers over 95% protection against the development of chronic infection (12).

Like many other developing countries, South Sudan also includes hepatitis B vaccine at 6, 10 and 14 weeks after birth as national immunization program (10). Delay in 6 weeks for vaccination will decrease the efficacy of the vaccine in the prevention of vertical transmission (13).

1.3 Significance of the study

Viral hepatitis is responsible for the death of 1.34 million people in 2015 and this number is comparable to deaths caused by tuberculosis and higher than those caused by HIV. However, the number of deaths due to viral hepatitis is increasing over time, while mortality caused by tuberculosis and HIV is decreasing. The epidemic caused by HBV affects mostly the WHO African Region and the Western Pacific

Since Perinatal transmission is the major route of HBV transmission in many parts of the world. It is the main mode of transmission in high-prevalence in Africa, particularly in endemic areas where up to 20% of women of childbearing age may have HBV. Hence, prevention of prenatal transmission remains an

important target in the struggle for global eradication of HBV infection.

Therefore, the finding of this study will help Ministry of Health, and other stakeholders working on the health sector to develop strategies for promoting community awareness and improving HBV vaccine coverage, and other preventive strategies.

2. Literature review

2.1 Global HBsAg endemicity

Globally, in 2015, an estimated 257 million people were living with chronic HBV predominantly in low- and middle-income countries. The leading region infected for HBV are African and Western Pacific regions accounted for 68% of those globally infected. It was estimated that prevalence of HBV infection in the general population was 3.5%. Among those born before the hepatitis B vaccine became available, the proportion of persons living with chronic HBV infection remains high. Prevalence was the highest in the African (6.1%) and Western Pacific regions (6.2%). Assuming that women of reproductive age constitute 25.3% of the world's population (United Nations data), adults chronically infected may include 65 million women of childbearing age who can potentially transmit HBV to their babies (4). Prevalence of HBV varies from region to region. WHO categorized into three regions; high prevalence, moderate prevalence and low prevalence. The high prevalence ($\geq 8\%$) includes sub-Saharan Africa, South-East Asia, the Eastern Mediterranean countries, south and western Pacific islands, the interior of the Amazon basin and certain parts of the Caribbean. The moderate prevalence (2–7%) includes south-central and south-west Asia, eastern and southern Europe, the Russian Federation and most of central and South America. Low prevalence ($< 2\%$) region includes Australia, New Zealand, northern and western Europe, and North America (14).

The European Centre for Disease Prevention and Control, showed that highest risk population for HBV or have a high disease

burden in EU/EEA are dialysis/haemodialysis patients, people living with HIV (PLHIV), PLHIV with multiple risks such as Men who have sex with Men (MSM) living with HIV, People Who Injected Drugs (PWID) living with HIV, PLHIV in prison (15).

In Europe there is steady decrease of report in the acute cases of HBV and the likely reason for decreased report is the impact of vaccination campaigns (16). However, the total percentage of people infected with HBV varies between different countries with in Europe. For HBV, the prevalence in the general population ranged from 0.1% in Ireland to 4.4% in Romania. Highest prevalence of HBV have been reported in Greece and Romania, 3.3% and 4.4% respectively, while the vast majority of other European countries have HBV prevalence around or below 1% (17).

European Centre for Disease Prevention and Control, in 2013, reported that data on transmission of hepatitis B were complete for only 21.3% of cases. Among cases with complete information, heterosexual transmission (30.5%), nosocomial transmission (18.9%), injecting drug use (13.2%) and transmission among men who have sex with men (9.4%) were most commonly reported for acute infections. Perinatal transmission was the most common route (43.5%) for chronic cases (18).

2.2 Prevalence of hepatitis B

Here are some of the literatures on the sero-prevalence of HBsAg in different countries especially African countries and some other part of the world was summarized in the following as follows:

The finding of the studies conducted in different part of Africa showed that the prevalence of HBsAg range from 0.8% to 11.8%. For instance, the study conducted in South Sudan, Juba [6.3%] (10), Democratic Republic of the Congo in Lubumbashi [6.69%] (19), South Africa[0.8%](20), Ethiopia, Bishoftu [5.4%] (21), Ethiopia, Hawassa [7.8%](22), Eritrea, Asmara [3.2 %](23), Cameroon , in the rural milieu

[10.2%] (24), Sudan ,Khartoum [7.5%] (25), Uganda, Kampala [11.8%](26), Kenya , Nairobi and 8 other regions [9.3%](27), South Sudan , Juba [11%](28). Ethiopia, Gambella 7.9% (32), Ethiopia Harrar City[6.3%] (33), which indicates intermediate endemicity. The finding of similar study conducted in Brazil, São Luís indicated that the prevalence of HBsAg was 7.4% (29).

On the contrary, the prevalence of HBsAg western countries is low, that is < 2%. Here are some of the countries with low prevalence of HBsAg. Namely a study conducted across four hospitals in London over 2 years (2009-2010) attending ANC showed that only 1.05% were positive for HBsAg.(31) , Italy ,in the region of Apulia the prevalence of HBsAg were 0.5%, in Ireland, the prevalence in the general population were 0.1%(17).

2.3 Associated risk factor for HBsAg among pregnant woman

In most African countries most pregnant woman were not aware of their serologic status with HBV. The finding of research conducted on seroprevalence and risk factors for hepatitis B infection in pregnant women in Lubumbashi, Democratic Republic of the Congo showed that all pregnant women were previously unaware of their serologic status with HBV. In this study the highest prevalence of hepatitis B was observed in the age group 31-40(10.53%), though it was not statistically significant. However, HBV was significantly higher in HIV-positive pregnant women, who presented a risk of nearly 9 times higher compared to HIV-negative pregnant women (19).

A retrospective study conducted at the antenatal clinic of one Military Hospital, Tshwane, South Africa for the period January 2008-December 2013. A total of 2,368 patients were enrolled as both their HBV and HIV serology results were available. The finding revealed that an overall HBV prevalence was 0.8%. However hepatitis B surface antigen (HBsAg) prevalence was significantly higher (2.1%) among HIV co-infected compared with HIV-uninfected patients (0.4%) (20).

A study conducted in Ethiopia, on Hepatitis B virus infection and risk factors among pregnant women public hospital, revealed that none of the study participants were aware of their HBV sero-status. Risk factors such as history of abortion, surgery and family history for hepatitis were significantly associated with Sero-positivity of HBsAg (21).

The finding of the study conducted in the Southern Ethiopia on prevalence and associated risk factor of HBV infection among pregnant woman in 2015, showed that educational status of the study participant has a significant association with HBV infection, in which those with no formal education were more likely to be infected than those who had completed secondary school. Despite HBsAg was detected more often in pregnant women with multiple exposure factors (8.8%) than in pregnant women who had not experienced possible risk factors (4%), this difference was not statistically significant (22).

The finding of another study conducted in Eritrea, showed that the overall HBsAg prevalence among pregnant women was 3.2%. In this research, the researchers tried to see whether there were significant association with socio-demographic characteristics and HBV infection. No significant association observed in relation to marital status, occupation, spouse occupation or religion. However, a significant relationship between the rate of HBV infection and the level of education were observed. It showed that illiterate were 2 times prone to HBV infection compared to those who had attained secondary or higher education (23).

The finding of a study conducted in rural district of the Far North Region of Cameroon revealed that Only 4 women (1.2%) had been vaccinated against HBV. Thirty-three women (10.2%) were HBsAg-positive. Five (1.5%) women were co-infected with HIV and HBV. This research identified risk factors such as concurrent infection with HIV and blood transfusion is highly associated with HBV infection (24)

A study conducted in Sudan, neighboring country, among a pregnant woman attending Khartoum teaching hospital showed that prevalence of HBV infection was 7.5 %. This study indicated that there were significant relationship between some risk factors and HBV infection. These are history of surgery with positive HBsAg, history of jaundice with positive HBsAg and histories of jaundice in their husbands, and with positive HBsAg. Others risk factors such as parity and age group have no significant association (25)

The finding of the study conducted in Uganda revealed that history of scarification, number of sexual partners, history of blood transfusion or polygamy had no statistically significant relationship with HBsAg positivity, however women 20 years of age or younger were 2.5-fold more likely to test positive for HBsAg than those aged above 20 years (26). On the contrary the finding of the study conducted in Kenya showed that scarification's, blood transfusion, alcohol and STD were statistically significant association with HBV transmission (27)

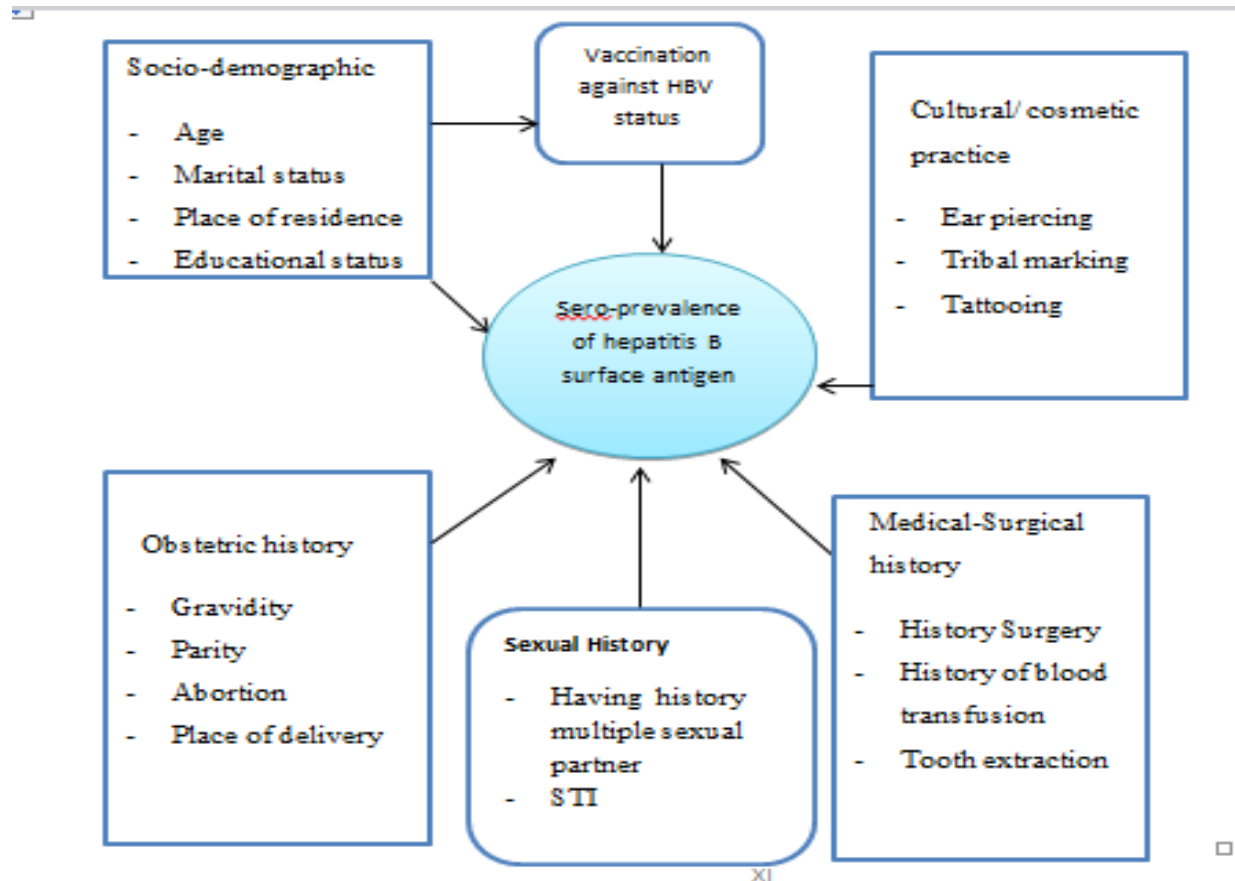
Very Few studies have been conducted in South Sudan on prevalence and associated factors of HBV infection among pregnant woman. Among these studies (10, 28), a study conducted by Akway M. Cham et.al revealed that, age range between 15-34 years has shown possible link to HBV infection than other ranges. However surgical procedure, blood transfusion, cultural background and marital status did not show any significant association with HBV infection (10). Similarly, another study conducted in Juba teaching hospital revealed loss of marital partner and history of jaundice was statistically significant. Other risk factors such as occupation, education, multiple sexual partners, blood transfusion and history of surgery were not significantly associated with positive HBsAg (28).

A study conducted in Gambella, south western Ethiopia, History of abortion occupation and

Multiple sexual partners had statistical significant association with HBsAg seropositivity. (32). Another study conducted in Harrar city, Ethiopia, revealed that blood transfusion history of surgical procedure history of sexually transmitted infection and history of tooth extraction were independent predictors of HBV infection(33).

This is a set of interactions between socio-demographic factors and risk factors such as cultural practice, obstetric history and medical surgical history which contribute for increased seroprevalence of HBsAg. This conceptual frame work was adopted from different literature and modified accordingly.

2.4 Conceptual framework



3. Objectives

3.1 General objective

To assess the seroprevalence Hepatitis B surface antigen and its associated factors among pregnant women attending Rubkona Primary Health Care Centre (South Sudan).

3.2 Specific objectives

1. To determine the prevalence of Hepatitis B surface antigen among pregnant women attending Rubkona primary health care Centre.

2. To identify factors associated with Hepatitis B surface antigen among pregnant women attending Rubkona primary health care Centre.

4. Methods and Materials

4.1 Study Area and Period

Rubkona is the most important county in Unity State, since it had been the capital of a comparatively larger state; it remained the center for trade, communication and program intervention. This county has the largest internally displaced persons' camp in the

country, accommodating 120,000 people. It borders Panrieng County to the north, Koch County to the south, Mayom County to the west and Guit County to the south-east. The Bhar El Arab River, which is part of the Nile River system, flows through it from east to west towards Mayom County. This river is partly navigable. The county is composed of floodplain, and becomes inundated or inaccessible during the rainy season. It has one health facility which serves the entire population, called Rubkona Primary Health Care Center (PHCC) (30). Study period was from December 1 to July 29, 2020.

4.2 Study design

A cross sectional study was conducted among pregnant women attending antenatal Clinic in Rubkona primary Health care center in Rubkona County, South Sudan.

4.3 Population

4.3.1 Source of population

All pregnant women who are living in Rubkona County were the source of population.

4.3.2 Study Population

All Pregnant women who are attending antenatal care at Rubkona Primary Health Care Center during the study period were the study Population.

4.4 Eligibility

4.4.1 Inclusion Criteria

All Pregnant women who are attending antenatal clinic in Rubkona Primary Health Care Center during the study period were included.

4.4.2 Exclusion Criteria

Those women who are unable to communicate or seriously ill at the time of data collection

4.5 Sample Size determination

The single population proportion formula was used to determine the sample size by considering previous prevalence in Juba which was 6.3% (10). Since the prevalence of the

previous study is 6.3% which was less than 10% the recommended d (tolerable margin of error) should be half of it. That is 3.15% level of significance / margin of error. If the prevalence of the previous study is below 10% and above 90% we should take half of the Prevalence to be d (margin of error)

The following assumptions were made during sample size calculation.

Z = Standard deviation of the normal distribution = 1.96 (confidence level at 95%)

P = prevalence 6.3 % (prevalence of HBsAg in serological survey in Juba [24])

d = Tolerable error / level of significance = 3.15%.

X = 5 % non-response rate

N = Minimum sample size

Sample size = n (Minimum sample size) + X (non-respondent)

$$n = \frac{Z^2 P (1-P)}{d^2}$$

$$d^2$$

$$n = \frac{1.96^2 \cdot 0.063(1-0.063)}{0.0315^2}$$

$$0.0315^2$$

$$n = \frac{3.8416 \times 0.063(0.937)}{0.00099}$$

$$0.00099$$

$$N = n + x$$

$$N = 229 + 5\% \text{ Non-response rate}$$

$$N = 229 + 11.45$$

$$N = 241$$

$$\text{Sample size (N)} = 241$$

4.6 Sampling technique

Systematic random sampling method was used to recruit study participants. The sampling frame will include all pregnant women attending ANC Rubkona PHCC. Then, sampling interval (K unit) was calculated based on ANC records of the last month. After calculating K unit, a

random starting point was then selected by lottery method. Finally, select every K^{th} unit after that first number.

4.7 Study variables

4.7.1 Dependent variable

Hepatitis B Surface Antigen Prevalence

4.7.2 Independent variables

Socio demographic characteristics

- Age
- Marital status
- Place of residence
- Educational status

Obstetric history

- Gravidity
- Parity
- Abortion
- Place of delivery

Cultural/cosmetic practice

- Ear piercing
- Ethnic scar
- Tattooing

Medical-Surgical history

- History Surgery
- History of blood transfusion
- Tooth extraction

Sexual history

- Having history multiple sexual partner
- STI

Vaccination

- Vaccination against HBV status

4.8 Measurement and data collection

4.8.1 Data collection

The data for the study were derived from serological testing and questionnaires. Both the questionnaire and blood test were done after

having received a clear explanation of the objective of the study and written consent from the participants. Socio demographic and pertinent data on risk of acquiring HBsAg were collected using a standard structured questionnaire by health professional.

4.8.2 Specimen collection and Processing

After obtaining written consent, 5 ml of venous blood was collected in plane tubes under aseptic conditions from peripheral vein by experienced laboratory personnel from all consenting pregnant women. Proper handling and labeling of the specimen will follow.

4.9 Quality Assurance

Trained data collector was collect Information on socio-demographic and other pertinent data using structured questionnaire. This questionnaire was prepared first in English and translated to Arabic language, and was finally back translated to English by linguistic professionals in order to ensure its consistency. Pre-testing of 5% the questionnaire was done prior to the study. The questions were standardized during the pre-test to ensure they provided desired answers. Data completeness and consistence were checked daily by principal investigator while in the field. All the data were double entered to ensure the data quality.

Quality control of serological test: Known positive and negative controls were run in parallel with test samples. All laboratory procedures were carried out following standard operating procedures (SOPs). The quality assurances of pre-analytical, analytical and post-analytical stages were applied.

Pre-analytical stage

First the specimens were collected by trained lab technician from women and labeled by the patient unique identification number. Then samples were centrifuged; the serum was evaluated and separated; appropriately and stored until transported to the laboratory. The transported samples were stored at the optimum temperature until they were processed.

Analytical stage

The blood test for HBsAg was performed by trained laboratory technologist. All samples were tested, using Enzyme Linked Immunosorbent Assay (ELISA) for HBsAg. The standard laboratory procedures were also followed and the results were checked by the supervisors.

Post-analytical stage

The results were recorded with the patients' unique identification number; the results were reported to the principal investigator.

4.10 Data Processing and Analysis

After the data collection, each questionnaire was checked for its completeness. Data entry, cleaning and coding done by Epi-Info version 3.5.4 statistical software package and was exported to SPSS window version 25 for analysis. Bivariate logistic regression was carried out to identify the predictors associated with HBV infection. All variables with p-value of ≤ 0.25 in bivariate logistic regression were taken into multivariable model. Variables having p value ≤ 0.05 in the multivariate analysis were taken as significant predictors. Crude odd ratios (COR) and adjusted odds ratios (AOR) with their 95% confidence intervals (CI) were calculated. Tables, cross tabulations were used to present the data.

4.11 Ethical considerations

Ethical clearance was obtained from the Research and Publication Committee of rift valley University Abichu campus postgraduate studies coordinating office. Permission was obtained from Rubkona Primary Health Center. The respondent's written consent, privacy and confidentiality was the primary concern of the study. Written consent was obtained from each participant. Confidentiality of the information was kept anonymously.

5. Result

5.1 Socio-demographic characteristics

In this study a total of 234/241 pregnant women attending Antenatal care were participated with a response rate of 97.09 %. The mean (\pm SD) age of the participants was 25.5 (\pm 5.64) years. The majority 101 (43.2%) of the participants were found in 21-25 age groups, followed by 26-30; 50(21.4%), and age range between 17-43 years.

Majority of the respondent were primary school, 94 (40.2%), followed by can't read and write, 89(38%). Regarding occupational status of respondents, 132 (56.4%) of them were House wife at the time of data collection, followed by private employed 50 (21.4 %). Pertaining to the marital status of the participants, 232(99.1%) were married.

Table 1- Socio-demographic characteristics of pregnant women who attend antenatal care at Rubkona Primary Health Care Center, South Sudan, N=234

Socio-demographic characteristics		Number	Percent
Age	Less than 21	43	18.4
	21 -25 years	101	43.2
	26 -30 years	50	21.4
	31 -35 years	26	11.1
	36 -40 years	11	5.7
	41 and above	3	1.3
Total		234	100.0
Occupation	House wife	132	56.4
	Private employed	50	21.4
	Government employed	13	5.6
	Daily labourer	39	16.7
Total		234	100.0

Socio-demographic characteristics		Number	Percent
Education	Can't read and write	89	38
	Primary education	94	40.2
	Secondary education	42	18
	Diploma level and above	9	3.8
	Total	234	100.0
Marital status	Married	232	99.1
	Widowed	2	0.9
	Total	234	100.0

5.2 Sero-prevalence of HBV infection

The overall sero-prevalence of HBV infection was, 16 (6.8 %), 95% CI; 3.8-10.3). Out Of these, 7(14%) were belong to age group 26- 30 years. Pertaining to educational status versus positivity 9 (10.2 %) of respondents can't read and write followed by primary education, 6(6.3%), and 1(2.3%) of respondents with secondary education were positive for HBsAg.

Based on their occupation, 10 (7.5%) of pregnant Women who were house wives and 3(6%) of those who were private employee were positive for HBsAg. Other risk factors such as abortion, history of jaundice and multiple sexual partner were 6(25%), 6(30%) and 10(4.67%) were positive for HBsAg respectively.

Table 2- prevalence of HBV infection among pregnant women who attend antenatal care at Rubkona Primary Health Care Center, South Sudan, 2020 N=234

Variables		HBsAg status Positive number
Age	Less than 21	1(2.32%)
	21 -25 years	4(3.96%)
	26 -30 years	7(14.0%)
	31 -35 years	3(11.53%)
	36 -40 years	1(9.09%)
	41 and above	0(%)
Occupation	House wife	10 (7.5%)
	Private employed	3(6%)
	Government employed	1 (7.69%)
	Daily labourer	2 (5.12%)
Educational status	Can't read and write	9(10.2%)
	Primary education	6(6.3%)
	Secondary education	1(2.3%)
	Diploma level and above	0(0%)
Gravidity	Primigravida	2(3.70%)
	Multigravida	14(7.77%)
Abortion	Yes	6(25%)
	No	10(4.76%)
History of Jaundice	Yes	6(30%)
	No	10(4.67%)
Multiple sexual partner	Yes	5(22.72%)
	No	11(5.18%)
History of sharp injury	Yes	10(10.75%)
	No	6(4.25%)
History of blood transfusion	Yes	2(22.22%)
	No	14(6.22%)
History of surgery	Yes	2(18.18%)
	No	14(6.27%)

5.3 Factors associated with Hepatitis B virus infection

Bivariate and multivariate logistic regressions were done to assess the predictors of Hepatitis

infection. All variables in the bivariate analysis at p -value < 0.2 were entered to the multivariate analysis. Among the variables that were included in the multivariate analysis model abortion, History of jaundice and multiple sexual partners were significantly associated with HBsAg sero-status

Pregnant women who had history jaundice (Yellowing of the skin and the whites of the eyes) were almost eleven times more likely to have sero-positive for HBV infection compare to those with no history of jaundice [AOR=

10.91: 95%CI (2.6-45.2)]. Pertaining abortion history, pregnant women who had history of abortion were five times more likely to have sero-positive for HBV infection as their counterpart [AOR= 5.5: 95%CI (1.5-23.5)]. Similarly the odds of being sero-positive for HBV infection among pregnant women who had history multiple sexual partner, were nine times greater than those pregnant women who had single partner [(AOR=9.5:95%CI(2.3-39.7)].

Table 3- Factors associated with Hepatitis B virus infection among pregnant women who attend antenatal care at Rubkona Primary Health Care Center, South Sudan, 2020 N=234

S N	Variables		HBsAg test result		COR	AOR	P-value
			Positive	Negative			
1	History of sharp injury /cut	Yes	10(10.75%)	83(89.25%)	2.7(0.9-7.7)	3.2(0.97-10.83)	0.056
		No	6(4.25%)	135(95.75%)	1	1	
2	History of Jaundice	Yes	6(30%)	14(70%)	8.74(2.7-27.5)	10.91(2.6-45.2)	0.001**
		No	10(4.67%)	204(95.33%)	1	1	
3	History of abortion	Yes	6(25%)	18(75%)	6.7(2.2-20.4)	5.5(1.5-23.5)	0.012**
		No	10(4.76%)	200(95.24%)	1	1	
4	History of surgery	Yes	2(18.18%)	9(81.82%)	3.32 (0.6-16.8)	0.9(0.05 21.3)	0.99
		No	14(6.27%)	209(93.73%)	1	1	
5	History of multiple sexual partner	Yes	5(22.72%)	17(77.28%)	5.3(1.67-17.27)	9.5(2.3-39.7)	0.002**
		No	11(5.18%)	201(94.82%)	1	1	
6	History of blood transfusion	Yes	2(22.22%)	7(77.78%)	4.31(0.8-22.7)	2.5(0.1-51.4)	0.56
		No	14(6.22%)	211(93.78%)	1	1	

Table at p - value < 0.05

6. Discussion

The finding of this study showed that the overall Seroprevalence of HBV infection among pregnant women who attend antenatal care at Rubkona County, Primary Health Care Center, was 16 (6.8 %). According to W.H.O classification, the prevalence of HBV infection in this study area can be categorized as moderate prevalence (2–7%) (14).The overall prevalence of this study was similar with the finding of study conducted in South Sudan, Juba [6.3%] (10),Democratic Republic of the Congo in Lubumbashi [6.69%] (19) and Ethiopia, Harrar City[6.3%] (33),

In contrast, this finding is higher than that reports from Ethiopia, Bishoftu [5.4%] (21), Eritrea, Asmara [3.2 %] (23), and South Africa [0.8%] (20). However the finding of this study is lower than that finding reported in Ethiopia (Hawassa) [7.8%] (22), Uganda, Kampala [11.8%] (26), Cameroon in the rural milieu [10.2%] (24), Sudan (Khartoum) [7.5%] (25), Kenya (Nairobi) and 8 other regions [9.3%](27), South Sudan (Juba) [11%](28). Ethiopia (Gambella)[7.9%] (32).The likely reason for such discrepancy might be due to the differences in study design, sample size difference, socio-cultural environment, traditional practices, sexual practices, and period of study difference and supportive health policy in prevention.

In this study, it revealed that history of multiple sexual partners, history of jaundice, and history of abortion are highly associated with Seroprevalence of HBV infection, however; social demographic factors and rest of associated factors are not significant to this study. The odds of having HBV among those who had multiple partners was greater than those pregnant women who had single partner.

This finding is in line with the finding which were studied in (Asmara), Eritrea, (Juba) South Sudan, (Gambella), South western of Ethiopia, (Harrar) Ethiopia (23,28,32 and 33), in which age, occupation, marital status have no significant.

This finding is also congruent or corresponding with the study conducted in (Bishoftu General Hospital), Ethiopia, (Gambella), South western Ethiopia (21, 32), in term of abortion. On the other hands, a study conducted in Uganda (10) and in South Sudan (10, 28) revealed that, number of sexual partners, or polygamy had no statistically significant.

The likely explanation for higher odds in abortion, is when a woman has experienced abortion in the rural area, might have higher chance of having unsafe abortion which might predispose to HBV infection. However, a research conducted by Anthony Laku Stephen Kirbak et al., contradicted this finding. The likely reason for the discrepancy might be the study area, where Juba is a capital city whereas Rubkona is a rural area. Hence education, peace stability and ANC coverage in Juba is better than Rubkona.

Among the risk factors that were identified to have relationship with prevalence of HBV, history of jaundice is also found to be statistically significant with HBV positivity. This finding agrees with a study in neighboring country (Khartoum), Sudan, and (Juba) South Sudan (25, 28).

Unlike the current finding study, an others studies were conducted in Southern Ethiopia, Eritrea, South Sudan which had had supported the educational associated risk factor on HBV infection among pregnant woman (.22, 23, 28)

respectively. The difference could be due to: in this study, majority are primary and less than but in those studies majority of, were high school and colleges and above than my study.

7. Limitations of the study

The study was conducted in antenatal clinic (institution based) and therefore results of the research may not be representative of the entire pregnant women in the community of Rubkona County.

8. Conclusion and recommendation

8.1 Conclusion

Hepatitis B is identified to be a major health problem in this community. According to W.H.O classification, the prevalence of HBV infection in this study area can be categorized as moderate prevalence (2–7%). In this study, Abortion, history of multiple sexual partners and jaundice are found to be the risk factors associated with HBV infection. The associated risk factors prevalence of hepatitis B virus were found to be highly between ages of 26-30 7(14.0%) years old, and most of them were house wives 10 (7.5%)

8.2 Recommendation

Recommendation

Based on the findings of this study the following recommendations shall be forwarded to Ministry of Health, and other stakeholders working on the health sector to develop strategies of:

- Vaccination of all neonates against hepatitis B virus at birth if possible will reduce the virus.
- Increasing the screening on both female and male will let the declination of the virus.
- Increase awareness on screening, vaccination must be introduced through health education during antenatal care follow up by the concerned bodies
- Since this study is the first in its type the study area, further study should be

done to identify the risk factors related to the HBV

to increase their awareness towards hepatitis B infection

- Extensive health education campaign should be provided to general population and especially to pregnant

9 Declaration

The information provided about above work done are true in my best knowledge.

REFERENCE

1. American Public Health Association. Control of communicable diseases manual. 18th ed. Washington 2005
2. Fact sheet – Hepatitis B in the WHO European Region, WHO 2017.
3. Guidelines for the prevention, care and treatment of persons with chronic hepatitis b infection march 2015 available at website (www.who.int/about/licensing/copyright_form/en/index.html)
4. Global Hepatitis Report 2017. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.
5. Abdi F, GhaffariNovin M, Afrakhteh M, Khorvash F. Hepatitis B and pregnancy: An update review article. World J ObstetGynecol 2015; 4(1): 1-8 Available from: URL: <http://www.wjgnet.com/2218-6220/full/v4/i1/1.htm> DOI: <http://dx.doi.org/10.5317/wjog.v4.i1.1>
6. Polaris Observatory C. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modeling study. Lancet Gastroenterol Hepatol. 2018;26:26. PMID: 29599078. [https://dx.doi.org/10.1016/S2468-1253\(18\)30056-6](https://dx.doi.org/10.1016/S2468-1253(18)30056-6)
7. Matthews PC, Geretti AM, Goulder PJ, Klenerman P. Epidemiology and impact of HIV coinfection with hepatitis B and hepatitis C viruses in sub-Saharan Africa. J Clin Virol 2014; 61: 20–33.).
8. Kourtis AP, Bulterys M, Hu DJ, Jamieson DJ. HIV–HBVco-infection—a global challenge. N Engl J Med 2012; 366: 1749–52.
9. Hoffmann CJ, Thio CL. Clinical implications of HIV and hepatitis Bco-infection in Asia and Africa. Lancet Infect Dis 2007; 7: 402–09.
10. Akway M. Cham, , Sekwat Saviour Clever, Rozeta Julio Celestino, Remo Charles Cornelius Eida Clement Thomas The Burden Of Hepatitis B Among Pregnant Women Attending Antenatal Care (Anc) Services In Juba Teaching Hospital (Jth)-Juba South Sudan , IJRDO- Journal of Health Sciences and Nursing Volume-2 | Issue-12 | December,2017
11. Beasley RP, Hwang LY, Lee GC, Lan CC, Roan CH, Huang FY, et al. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet. 1983;2(8359):1099–102.
12. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepatol. 2004;11:97-107.
13. Anthony Laku Stephen Kirbak et al. Sero-prevalence for Hepatitis B virus among pregnant women attending antenatal clinic in Juba Teaching Hospital, Republic of South Sudan, Pan African Medical Journal. 2017; 26:72 doi:10.11604/pamj.2017.26.72.11410, available online at: <http://www.panafrican-med-journal.com/content/article/26/72/full/>

14. World Health Organization. Hepatitis B vaccines. WHO position paper. Weekly Epidemiological Record [Internet]. 2004; 79(28):[253-64 pp.]. Available from: http://www.who.int/immunization_delivery/adc/hepb_wer.pdf.
15. European Centre for Disease Prevention and Control. Hepatitis B and C epidemiology in selected population groups in the EU/EEA. Stockholm: ECDC; 2018.
16. European Centre for Disease Prevention and Control. Hepatitis B surveillance in Europe – 2013. Stockholm: ECDC; 2015. S
17. European Centre for Disease Prevention and Control. Systematic review on hepatitis B and C prevalence in the EU/EEA. Stockholm: ECDC; 2016.
18. European Centre for Disease Prevention and Control. Hepatitis B surveillance in Europe – 2013. Stockholm: ECDC; 2015.
19. Tshanda NM, KitengeFwM, Kakoma JB. Preliminary study of seroprevalence and risk factors for hepatitis B infection in pregnant women in Lubumbashi, Democratic Republic of the Congo. *Afr J Health Issues*. 2018 Mar 27; 2(1): 9. DOI: <http://dx.doi.org/10.26875/ajhi212018ix>
20. Diale, Q., Pattinson, R., Chokoe, R., Masenyetse, L. and Mayaphi, S. "Antenatal screening for hepatitis B virus in HIV-infected and uninfected pregnant women in the Tshwane district of South Africa." *S Afr Med J* 2015 106(1): 97-100.
21. Zelalem Desalegn et al. Survey of Hepatitis B virus infection and risk factors among pregnant women at public hospital in Ethiopia *International Journal of Biomedical Research* 2016; 7(7): 450-456.
22. Metaferia Y et al.: Seroprevalence and associated risk factors of hepatitis B virus among pregnant women in southern Ethiopia: a hospital-based cross-sectional study. *Epidemiology and Health* Volume: 38, Article ID: e2016027, 7 pages <http://dx.doi.org/10.4178/epih.e2016027>
23. Fessehaye N, Berhane A, Ahmed H, Mohamed S, Tecle F et al. (2018) Prevalence of Hepatitis B Virus Infection and Associated Seromarkers among Pregnant Women in Eritrea. *J Hum VirolRetrovirol* 6(1): 00191. DOI: 10.15406/jhvr.2018.06.00191
24. Noubiap, J. J., Nansseu, J. R., Ndoula, S. T., Bigna, J. J., Jingi, A. M. and Fokom-Domgue, J. "Prevalence, infectivity 31 and correlates of hepatitis B virus infection among pregnant women in a rural district of the Far North Region of Cameroon." *BMC Public Health* 2015 15: 454.
25. Mohammed Hammad A., Mohammed B., Koko B. Prevalence of Hepatitis B Infection among Pregnant Women at Khartoum Teaching Hospital, Sudan. *Journal of US-China Medical Science* 12 (2015) 58-63 doi: 10.17265/1548-6648/2015.02.003
26. Bayo P, Ochola E, Oleo C, et al. High prevalence of hepatitis B virus infection among pregnant women attending antenatalcare: a cross-sectional study in two hospitals in northern Uganda. *BMJ Open* 2014;4: e005889. doi:10.1136/ bmjopen-2014-
27. Gatheru, Z., Murila, F., Mbuthia, J., Okoth, F., Kanyingi, F., Mugo, F., Esamai, F., Alavi, Z., Otieno, J., Kiambati, H., Wanjuki, N. and Obimbo, M.M. (2018) Factors Associated with Hepatitis B Surface Antigen Seroprevalence amongst Pregnant Women in Kenya. *Open Journal of Obstetrics and Gynecology*, 8, 456-467.
28. Anthony Laku Stephen Kirbak et al. Sero-prevalence for Hepatitis B virus among pregnant women attending antenatal clinic in Juba Teaching Hospital, Republic of South Sudan, Pan

African Medical Journal. 2017; 26:72 doi:10.11604/pamj.2017.26.72.11410, available online at: <http://www.panafrican-med-journal.com/content/article/26/72/full/>

29. Marinilde T S, Tainá L R Max DC, et al Prevalence of hepatitis B among pregnant women assisted at the public maternity hospitals of São Luís, Maranhão, Brazil, The Brazilian journal of infectious diseases: an official publication of the Brazilian Society of Infectious Diseases • November 2012
30. Polio global eradication initiative, published by UNICEF South Sudan, May 2018
31. Godbole, G., Irish, D., Basarab, M., Mahungu, T., Fox-Lewis, A., Thorne, C., Jacobs, M., Dusheiko, G., Rosenberg, W.M., Suri, D., et al. "Management of hepatitis B in pregnant women and infants: a multicentre audit from four London hospitals." BMC Pregnancy Childbirth 2013 13: 222.
32. Abayneh T , Misanew T, Desta H, Chaltu F and Gemechu J. Sero-prevalence of hepatitis B virus and associated factors among pregnant women in Gambella hospital, South Western Ethiopia: facility based cross-sectional study. BMC infectious disease, July 2019
33. Tiruye G, Shiferaw K, Tadesse F (2018) Seroprevalence of Hepatitis B Virus Infection and Associated Factors among Pregnant Women Attended Antenatal Care Services in Harar City, Eastern Ethiopia. J Women's Health Care 7: 436. doi:10.4172/2167-0420.100043

EFFECT OF MENSTRUAL IRREGULARITY ON ACADEMIC PERFORMANCE OF UNDERGRADUATE STUDENTS OF DEBRE BERHAN UNIVERSITY: A COMPARATIVE CROSS SECTIONAL STUDY

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Abstract

Background: Menstrual period is a critical time in the life of females. For the first few years after menarche, irregular cycle is common due to premature hypothalamic-pituitary ovarian (HPO) axis physiologically. If persistent, it becomes a major problem in student's life. But few studies conducted on effect of menstrual cycle irregularity on academic performance among university students were descriptive. However assessing the effect of menstrual irregularity on academic performance by using average grade point approach is critically important to magnify its effect.

Objective: To compare effects of menstrual irregularity on academic performance among undergraduate students of Debre Berhan University, Ethiopia 2020.

Methods: A comparative cross sectional study was conducted among 404 students in Debre Berhan University, Ethiopia, 2020. A standard tool of menstrual cycle regularity which is prepared by international federation of gynecologist and obstetrics (IFGO) was used to screen students who have menstrual irregularity. Independent sample t- test was done to compare the mean difference of academic performance between the two groups of students.

Results: The age of study participants ranges between 18 and 26 years with a mean age of 20.69 ± 1.43 years. The mean age at menarche was 14.9 ± 1.67 ranging from 9 to 18 years. Students who had menstrual irregularity had mean average grade point (AGP) of (2.78 ± 0.57) and students who had regular menstruation had mean AGP of (2.97 ± 0.53) . Students who had menstrual irregularity had lower mean average grade point by a mean difference of 0.19 (95%CI: 0.09-0.30).

Conclusion: This study found that students who had menstrual irregularity had significantly lower mean AGP as compared with students who had regular menstruation.

Key Words: Menstruation; Academic performance, menstrual irregularity

Introduction

Regular menstrual cycle (counting from the first day of one menstrual period to the first day of the next cycle) is 21 to 35 days and lasts from 3 to 7 days duration with volume of blood loss 5-80 ml (1). Menstrual irregularity refer to any kind of changes occurring in regularity of onset, frequency of onset, duration of flow and volume from regular menstrual cycle (2-4).

Menstrual period has a notable effect on the academic performance of female students (5). It influences social life, diet, exercise, amount of sleep, sleep quality, study time, concentration, group activities, preparation and performance on exam and attendance (6, 7). Different studies showed that academic performance of women varies during their menstrual cycle, in a way that the mental status is decreased during and several days before the period (8, 9). In addition, another study conducted in Saudi Arabia revealed that there was an increase rate of absenteeism and loss of concentration in academic work which might have impact on school performances as well as the achievement of their life goals (10). Study conducted in Mansoura University revealed that more than two third of nursing students had sleeping desire during lectures (11).

The majority of researches regarding effect of menstrual irregularity have been focused on daily activities and descriptive. However assessing the effect of menstrual irregularity on academic performance by using average grade point approach is critically important. Therefore, the aim of this study was to compare the effect of menstrual irregularity on academic performance by using average grade result of the last semester among Debre Berhan university students.

Methods

Study Design, settings and participants

Institutional based comparative cross sectional study design was conducted in Debre Berhan University from February 11 to March 10 /2020. Debre Berhan is located at 130 Kms from Addis

Ababa (capital city of Ethiopia) and at 695 kms from Bahir Dar (the capital city of Amhara Region). There were about 5387 female students during the study period. Undergraduate regular students who were pregnant, who were within one year after delivery and lactating, who had treatment history for menstrual irregularity during the year of study and who were critically ill during data collection period were excluded from the study.

Sample size and sampling procedures

The sample size was calculated using a formula for estimation of double population proportion with the assumption of small to medium effect sizes (Cohen's $d = 0.05$), 95 % confidence interval and a power of 80%. 202 students who have irregular menstrual cycle and 202 students who had regular menstrual cycle were included in the study. The study subjects were selected using simple random sampling (lottery method) technique from the list of the students.

Data collection tools and procedures

A pretested self-administered questioner was used to collect the data. The questionnaire includes socio demographic questions, menstrual cycle pattern related questions, menstrual related symptoms questions, problems faced during menses questions and average grade point of the last semester taken from the students register. The questionnaire was first prepared in English, and translated into Amharic language. A person who was expert in both languages checked the questionnaires' consistency. Two data collectors (graduated public health) and one supervisor (master of public health student) was participated throughout the data collection process.

Measures

Outcome

The investigators want to compare the average grade point of students who have menstrual cycle irregularity with students who have menstrual cycle regularity. In order to assure the comparison similar in academic year only

the last one semester average grade point was taken for all students who have regular and irregular menstrual cycle.

Predictors

Whether menstrual cycle is regular or irregular should be determined by using standards of menstrual irregularity definition which was prepared by international federation of gynecology and obstetrics (IFGO) 2018. Therefore, in the present study regular menstrual cycle was defined as if frequency of menses is 24–38 days, duration of bleeding less than or equal to 8 days, cycle to cycle variation over the last one year be less than 10 days and if the individual perception on amount is normal(12). On the other hand menstrual irregularity refers to anything outside regular menstrual cycle limit.

Data analysis

Epi-data version 3.1 was used for data entry and exported to SPSS version 21 software for

Table1- Socio-demographic characteristics of undergraduate female students of DBU, Ethiopia participated in the study, 2020 (N=404).

Variables		Frequency	Percent %
Age	18-20	209	51.73
	21-22	179	44.31
	23-26	16	3.96
Ethnicity	Amhara	298	73.76
	Oromo	68	16.83
	SNNP	16	3.96
	Tigrie	17	4.21
	Others	5	1.24
Residence before university admission	Urban	201	49.75
	Rural	203	50.25
Religion	Orthodox	346	85.64
	Protestant	30	7.43
	Muslim	27	6.68
	Others	1	0.25
Marital status	Single	376	93.07
	Married	26	6.44
	Others	2	0.50
Birr sent from family per month	<3USD	88	21.78
	3-6USD	116	28.71
	≥6USD	200	49.50

analysis. Normality test (by using Kolmogorov-Simonov and Shapiro-wilk test), homogeneity of variance (by using Levene's test) were done. Out layer detection was done by using box and whisker plot. Descriptive statistics such as frequency and percentage were computed for categorical variables. Continuous variables were presented as mean \pm standard deviation or median. Independent sample t- test was done to compare the mean difference of academic performance between the two groups of students.

Results

Socio-demographic characteristics

The average age of the students was **20.69 \pm 1.43** years old with a range of 18-26. **Half** of respondents 203 (50.20%) came from rural area and 346 (85.60%) of study participants were orthodox Christian followers (Table1).

Menstruation and menstrual irregularity

The mean menarcheal age of study subjects was **14.90 \pm 1.67** ranging from 9 to 18 years.

The average duration of the menstrual cycle lasted between 2-12 days with a mean of **4.84 \pm 1.81** days.

Among menstrual irregularities, (those who have menstrual cycle length <24 days, menstrual cycle length > 38 days, inter

menstrual difference > 10 days, perception on menstrual blood flow light, perception on menstrual blood flow heavy and menstrual

blood flow duration > 8 days), irregular onset was the major problem 123 (30.45%). Light menstrual flow, heavy menstrual flow, prolonged period, frequent period and infrequent period accounts in 116 (28.71%), 80 (19.80%), 24 (5.94%), 23 (5.69%) and 3 (0.74%), respectively (Table 2).

Table 2- Pattern of menstrual cycle among undergraduate students of DBU, Ethiopia, participated in the study, 2020(N= 404).

Variables		Frequency	Percent (%)
Length of menstrual cycle	<24 days	23	5.69
	24-38 days	204	50.50
	>38 days	3	0.74
Regularity of onset / Inter menstrual difference	Regular (<10 days)	51	12.62
	Irregular (≥ 10 days)	123	30.45
Menstrual blood flow duration	≤8 days	380	94.10
	>8 days	24	5.94
Perception on menstrual blood flow	Light	116	28.71
	Normal	208	51.49
	Heavy	80	19.80
Over all menstrual cycle	Irregular	202	50
	Regular	202	50

Menstrual related symptoms

The majority experienced symptoms during their menstrual period were

abdominal cramps 285 (70.54%), back pain 139 (34.41%), disappointment 94(23.27%) (Table 3).

Table 3- Menstrual related symptoms occur during menses among undergraduate students of DBU, Ethiopia, participated in the study, 2020 (N=404)

Symptom	Number	Percentage (%)
abdominal cramps	285	70.54
back pain	139	34.41
Disappointment	94	23.27
Headache	85	21.04
Nausea	69	17.08
Depression	67	16.58
Vomiting	34	8.41

Related with symptoms, 180 (44.55%) students passed the time without any problem. Others 224(55.45%) students faced different problems.

From them 126 (56.25%) had problem of attention in the class (Table 4).

Table 4- Problems face during menses among undergraduate students of DBU, Ethiopia participated in the study2020 (N=224).

Problem	Categories	Number	Percent (%)
No attention in the class	Yes	126	56.25
	No	98	43.75
Absent from class	Yes	71	31.69
	No	153	68.30
Decrease class activity	Yes	75	33.48
	No	149	66.52
Low grade achievement	Yes	104	46.43
	No	120	53.57
Absent from exam	Yes	12	5.36
	No	212	94.64

Effect of menstrual irregularity on academic Performance

The mean average grade point (AGP) of study participants was 2.88 with (SD±0.57). Furthermore, 45% of students had an average grade point above the mean. Students who had menstrual irregularity had mean AGP of (2.78±0.57) and students who had regular

menstruation had mean AGP of (2.97±0.53) (Table 5).

This study found that students who had menstrual irregularity had significantly lower mean average grade point by a mean difference of 0.19 (95%CI: 0.08-0.30) as compared with students who had regular menstruation (Table 5).

Table 5- Effect of menstrual irregularity on academic performance of undergraduate students of DBU, Ethiopia 2020

Variable		Frequency	Mean AGP	Mean difference	95%CI of mean AGP	p value	Std. Deviation	Mean±Std
Menstruation	Regular	202	2.97	0.19	(0.08-0.30)	0.001	0.53	2.97±0.57
	Irregular	202	2.78				0.57	2.78±0.57

Discussion

The implications of reproductive health on quality of life and other activities are many. This study examines the association between menstrual cycle and academic performance among undergraduate students of Debre Berhan University. In the present study 56.25% of students had no attention in the class, 31.69% were absent from class, 33.48% decrease class activity, 46.43% had low grade achievement and 5.36% were absent from exam during menses. Moreover, students which had menstrual irregularity had significantly lower mean average grade result as compared with students who had regular menstruation.

This is in accordance to the Arabian study, which showed that academic performance was affected by menstruation in several ways,

mainly study time (76%), concentration (65.8%), participation in group activities (58.1%), examination performance (51.8%) and class attendance (40.8%) (12).

Another study conducted in Mansoura University revealed that 79.5% of nursing students had sleeping desire during lectures, 78.7% experienced restrictions in practical performance, 77.5% had difficulty in concentration and understanding and 75.1%, decreased participation in discussion. In addition study conducted in Kuwait study 75.5% of the participants perceived menstrual discomfort as having negative effects on their participation (13). A study conducted on the effects of the different phases of the menstrual cycle on physical working capacity showed that the female resting heart and respiratory rates were decrease during the menstrual cycle which

have adverse effects on physical work output and sport participation (14).

Our study has limitations to consider when interpreting results. First most data on the participant's questioner were self-reported even Average grade result so social desirability bias may be present.

Conclusion

It can be concluded that menstrual irregularity has a major impact on students 'academic performance. This study found that students who had menstrual irregularity had significantly lower mean average grade point as compared with students who had regular menstruation.

But this study gives insight about the effect of menstrual irregularity on academic performance, further research should be conducted to study the effect of menstrual irregularity on academic performance on university students.

Abbreviations

AGP: Average Grade Point DBU: Debre Berhan University IFGO: International Federation of Gynecologists and Obstetrics;

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REFERENCES

1. Sharma S, Deuja S, Saha C. Menstrual pattern among adolescent girls of Pokhara Valley: a cross sectional study. *BMC women's health*. 2016;16(1):1-6.
2. Larramendy M, Soloneski S. Chromosomal Abnormalities: A Hallmark Manifestation of Genomic Instability: BoD—Books on Demand; 2017.
3. Mahmood M, Jabeen N. Pattern of menstrual irregularities amongst women presenting to gynaecological outpatient department. *Journal of Islamabad Medical and Dental College*. 2013;2(1):9-12.
4. Nam GE, Han K, Lee G. Association between sleep duration and menstrual cycle irregularity in Korean female adolescents. *Sleep medicine*. 2017;35:62-6.
5. Boyle GJ. Effects of menstrual cycle moods and symptoms on academic performance: a study of senior secondary school students. *British journal of educational psychology*. 1997;67(1):37-49.
6. Gujarathi J, Jani D, Murthi A. Prevalance of menstrual disorders in hostellers and its effect on education-a cross sectional study. *World Journal of Pharmaceutical Research*. 2014;3(2):4060-5.
7. Khamdan H, Aldallal K, Almoosa E, Al Omani N, Haider A, Abbas Z. The impact of menstrual period on physical condition, academic performance and habits of medical students. *J Womens Health Care*. 2014;3:185.
8. Bodat S, Ghate MM, Majumdar JR. School absenteeism during menstruation among rural adolescent girls in Pune. *Natl J Community Med*. 2013;4(2):212-6.
9. Koushkie Jahromi M, Gaeini A, Rahimi Z. Influence of a physical fitness course on menstrual cycle characteristics. *Gynecological endocrinology*. 2008;24(11):659-62.
10. Karout N. Prevalence and pattern of menstrual problems and relationship with some factors among Saudi nursing students. *Journal of Nursing Education and practice*. 2015;5(12):1-8.
11. Elnagar RR, Elmashed HAM, Kheder NFH. Menstrual disorders: incidence and its effect on students' academic performance. *International Journal of Nursing Didactics*. 2017;7(3):27-37.

12. Munro MG, Critchley HO, Fraser IS, Committee FMD, Haththotuwa R, Kriplani A, et al. The two FIGO systems for normal and abnormal uterine bleeding symptoms and classification of causes of abnormal uterine bleeding in the reproductive years: 2018 revisions. *International Journal of Gynecology & Obstetrics*. 2018;143(3):393-408.
13. Shehadeh JH, Hamdan-Mansour AM. Prevalence and association of premenstrual syndrome and premenstrual dysphoric disorder with academic performance among female university students. *Perspect Psychiatr Care*. 2018;54(2):176-84.
14. Girija B, Veeraiah S. Effect of different phases of menstrual cycle on physical working capacity in Indian population. *Indian j physiol pharmacol*. 2011;55(2):165-9.

UNDERNUTRITION AMONG PREGNANT ADOLESCENT, A SCOPING REVIEW

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Abstract:

Background: In pregnant adolescents, it is hypothesized that there is 'nutrient partitioning', a competition for nutrients between the still growing adolescent mother and her rapidly developing fetus resulting in a compromised nutrition status of both. This scoping review examined the prevalence of undernutrition, associated factors and outcomes of adolescent pregnancy.

Methods: We used a five stages framework suggested by Arksey & O'Male (2005) to carry out this scoping review. Published articles, reviews and reports were identified through a complete search. We included articles published in English language from 2000 to 2020. We summarized prevalence, associated factors and health outcomes of pregnancy during adolescence.

Results: 25 studies met the inclusion criteria. 32% of the studies are on dietary intake, 20% of them reported nutritional status and associated factors and 48% studies discussed effect of poor nutrition on outcome of Pregnancy during adolescence. Only 4 of the studies are community based and 21 are facility based. Magnitude of under-nutrition among pregnant adolescent girls ranged from 23.5% to 34%; Social determinants of health such as poor access to antenatal care visits, low educational status of partners, poor dietary intake, early marriage, rural residency, young age and having multiple pregnancies are associated with poor nutritional status. Pregnant adolescents have also more risks of poor pregnancy outcomes compared with pregnant adults' women. These include fetal complications like prematurity, low or very low birth weight, and perinatal mortality, major congenital defects; hypertensive pregnancy disorders, abortion, urinary infections, and premature rupture of the fetal membranes,.

Conclusion: A higher magnitude of under-nutrition, less dietary intake and more risks of poor pregnancy outcomes were observed from reviewed studies. This review demonstrated absence of comprehensive literature which might be explored through a population-based prospective study.

Key words: Pregnant Adolescents, malnutrition, dietary intake, scoping review

Introduction

Nutrition has magnificent impact in human life and its requirement varies with respect to age, gender and during physiological changes such as pregnancy. Pregnancy is a key phase in human life and mother needs optimal nutrients of superior qualities to support the developing fetus.^{1,2} Inadequate maternal nutrition results in increased risks of short-term consequences such as; Intra Uterine Growth Restriction (IUGR), low birth weight, preterm birth, prenatal and infant mortality and morbidity. It has also been associated with patho-physiologic or metabolic consequences³. It results poor growth and development, affects quality of life during adolescence and adulthood.⁴

Pregnant adolescents might face additional challenges and are at higher risk for a number of problems when compared to adult pregnant women. They are at greater risk for certain health conditions, such as pregnancy induced hypertension, pre-eclampsia, preterm delivery, low birth weight, and inadequate weight gain, related to their age and developmental stage, and have limited knowledge of their bodies, reproduction, pregnancy, and birth.^{5,6}

Pregnancies during adolescence holds 23% of the burden of disease arising from pregnancy and childbirth, although it only represents 11% of all births worldwide.⁷ They incur increased risks for a number of adverse growth and developmental outcomes, in both the offspring and the mother,^{8,9,10} that are known to impact adversely on long term morbidity and mortality risk.^{9,11} Ninety five percent of the 16 million adolescent pregnancies that occur each year are in low and middle income countries (LMICs)⁷ and this is where the burden of small for gestational Age and stunting is concentrated.¹²

It is hypothesized that there is competition for nutrients between the still growing adolescent mother and her rapidly developing fetus, also known as 'nutrient partitioning', which may result in the growth and development of the mother and/or fetus being compromised. An alternative explanation, which may work in tandem with nutrient partitioning, is that optimal fetal development is being traded-off as a result of gynecological immaturity to allow safe delivery.^{5,13}

Aim of the reviews: Nutritional status of pregnant adolescents has been less examined; therefore this scoping review synthesized the current information and show research gaps. This review was done with the aims of producing a profile of the research on nutritional status of pregnant Adolescents that can serve as a foundation for more study based on gaps.

Material and methods

Study setting and design

A scoping review was done on worldwide studies.

Data sources and search strategies

The primary outcome of this review was malnutrition measured at any time of pregnancy. All types of studies published globally and written in English were searched systematically. Publications from January 1st, 2000 to April 1st, 2020 were considered in the following databases and search Engines: MEDLINE (via PubMed), Hinari, Embase, Google scholar and Google. Moreover retrieving references from a list of eligible studies were done. Example of search strategy in pubmed (((("nutritional status") OR malnutrition) OR Undernutrition)) AND (((("Pregnant Adolescents") OR "Teenage

pregnancy") OR (Pregnan* adolescen*) OR ("Pregnancy in adolescence") filter: Publication date from 2000/01/01 to 2020/04/01; Studies on- Humans; language- English

Inclusion criteria

Types of participants - Pregnant Adolescents

Concept -The core concept examined by this scoping review was Nutritional status of pregnant adolescents (nutritional status, dietary intake and outcome of pregnancy during Adolescence).

Context -Worldwide

Types of sources - The source of information can include any existing literature e.g. all types of Primary research studies, systematic reviews and meta-analyses were considered.

Exclusion criteria -studies published other than English language

Data extraction

Initially, searching was conducted through identified data bases, search engines, and reference lists of selected studies. Second, studies conducted before 2000 and unrelated articles based on their title and abstract were excluded. Thirdly, those potentially eligible for inclusion were imported to Endnote v-9 and duplicates were removed. Fourth, two independent reviewers conducted abstract and full-text review and data abstraction. In case of disagreement between the two reviewers, discussion was made to reach to agreement. Finally, data were extracted on structured data extraction form and presented using tables. Information's extracted include: Name of

Principal Investigator, year of publication, country in which the study was conducted, sample size, tool used to screen malnutrition, cut-off point for screening tool, prevalence and other major findings . JBI Template study details, characteristics and results extraction instrument was used.

Methods and analysis:

A scoping review was performed based on the York methodology outlined by Arksey and O' Malley⁴⁷ .The 'York framework' suggested five stages that we have followed for this review:

Stage 1: Identifying the research question-

Stage 2: Identifying the relevant studies-

Stage 3: Study selection

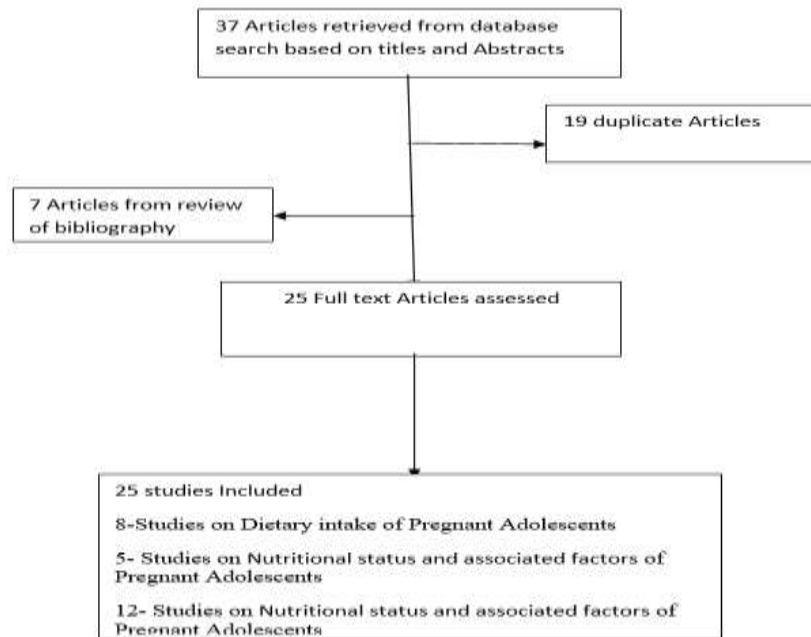
Stage 4: Charting the data-

Stage 5: Collating, summarizing and reporting the results

Stage 6: Consultation

Results

Application of inclusion and exclusion criteria resulted in overall selection of 25 papers for this review. Literatures selected were from a 20 year period with the oldest from 2000 to the most recent in 2020. 32% of the Studies are on Dietary intake of Pregnant Adolescents, 20% are on Nutritional status and associated factors of Pregnant Adolescents and 48% of the Studies are on Effect or outcome of Teenage Pregnancy. Studies that are exclusively containing Pregnant Adolescents and/or their newborn are taken. (Fig-1)



Nutritional status and associated factors among pregnant adolescents

age and multiple pregnancy are some of associated factors identified. (Table 1)

The magnitude of undernutrition ranged from 23.5% to 34%. Social determinants such as poor access to antenatal care visits, low educational status of partners, poor dietary intake, early marriage, rural residency, young

Table-1 Studies on Dietary intake of Pregnant Adolescents included in the Review

Country	Study	Purpose of the study	Study Design	Participants' Characteristics And sample size	Classification Criteria	Main findings
Developed countries	Katie Marvin, et al,2016	to examine what is known about the nutritional status of adolescent pregnant women	Systematic Review	Pregnant Adolescents (from countries considered as having very high levels of human development by the United Nations Human Development Index	Different measurements of nutrient intakes or biological markers of nutritional status	Intakes of energy, fiber & key micronutrients were below recommended levels. Low Vit D, selenium levels were found to be low (61.8 µg/L,)
Industrialized countries	Victoria H et al,2007	* to review evidence relating to the dietary assessment of pregnant adolescents living in industrialized countries	Systematic Review			Nutrient intakes of pregnant adolescents were below the DRI for energy, iron, folate, calcium, vit-E
USA	Sunmin L,et al,2014		Longitudinal Study	Participants were aged 13–20 years, with a mean age of Between 16 and 17 years.	-24-hr dietary recalls with –dietary reference intakes.	Majority do not meet Req intake for vit D(93%), vit-E (94%) Mg(90%) Fe(76%) Ca(74%).
USA		*To				

Country	Study	Purpose of the study	Study Design	Participants' Characteristics And sample size	Classification Criteria	Main findings
	Bridget E,2012	<p>characterize dietary intake and –</p> <p>* to determine the degree to which prenatal supplement use compensates for dietary deficits.</p> <p>To examine relations between maternal calcium intake, 25-hydroxyvitamin D [25(OH)D] status, and fetal bone growth across pregnancy.</p>	Longitudinal Study (Prospective)	<p>156 pregnant Adolescents</p> <p>171 pregnant adolescents</p>		<p>Fetal femur and humerus z scores and neonatal birth length were significantly greater ($P < 0.03$) in adolescents consuming ≥ 1050 mg than in those consuming < 1050 mg Ca/d.</p> <p>Maternal 25(OH)D > 50 nmol/L was significantly positively associated with fetal femur & humerus z scores</p>
Bangladesh	Nguyen PH,et al,2017	to examines d/f in services received by pregnant adolescents & non-adolescents & compares MCH nutrition and health conditions	Cross-sectional	2,000 recently delivered women with infants < 6 months of Age	–	<p>* Infants of adolescent had lower Ht/Age z-score lower Wt/Age & higher underweight prevalence*Same ANC coverage*adolescents had lower BMI</p> <p>*Adolescents recover with greater difficulty after childbirth.</p> <p>*Dietary intakes of pregnant adolescent girls & women were similar</p> <p>*Probabilities of adequacy ~ 0.30 for Vit B2, vitB-12, ca,& zinc; 0.12–0.15 for folate; 0.16–0.19 for vit-A; & low for iron at 0.01. The WDDS-10 was associated with MPA in both groups & predicted MPA equally well at population level (SD of residuals 0.11 for both).Use of the 5-food groups cutoff for</p>
Bangladesh	Phuong H,2018	<p>1) compare adequacy of micronutrient intakes b/n pregnant adolescent girls & women,</p> <p>2) examine performance of WDDS-10 in predicting mean probability of adequacy of 11</p>	Cross-sectional	pregnant women	Multiple-pass 24-h recall	

Country	Study	Purpose of the study	Study Design	Participants' Characteristics And sample size	Classification Criteria	Main findings
Bangladesh	Kawasri,2007	micronutrients, 3) assess how well the MDD-W cutoff of 5 groups performed in pregnant adolescent girls & women	Retrospective Cohort	539 first birth-order children of Adolescents		MDD-W to classify individual diets into MPA >0.6, however, resulted in a low correct classification (40%). *Children of adolescent mothers are likely to be more malnourished & had longer duration of hospitalization
Brazil	Maria E,et al,2013	To assess clinical & nutritional features & socioeconomic characteristics of the 1 st birth-order children (1–48 months) of adolescent mothers. The study investigated the effect of calcium plus vitamin D supplementation during pregnancy on bone mass during lactation in Brazilian adolescent mothers with low-calcium diets (~600 mg/d).	RCT/ Randomized control trail	Pregnant adolescents (14–19 y)		Calcium plus vit- D supplementation during pregnancy of adolescents with low ca intake results in higher lumbar spine bone mass and a reduced rate of femoral neck bone loss during lactation

DRI-daily required intake; EAR, estimated average requirement; EER, estimated energy; requirement; MDD-W, Minimum Dietary Diversity for Women; MPA, mean probability of adequacy; WDDS-10, 10-food group Women Dietary Diversity Score; ANC-Anti-natal care;

Outcome of Teenage Pregnancy

Different studies showed more risks of poor pregnancy outcomes among Pregnant Adolescents compared with pregnant Adults .These include Fetal Complications like prematurity, low or very low birth weight, and

perinatal mortality and major congenital defects; which are fetal complications and hypertensive pregnancy disorders, abortion, urinary infections, and premature rupture of the fetal membranes, which are maternal complications.(Table2)

Table 2- Nutritional status and associated factors among pregnant Adolescents

Country	Study	Purpose of the study	Participants' Characteristics And sample size	Classification Criteria	Main findings (Prevalence)	Risk factors
Malawi	Boniface F. et al, 2006	to describe the anthropometry of pregnant adolescent girls and young adult pregnant women	991 women (Of these, 190 were adolescent (12-19 years).	MUAC less than 23 cm, or BMI less than 18.5 kg/m ² (WHO)	25.8% Undernutrition (Adolescent) 10.5% (Adult women)	young age maternal weight iron status malaria parasitemia
Nigeria	Mondy Daniel et al, 2019	to assess the breastfeeding practices of teenage mothers & determine its association with the nutritional indices of their under-five children.	300 mother-child pair	WHO standard classification was used to categorize the nutritional status. (WHO) Anthro software.	*87% initiated breastfeeding < 1 h after birth while 31.9% breastfed their children exclusively for 6 months. *Stunting/18.6%, Wasting/25.3%, underweight/29.5% among the under-fives	Education of teenage mothers on breastfeeding initiation and duration
Sub-Saharan Africa	Ibrahim Y, et al, 2018	to identify factors influencing adolescent pregnancies	11,651 pregnant from 24 study(systematic review)	Pregnancy test	-	Peer influence, , poverty, early marriage, , parental neglect, absence of free education, lack of sexuality education, early sexual debut

Country	Study	Purpose of the study	Participants' Characteristics And sample size	Classification Criteria	Main findings (Prevalence)	Risk factors
						& inappropriate forms of recreation
Ethiopia	Belete Y= t al	to determine prevalence of under nutrition and associated factors among adolescent pregnant women	424 adolescent pregnant women	MUAC of <22 cm (WHO)	34.0% Undernutrition (Adolescent)	Women married before 15 years No ANC visits <3 pregnancy Having support Workload
Africa (Systematic Review)	Hana and Abel, 2019	to review burden of malnutrition, (protein energy malnutrition), during pregnancy in Africa	20,672 pregnant Adolescents	MUAC BMI	Pooled Prevalence =23.5% (95%CI: 17.72–29.32; I ² = 98.5%)	Rural residency , Low educational status of partners , multiple pregnancy and poor nutritional indicators

MUAC-mid upper arm circumference; BMI- body mass index; CI-confidence interval

Effect or outcome of Teenage Pregnancy

Table 3- Effect or outcome of Teenage Pregnancy

Country	Study	Purpose of the study	Participants' Characteristics and sample size	Main findings (Effects-
Sri Lanka	Goonewardene and Deeyagaha	To compare obstetric complications between teenagers and older women.	two groups of pregnant teenagers (13–16 years, n = 95 and 17–19 years, n = 250) were compared with a control group of pregnant women (20–24 years, n = 275).	*Teenagers were from lower socioeconomic strata & the younger teenagers were less educated than the controls. Teenagers had higher risk of anaemia. The younger teenagers had a significantly higher risk of gestational HTN and pre-eclampsia The older teenagers had higher risk of delivery before 34 weeks of gestation There were no significant differences in the mode of delivery. *Teenagers had a much higher proportion (54%) of unplanned pregnancies compared to the controls (16 %).
Singapore	Gavrielle K, et al, 2015	to determine the effect young maternal age and single motherhood has on neonatal outcomes.	267 infants born to mothers aged ≤ 21 years, from Jan 2011 to Dec 2012	*Unsatisfactory antenatal care was more prevalent among the young single mothers than among the young married mothers. *The infants of the young single mothers had a lower mean birth weight . *Young maternal age was linked to a higher incidence of prematurity), major congenital defects), and a perinatal mortality of 18.7 per 1,000 births
Worldwide	Walter F. et al, 2015	To analyze complications related to adolescent pregnancy.	Pregnant Adolescents	*Fetal Complications found were prematurity, low or very low birth weight, and perinatal mortality. *maternal complications hypertensive pregnancy disorders, abortion, urinary infections, and premature rupture of the fetal membranes.
Worldwide	Andrea Nove, et al, 2014	to quantify the risk of maternal death in adolescents by estimating maternal mortality ratios for women aged 15–19 yrs and to compare these ratios with those for women in other 5-year age groups	data from 144 countries and territories (65 with vital registration data and 79 with nationally representative survey data)	Slightly increased risk of mortality in adolescents compared with women aged 20–24 years (260 [uncertainty 100–410] vs 190 [120–260] maternal deaths per 100 000 livebirths for all 144 countries combined)

England	Rachel R, et al,2018	to explore knowledge & understanding of nutrition advice during adolescent pregnancy, & identify barriers & facilitators to dietary change & supplementation use	34 young women (adolescents aged 16–19 years) and 20 health professionals (Qualitative study)	Young women made small changes to their dietary intake despite limited knowledge & social constraints. Forgetting was the main reason for poor adherence. Health professionals provided nutrition information but often lack the time & resources to tailor this appropriately.
India	Kumar A, et al,2007	to evaluate the obstetric, fetal & neonatal outcomes of teenage pregnancy in a tertiary care teaching hospital.	All teenage mothers as cases and age group of 20-30 year were selected as controls	Teenage pregnancy was associated with a significantly higher risk of PIH, PET, eclampsia, premature onset of labor, fetal deaths and premature delivery. Increased neonatal morbidity and mortality were also seen in babies delivered to teenage mothers. Younger teenager group (≤ 17 years) was most vulnerable to adverse obstetric and neonatal outcomes.
Bangkok	Sathja T, et al,2007	to compare pregnancy outcomes in adolescent females aged 19 and younger with those of adult women aged 20–34 years	401 randomly selected adolescent females and 815 adult mothers	Adolescent Mothers had higher rates of anemia (OR 0.44, CI 0.26, 0.75), preterm deliveries (OR 1.21, CI 1.01, 1.75), and lower mean birth weight babies (2931 g and 3077 g, $p < .001$).
USA	XI-kuan C, et al,2007	to determine whether teenage pregnancy was associated with increased risks of adverse birth outcomes	3886364 Nulipara's data were derived from the 1995–2000 nationally linked birth/infant death data set of the USA, compiled by NCH Statistics and Centers for Disease Control and Prevention. young adolescents (≤ 18 years), older adolescents (18–20 years), and adults (> 20 years).	The rates of very pre-term delivery, pre-term delivery, very LBW, LBW, SGA, very low Apgar score, low Apgar score and neonatal mortality were higher in teenage pregnancies.
Malawi	Alyssa F, et al, 2017	to determine outcomes of young adolescents (≤ 18 years), older adolescents (18–20 years), and adults (> 20 years) To investigate the risk of adverse pregnancy outcomes among	A total of 124 446 mothers and their infants	Young adolescents delivered with a lower fundal height than adults. Among newborns, length for age was lowest in young adolescents, greater in older adolescents, and greatest in adults. Compared with mothers aged 20–24 years, adolescent mothers aged 10–19 years had higher risks of eclampsia, puerperal
Multicounty				

study 29 countries. (Africa, Latin America, Asia and the Middle East)	Ganchimeg T,et al,2014	adolescents in 29 countries to investigate the influence of maternal adolescence on pregnancy outcomes	775 women giving birth in 3 mother-child health centers	endometritis, systemic infections, low birth weight, preterm delivery and severe neonatal conditions. The increased risk of intra-hospital early neonatal death among infants born to adolescent mothers was reduced and statistically insignificant
Gabon (Central Africa)	Florian K, et al,2010	The study estimates and compares the incidence of adverse obstetric and perinatal outcomes of teenage women with older women	obstetric and perinatal outcomes of all teenage (ages < 19 years) pregnant women with those of older pregnant women (ages >19 years)	Young maternal age showed a significant association with the risk for low birth weight.
South Africa	Monjurul H, et al,2010			adolescent women were shown to attend significantly less antenatal care visits The rate of gestational age at delivery , vaginal and forceps deliveries, foetal presentation at birth, multiple pregnancies, low birth-weight and live births deliveries and mean Apgar scores were similar for both groups. The caesarean delivery rate (20%) and macerated stillbirth rate (1.1%) were significantly lower ($p < 0.05$) for teenagers than for older women.

OR-odds Ratio; CI-confidence interval; PIH-Pregnancy induced hypertension, PET- pre-eclamptic toxemia; LBW-low birth weight, SGA-small for gestational Age; Apgar-Appearance, pulse, Glimace, Activity and respiration.

Discussion

We observed greater data scarcity from community-based studies as the great majority of the studies were sampled from facility-based hospital record review (secondary data) which has many limitations. Therefore, information from this scoping review may not represent the true picture of nutrition problem among pregnant adolescents.

Different studies^{40,41,42} including a systematic and scoping review on maternal health service utilization of adolescent women in sub-Saharan showed as a significant number of adolescents do not access and use maternity services from health facilities during pregnancy.

Among studies which compared effect or outcome of teenage pregnancy and pregnancy during adulthood only one study³⁴ reported comparable risk and all others showed a greater risk of poor pregnancy outcome among teenage pregnancy than adulthood pregnancy.

Estimation of adverse risks could be biased as most studies were implemented in health institutions, mainly located in urban Areas, which had the capacity to perform caesarean sections and other better services. This results in underestimation of adverse risks, which may limit the generalizability of findings.

Africa, especially Sub-Saharan Africa has the highest rate of adolescent pregnancy^{35,37,43} in the world however most studies are in developed countries. Developed countries, such as the United States gave more attention to this group. There is a huge gap of literature on developing countries; more researches need to be done to know the real causes for poor pregnancy outcomes of pregnancy during Adolescence. Moreover, we need studies to gain deeper understanding of the nutrient requirements needed during and before adolescent pregnancy to help in giving them with a necessary diet.

Compared to adult pregnant women, very few studies have assessed the nutritional status of pregnant Adolescents. Gaps on very important nutrition study among pregnant Adolescents have been identified; these include Anemia, iodine deficiency, dietary diversity score, longitudinal study on first 1000 days and Undernutrition / PEM with better methods than MUAC/BMI. More over health and nutrition education, macronutrient and micronutrient supplementation and school feeding programs interventions are also crucial.

Adolescent girls are at a greater risk of iron deficiency anemia due to fast growth during adolescence and onset of menarche.^{44,45} During pregnancy there is increased demand for iron for expansion of maternal tissues and fetal growth, which makes pregnant adolescents a highly vulnerable group. However, we found a literature gap on Anemia among pregnant Adolescents which this important problem.

Globally, changes are happening in food and nutrition-related policy environment with many countries taking actions to improve food and nutrition environment to promote healthy diets and nutrition.⁴⁶ However; pregnant adolescent's nutrition issue is not adequately addressed. They demand special attention, countries should incorporate strong interventions regarding these important population group.

The review has some limitations. Information reviewed may not be exhaustive, because of inaccessibility of some databases and grey literature sources. However, this review provides helpful insights for future research

Abbreviations

ANC	Anti-natal care
BMI	Body Mass Index
CI	Confidence Interval
DRI	Daily Required Intake
EAR	Estimated Average Requirement

needs on study regarding nutritional status of Pregnant Adolescents.

Conclusion

Magnitude of undernutrition ranged from 23.5% to 34%; No Antenatal care visits, Low educational status of partners, poor dietary intake, Women married before 15 years, rural residency, young age and multiple pregnancy are some of the associated factors identified. Pregnant Adolescents have less dietary intake and more risks of poor pregnancy outcomes compared with pregnant Adults .These include Fetal Complications like prematurity, low or very low birth weight, and perinatal mortality and major congenital defects; which are fetal complications and hypertensive pregnancy disorders, abortion, urinary infections, and premature rupture of the fetal membranes, which are maternal complications. This review demonstrated absence of comprehensive literature which might be explored through a population-based prospective study.

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Disclosure

The authors report no conflicts of interest in this work.

Additional Files

- The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

EER	Estimated Energy Requirement
IUGR	Intra Uterine Growth Restriction
LBW	Low Birth Weight
LMICs	Low and Middle Income Countries
MDD-W	Minimum Dietary Diversity for Women
MPA	Mean Probability of Adequacy

MUAC	Mid Upper Arm	Circumference	WDDS-10	10-food group Women Dietary Diversity Score
WHO	World Health Organization			

REFERENCES

1. Abu-Saad, K. and Fraser, D. Maternal Nutrition and Birth Outcomes. *Epidemiologic Reviews*. 2010. V32 (1) 5-25.
2. Andrea Nove, Zoë Matthews, Sarah Neal, Alma Virginia Camacho, 2014, Maternal mortality in adolescents compared with women of other ages: evidence from 144 countries, www.thelancet.com/lancetgh Vol 2, [http://dx.doi.org/10.1016/S2214-109X\(13\)70179-7](http://dx.doi.org/10.1016/S2214-109X(13)70179-7)
3. Arksey H & O'Malley L (2005). Scoping studies: towards a methodological framework. *Int J Soc Res Methodol* 8: 19-32.
4. Belete Y, Negga B, Firehiwot M (2016) Under Nutrition and Associated Factors among Adolescent Pregnant Women in Shashemenne District, West Arsi Zone, Ethiopia: A Community-based Study. *J Nutr Food Sci* 6: 454. doi:10.4172/2155-9600.1000454
5. BONIFACE F. KALANDA, FRANCINE H. VERHOEFF & BERNARD J. BRABIN, 2006, chronic malnutrition in pregnant adolescents in rural Malawi: An anthropometric study, *Acta Obstetrica et Gynecologica*. ; 85: 33_/39.
6. Bridget E Young, Thomas J McNanley, Elizabeth M Cooper, Allison W McIntyre, Frank Witter, Z Leah Harris, and Kimberly O, 2012, Maternal vitamin D status and calcium intake interact to affect fetal skeletal growth in utero in pregnant adolescents, 95(5): 1103–1112, *Am J Clin Nutr*.. doi: 10.3945/ajcn.111.023861
7. Emerging Risk Factors Collaboration .Adult height and the risk of cause-specific death and vascular morbidity in 1 million people: individual participant meta-analysis. *Int J Epidemiol* 2012;41:1419–33. [PMC free article] [PubMed] [Google Scholar]
8. Florian K., Sabine B., Ghyslain M. , Katharina S., Ayola A., Marielle K. Bouyou, Peter G. , and Michael R 2010, Adolescence As Risk Factor for Adverse Pregnancy Outcome in Central Africa – A Cross-Sectional Study, *PLoS One*, doi: 10.1371/journal.pone.0014367
9. Frieber A, Callaghan-Gillespie M, Papathakis PC, Manary MJ. Adolescent pregnancy and nutrition: a subgroup analysis from the Mamachiponde study in Malawi. *Annals of the New York Academy of Sciences*. 2018; 1416(1):140–6.
10. Ganchimeg T, Ota E, Morisaki N, Laopaiboon M, Lumbiganon P, Zhang J, et al. Pregnancy and childbirth outcomes among adolescent mothers: a World Health Organization multicountry study. *BJOG* 2014; 121(Suppl 1):40–8.
11. Gavrielle Kang, MBBS, Jia Yi Lim, BSocSci, Anita Sugam Kale, FRCOG, MBBS, and Le Ye Lee, MMBS, MRCPCH, 2015, Adverse effects of young maternal age on neonatal outcomes , *Singapore Med J* , ; 56(3): 157–163., doi: 10.11622/smedj.2014194
12. Girard AW, Olude O (2012) Nutrition education and counseling provided during pregnancy: effects on maternal, neonatal and child health outcomes. *Pediatric Perinat Epidemiol* 26: 191-204.
13. Hall Moran V. Nutritional status in pregnant adolescents: a systematic review of biochemical markers. *Maternal child nutri*. 2007;3(2):74–93.

14. Hoque M, Hoque S. A comparison of obstetrics and perinatal outcomes of teenagers and older women: Experiences from rural South Africa. *Afr J Prm Health Care Fam Med*. 2010; 2(1), Art. #171, 5 pages. DOI: 10.4102/phcfm.v2i1.171
15. Kaplanoglu M, Bulbul M, Konca C, Kaplanoglu D, Tabak MS, Ata B. Gynecologic age is an important risk factor for obstetric and perinatal outcomes in adolescent pregnancies. *Women Birth* 2015; In Press. [PubMed] [Google Scholar]
16. Kassa, G.M., Arowojolu, A.O., Odukogbe, A.A. et al. Prevalence and determinants of adolescent pregnancy in Africa: a systematic review and Meta-analysis. *Reprod Health* 15, 195 (2018). <https://doi.org/10.1186/s12978-018-0640-2>
17. Katie M., Victoria J. and Hora S., 2016, Nutrient intakes and nutritional biomarkers in pregnant adolescents: a systematic review of studies in developed countries, Marvin-Dowle et al. *BMC Pregnancy and Childbirth* (2016) 16:268, DOI 10.1186/s12884-016-1059-9
18. Kawsari A., Mohammad A., Abu S., Mohammed A, T Ahmed, Kawsari Abdullah, 2007, Health and nutritional status of children of adolescent mothers: experience from a diarrhoeal disease hospital in Bangladesh, Pages 396-400 V- 96, I- 3, doi_org/10.1111/j.1651-2227.2007.00117.x
19. Khabar Samay , 2017, Breaking the inter-generational cycle of malnutrition among Adolescent girls
20. Kozuki N, Lee AC, Silveira MF, Sania A, Vogel JP, Adair L, et al. The associations of parity and maternal age with small-for-gestational-age, preterm, and neonatal and infant mortality: a meta-analysis. *BMC Public Health* 2013;13(Suppl 3):S2. [PMC free article] [PubMed] [Google Scholar]
21. Kumar A, Singh T, Basu S, Pandey S, Bhargava V. Outcome of teenage pregnancy. *Indian J Pediatr*. 2007 Oct;74(10):927-31.
22. Lee AC, Katz J, Blencowe H, Cousens S, Kozuki N, Vogel JP, et al. National and regional estimates of term and preterm babies born small for gestational age in 138 low-income and middle-income countries in 2010. *Lancet Glob Health* 2013;1:e26–36. [PMC free article] [PubMed] [Google Scholar]
23. Luigi R, Orbitello B, Perini L, Pera V, Ciano R, et al. (2005) Effects of pregnancy on eating attitudes and disorders. A prospective study. *J Psychosom Res* 59: 175-179.
24. Maria Eduarda L Diogenes, Flávia F Bezerra, Elaine P Rezende, Marcia Fernanda Taveira, Isabel Pinhal, Carmen M Donangelo, Effect of calcium plus vitamin D supplementation during pregnancy in Brazilian adolescent mothers: a randomized, placebo-controlled trial, *The American Journal of Clinical Nutrition*, Volume 98, Issue 1, July 2013, Pages 82–91, <https://doi.org/10.3945/ajcn.112.056275>
25. Melkam Tesfaye, Tilahun Yemane, Wondimagegn Adisu, Yaregal Asres, Lealem Gedefaw, 2015, Anemia and iron deficiency among school adolescents: burden, severity, and determinant factors in southwest Ethiopia, *Adolesc Health Med Ther.*; 6: 189–196. . doi: 10.2147/AHMT.S94865, PMCID: PMC4687608
26. Mohammad A. A Malek , Abu S G Faruque , Mohammed A Salam , T Ahmed, Health and nutritional status of children of adolescent mothers: experience from a diarrhoeal disease hospital in Bangladesh, 2007, <https://doi.org/10.1111/j.1651-2227.2007.00117.x>
27. Mohammed Feyisso Shaka, Yohannes Addisu Wondimagegne, 2018; Anemia, a moderate public health concern among adolescents in South Ethiopia, *PLoS One*. 13(7): e0191467., doi: 10.1371/journal.pone.0191467 PMCID: PMC6049899

28. Nguyen PH, Sanghvi T, Tran LM, Afsana K, Mahmud Z, Aktar B, et al. (2017) The nutrition and health risks faced by pregnant adolescents: Insights from a cross-sectional study in Bangladesh. *PLoS ONE* 12(6): e0178878. <https://doi.org/10.1371/journal.pone.0178878>
29. Olodu, M.D., Adeyemi, A.G., Olowookere, S.A. et al. Nutritional status of under-five children born to teenage mothers in an urban setting, south-western Nigeria. *BMC Res Notes* 12, 116 (2019). http://login.research4life.org/tacsgr1doi_org/10.1186/s13104-019-4147-x
30. Onikepe O ,Kerry L M ,Mardieh L Dennis, Emma Radovich, Francesca L Cavallaro, Caroline A Lynch, Adesegun Fatusi,, Issiaka Sombie, Lenka Benova, 2017,Comparing the use and content of antenatal care in adolescent and older first-time mothers in 13 countries of west Africa: a cross-sectional analysis of Demographic and Health Surveys,www.thelancet.com/child-adolescent, [http://dx.doi.org/10.1016/S2352-4642\(17\)30025-1](http://dx.doi.org/10.1016/S2352-4642(17)30025-1)
31. Phuong H., Lieven H., Tina G, Lan Mai T,Edward A Frongillo, Purnima M.,and Marie T ,2018,Dietary Diversity Predicts the Adequacy of Micro nutrient Intake in Pregnant Adolescent Girls and Women in Bangladesh, but Use of the 5-Group Cutoff Poorly Identifies Individuals with Inadequate Intake, doi: <https://doi.org/10.1093/jn/nxy045>.
32. Rachel Rundle, Hora Soltani and Alexandra D ,2007, Exploring the views of young women and their healthcare professionals on dietary habits and supplementation practices in adolescent pregnancy: a qualitative study, Volume 44, Issue 7, , Pages 1158-1164 <https://doi.org/10.1016/j.ijnurstu.2006.05.016>
33. Rah JH, Christian P, Shamim AA, Arju UT, Labrique AB, Rashid M. Pregnancy and lactation hinder growth and nutritional status of adolescent girls in rural Bangladesh. *J Nutr* 2008;138:1505–11. [PubMed] [Google Scholar]
34. S. H. Mahavarkar, C. K. Madhu & V. D. Mule (2008) A comparative study of teenage pregnancy, *Journal of Obstetrics and Gynaecology*, 28:6, 604-607, DOI: 10.1080/01443610802281831
35. Semiha TA, Elçin B, Osman G (2010) Comparison of life quality of adolescent pregnant women with that of pregnant adults in Turkey. *Upsala Journal of Medical Sciences* 115: 275-281.
36. Shamsu-Deen Ziblim, Adadow Yidana, Abdul-Rashid Mohammed, 2018, Determinants of Antenatal Care Utilization among Adolescent Mothers in the Yendi Municipality of Northern Region, Ghana, *Ghana Journal of Geography* Vol. 10(1), pages 78–97 <https://dx.doi.org/10.4314/gjg.v10i1.5>
37. Sunmin Lee, Bridget E. Young, Elizabeth M. Cooper, FACNM, Eva Pressman, Ruth Anne Queenan,2014 Nutrient Inadequacy Is Prevalent in Pregnant Adolescents, and Prenatal Supplement Use May Not Fully Compensate for Dietary Deficiencies,V-6:3 , ICAN: Infant, Child, & Adolescent Nutrition, DOI: 10.1177/1941406414525993.
38. Tensae Mekonnen, Tinashe Dune ,Janette Perz ,2019,Maternal health service utilization of adolescent women in sub-Saharan Africa: a systematic scoping review, *BMC Pregnancy and Childbirth*,
39. ThatoS.,Rachukul,ChompunutSopajaree ,2007, Obstetrics and perinatal outcomes of Thai pregnant adolescents: A retrospective study, Pages 1158-1164, <https://doi.org/10.1016/j.ijnurstu.2006.05.016>

40. Walter F., Michele B, Eduardo S. Valério Borges da Fonseca, Lícia Maria Ricarte de Azevedo, and Carla Braz Evangelista, 2015;. Complications in adolescent pregnancy: systematic review of the literature, 13(4): 618–626
41. WHO, 2018, Global nutrition policy review 2016-2017: country progress in creating enabling policy environments for promoting healthy diets and nutrition.
42. WHO, 2004, Adolescent Pregnancy Issues in Adolescent Health and Development.
43. World Bank (2009), Repositioning Nutrition as central to development: A strategy for large scale action, The International Bank for Reconstruction and Development
44. World Health Organization, Adolescent pregnancy. Fact sheet No. 364 2014. [www.who.int/mediacentre/factsheets/fs364/en/].
45. World Health Organization, 2009 Adolescent nutrition, a neglected dimension. <https://apps.who.int/nut/ado.htm>
46. Xi-Kuan Chen, Shi Wu Wen, Nathalie Fleming, Kitaw Demissie, George G Rhoads, Mark Walker, 2007 Teenage pregnancy and adverse birth outcomes: a large population based retrospective cohort study, Volume 36, Issue 2, Pages 368–373, <https://doi.org/10.1093/ije/dyl284>
47. Yakubu I, Salisu WJ. Determinants of adolescent pregnancy in sub-Saharan Africa: a systematic review. *Reprod Health*. 2018;15(1):15.

ASSESSMENT OF TRADITIONAL MEDICINE UTILIZATION AND ITS DETERMINANTS AMONG PARENTS OF CHILDREN, IN GINDEBERET OROMIA, ETHIOPIA

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Abstract

Objective: In Ethiopia, up to 80% of the population uses traditional medicine. However, prevalence of traditional medicine use by parents for their children and associated factors were not well addressed. Therefore, this study was conducted to assess traditional medicine utilization by parents for their children and its determinants among parents of children, in Gindeberet Woreda Oromia, Ethiopia, 2017.

Methodology: A community-based cross-sectional study was employed. Both descriptive and inferential statistics were used to present the data. Finally, odds ratio, binary, and multiple logistic regression were used to analyze the association between dependent and independent variables.

Results: A total of 267 households were included in this study. Two hundred twenty-two (79.4%) of parents had used TM for their children. Only 8 (3%) of parents preferred traditional medicine over modern medicine. Major therapies used were Herbal medicine, massage, and religious therapy 34.4%, 25.9%, and 11.8% respectively. Medium monthly income (500-850) [AOR: 0.25 (0.08, 0.78)], cultural belief [AOR: 3.01 (1.16, 7.83)], religious belief [AOR: 3.17 (1.26, 7.93)] and short duration of illness [AOR: 3.11 (1.07, 9.02)] were associated with parental traditional medicine use for their children in this study.

Conclusion: Traditional medicine use by parents for their children was high despite the low preference by parents. Therefore, the integration of traditional medicine as part of modern medicine with due consideration of the efficacy of claimed interventions by relevant stakeholders is important. Finally, subsequent studies should be done by accounting both parental and children factors over different regions for a better understanding of traditional medicine utilization practice in children.

Keywords: Traditional Medicine, Children, Parents, Gindeberet Woreda, Ethiopia

1. Introduction

Traditional medicine (TM) is "the total of the knowledge, skills, and practices based on the theories, beliefs, and experiences of different indigenous cultures, whether explicable or not. It includes the use of plant, animal, and mineral-based medicines, spiritual therapies, manual techniques, and exercises, applied singularly or in combination (1). The importance of traditional medicine as a source of primary healthcare was first officially recognized by the World Health Organization (WHO) in the Primary Health Care Declaration of Alma Ata (1978) (2). However, the history of traditional medicine is as long as the history of human beings in this world. For example, the medicinal herb, *Artemisia Annua*, used in China for almost 2000 years and was found to be effective against resistant malaria (3). Studies showed that several countries in Africa, Asia, and Latin America use TM to meet some of their primary healthcare needs (4). For example, in Nigeria Quinine from plant *Cinchona bark* was used to manage the symptoms of malaria long before the disease was identified and from willow bark, a garden aspirin tablet has been a popular pain killer for longer than we have had access to tablet making machinery (5, 6).

A study showed that in addition to modern biomedicine, traditional medicine provides healthcare to 65-85% of the world's population in developing as well as developed nations (6). Similarly, about 80% of the population uses traditional medicine for primary healthcare in Africa and Ethiopia (1)(7). Since Africa's disease burden is growing rapidly, indigenous African medicine can bring affordable remedies within reach of millions who are unable to access modern care due to its cost, access, or other reasons like to be treated in a more culturally sympathetic and familiar way (8).

Ethiopia has a rich history of traditional medicine and indigenous practices (9)(10). Studies estimated that some 80% of Ethiopians are relying on traditional healers and remedies for

their healthcare needs which is vastly complex and diverse and varies greatly among different ethnic groups (11). The reason that attracts a majority of people in developed countries is its natural product and congruent with their values, beliefs, and philosophical orientations toward health and life (12). In Ethiopia, the practice of TM is mainly due to its closeness, easily affordable, readily available, cheap, and consistent with indigenous cultures or ethnic groups (13, 14). Traditional medicine practices in Ethiopia are not uniformly practiced and there are considerable diversity and significance between regions. They are also not governed by standard policies and regulations rather associated with cultural beliefs. Sometimes, it forms an integral part of a community's identity and value, and it is difficult to regulate through a nationwide framework concerning its safety and effectiveness (13, 15, 16). Observation guided cross-sectional study conducted to obtain ethno botanical data on Traditional knowledge of medicinal plants in Gindeberet district, Western Ethiopia showed that Gindeberet district is rich in its medicinal plant composition and the associated indigenous knowledge (17). However, the prevalence and utilization, as well as factors determining traditional medicine for all categories of population in general and pediatrics in particular are not adequately studied and documented. Determining the magnitude and rationale of childhood traditional medicine utilization has paramount importance. Therefore, this study aims to assess the prevalence and determinants of traditional medicine use of parents of children in Gindeberet Woreda.

2. Methods and materials

2.1. Study Area, design, and period

A community-based cross-sectional study design was employed in Gindeberet Woreda in March 2017. The study was conducted in Gindeberet Woreda which is found in West the west of Shewa, Oromia Region of Ethiopia. Gindeberet (formerly known as Kuttaayee-Liiban) is one of the woreda in the Oromia

Region of Ethiopia. The district is marginal within the central highlands of Ethiopia, being isolated geographically by lowland gorges and rivers which separate it from all but one neighboring district (Abuna Gindeberet), and physically, due to a poor road network. Part of the West Shewa Zone, Gindeberet, is bordered on the south by Jeldu, on the southwest by Ambo, on the west by the Guder River, which separates it from the Horo Guduru Welega Zone, on the north by the Abay River which borders with the Amhara Region, on the east by the Muger River which separates it from the North Shewa Zone, and on the southeast by Meta Robi. The major town in Gindeberet is called Kachisi (it is also called Kachisi). Abuna Gindeberet Woreda is separated from Gindeberet and 188 Km from Addis Ababa.

2.2. Population

The source population was all parents, who had children < 18 years of age who lived in Gindeberet woreda. While all randomly selected parents who had < 18 years of old children and who fulfilled the inclusion criteria were the study population.

2.3. Inclusion and Exclusion Criteria

Parents who were residents of Gindeberet Woreda for at least six months, parents who had children < 18 years who lived with them at least for six months and were available at the time of data collection were included. However, parents who lived in the Woreda for less than six months, parents who were seriously ill or unable to give the required information during the data collection period were excluded.

2.4. Sample Size and Sampling Procedure

2.4.1. Sample Size Determination

The sample size for this study was calculated using single population proportion formula considering the following assumption: prevalence of traditional medicine use for children 88.2% (4), with 5% marginal error, 95% CI ($\alpha = 0.05$). Based on this assumption 162 sample size was calculated as follows:

$$n = z\alpha/2^2 * p * \frac{q}{d^2}$$

$$= (1.96)^2 * 0.88 * \frac{0.12}{(0.05)^2}$$

$$= 162$$

Where: n=the required sample size

z= standard score corresponding to 95%CI

p=prevalence of parental traditional medicine use for children $q=1-p$

d=the margin of error 5%,

The design effect was assumed to be 1.5, owing to the use of two sampling techniques (simple random method and systematic sampling method) supposing that adequate sample size was obtained. Therefore $162 * 1.5 = 243$ and 10% of none response rate 24.3 and a total sample size of 267.

2.4.2. Sampling Procedure

In Gindeberet Woreda, there were 26 kebeles. Of which 5 kebeles [(Waro laku (551), Arada (551), Mishigi (592), Haroo Birbabo (1073), Miaya Qarsa (592)] were selected using a simple random selection method, and participant involvement was selected based on systematic random sampling technique depending on the presence of < 18 children in the household by pre-data collection assessment and numbering. For example for Haroo Birbabo Kebeles, each household was selected based on the numbers of households in that kebeles and the sample size calculated proportionally for that kebeles [Waro laku (44), Arada (44), Mishigi (47), Haroo Birbabo (85), Miaya Qarsa (47)] having < 18 years old children and n is the required sample size). So after random selection of the first household, every 12th household were selected. When this was not possible, the immediate next was considered.

$$k = \frac{N}{n} = \frac{1073}{85} = 12$$

Where k is the sampling interval, N=total number of households

2.5. Data Collection tools and Procedure

The data collection tool was a structured interviewer-administered questionnaire. Either the father or mother of the children was interviewed. But priorities were given to the mothers because mothers are close to their children than fathers. When the mother was not available by any means, the father was interviewed. The questionnaire was adapted from previous research done on similar topics (4) and was translated into the local language (Afan Oromo). Consistency and equivalency were checked by translating the Afan Oromo version back to English by another individual who was fluent in both languages. The questionnaire consists of six parts. The first part is comprised of socio-demographic characteristics of parents. The second part consisted of traditional medicine practice. The third part consist type of TM. The fourth part consists of the socio-cultural environment, the fifth part perception of illness or sickness of the parent, and the sixth part of the questionnaire regarding healthcare experience. Ten health extension workers (HEWs) and five BSc. Nurses for data collection and two supervisors were trained for one day before data collection time. The sessions of the training include the purpose and objectives of the survey, the content of the questionnaires, the meanings of each question and how to approach the respondents and conduct the interview. Data collectors were taking responsibility to interview either parent of children (primarily the mother), consistently record the result, and finally submitted the result to the investigator as scheduled.

2.6. Data Quality Control

Data collectors were trained on how to interview and record the responses. They were assigned out of their respective kebeles to minimize information bias. To assess the validity and reliability of the instrument, clarity of the questions, and respondent reaction to the question and interviewer, the pre-test was done in another kebeles at 5% of actual respondents. After the pre-test, unclear questions were

collected and interviewers and investigators adjusted themselves as required. But the data from the pre-test was not included in the analysis. When the head of household (mother/father) was unavailable during the data collection, period, repeated trials three times were done. During the data collection time, the supervisors and principal investigator to ensure the quality of data did regular monitoring and supervision of the overall activity.

2.7. Data Processing and Analysis

The data were coded, entered, and cleaned, in Epidata version 3.1 and transferred to SPSS version 21.0 for analysis. Descriptive and inferential statistics were used to present the data. Descriptive statistics like frequency and percentage were used to summarize the socio-demographic characteristics of the study participants. And inferential statistics like odds ratio, binary logistic regression, and multiple logistic regression were used to determine if there is an association between the dependent variable (traditional medicine utilization for children) and 23 different independent factors. Statistical significant variables in binary regression analysis i.e. (p -value < 0.2), were entered for multivariate analysis and P value of less than 0.05 is considered as significant at 95% CI.

2.8. Variables

2.8.2. Dependent Variable

Traditional medicine utilization for their children

2.8.2. Independent Variables

- Socio-demographic characteristics of parent
- Predisposing factors like family composition (age, sex, religion, economic level, education, family size, and resident), age of the child
- Socio cultural environment (accessibility, availability, cost, influence from peers/family, religious belief, cultural beliefs,

prevailing socio-cultural concept of illness)

- Perceived illness or sickness (perceived type and nature of illness, perception of illness as serious, and experience of using for the previous child)
- Health care Experience parents (parental CAM use, dissatisfaction with modern medicine, efficacy of CAM, and fear of side effects of modern medicine)

2.9. Ethical Consideration

Ethical clearance and approval to conduct this research were obtained from the Research and Ethical Review Committee of Yanet Health College. Permission to conduct the study was also requested from Gindeberet Woreda Administration. The ethical considerations were taken in to account throughout the study. Participants were informed as their participation was voluntary and that they could withdraw at any time of the study. In addition, the objective of the study was verified by the participants. They were informed about the confidentiality of the data collected. For those who were volunteers to participate, written consents were obtained. At the end of the interview, participants were informed about TM use and associated potential effects.

3. Results

3.1. Socio-demographic characteristics

The survey included a total of 267 households/parents voluntarily with a response rate of 100%. Out of 267 respondents who participated in this study, 172 (64.4%) were females. About 95 (41.2%) of the respondents were within the age groups of 27-32 years old. The majority, 248 (92.9%) were Orthodox religious followers. Most of them, 251 (94%) of the participants, were married. The rate of illiteracy is so high that greater than half of respondents, 142 (53.2%), cannot read and write because they had no access to formal education at all. It was found

that about 85 (31.8%) were living in urban areas with the remaining in rural. Some 136 (50.9%) of the parents have three to four children. More than half of the participants, 151 (56.6%), have low monthly income (<500 birr/month) (Table 1).

3.2. Prevalence of traditional medicine utilization for their children

The survey result showed that of the total 267 participants, 212 (79.4%) have ever used traditional medicine for their children. Thus, the total prevalence of parental traditional medicine practice for children is 212 (79.4%). Of them, 182 (85.9%) had used at least one form of TM for their children in the last 12 months. Based on this study about half (50%) of the parents obtained information about the benefit and efficacy of traditional medicine from their family members. About 16.0% of information source was neighbors (Table 2).

3.3. Type of traditional medicine utilization for their children

About 73 (34.4%), 55 (25.9%), and 25 (11.8%) of respondents used herbal medicine, massage, and religious/prayer therapy for children respectively. However, only 53 (25%) of the parents practiced traditional medicine for their children within the last six months (Table 3). On the other hand, 152 (71.7%) of the respondents have utilized traditional medicine for previous children. They were practicing herbal medicine 60 (39.5%), massage 31 (20.4%), and religious prayer therapy 25 (16.4%). Most of the 127 (83.6%) have been practicing traditional medicine when their children get sick and 77 (50.7%) got these services from the nearby healer (Table 3). In this study more than half of the parents, 141 (52.8%), prefer modern healthcare services compared to 118 (44.2%) who prefer both traditional medicine, as well as modern medicine. Yet, a few 8 (3%) of the parents prefer only traditional medicine (figure 1). Some 102 (48%) of the respondents need the services both on oral and dermal (figure 2) bases.

3.4. Reason for Parental Traditional Medicine Use for Children

The four reasons for parental traditional medicine use for children are grouped into the socio-cultural environment, parental perception of illness/sickness, the parental experience of traditional medicine for themselves, and socio-demographic characteristics. The popular reasons to practice traditional medicine for their children in this study were religious belief 121(57.1%) and easily accessible 118(55.7%) followed by cultural belief 114(53.8%) and cost 114(53.8%) of the TM. The survey revealed that about 89(42.2%) TM users communicate with their traditional practitioners when the need arises.

About 81(38.2%) respondents reported that the overall health status of their children before treatment was fair and 33(15.6%) very poor. Overall 182(85.9%) of them sought TM for acute illness which has less than 30 days duration. Of all, 88(41.5%) and 65(30.7%) of the parents reported that their children became good and very good after treatment, respectively (Table 4). The majority of parents 182(86%) used traditional medicine to treat illness and relief symptoms (figure 3). Parents practice traditional medicine for the symptom of gastrointestinal (27.1%), headache (20.1%), and fever (13.7%), (Figure 4).

More than half 60.3% of family members practice traditional medicine for themselves over the last 12 months. About 50.9% of mothers and 29.2% of both fathers and mothers are practicing traditional medicine. The main reasons for applying traditional medicine for themselves include satisfaction with Traditional Medicine (29.2%) and knowledge of traditional medicine (21.7%). They rated the traditional medicine efficacy as fair (39.8%) and good (34.2%). They also put the level of satisfaction after traditional medicine utilization as somewhat satisfied (36.0%) and completely satisfied (29.8%). Generally, (41.9) of the parents rated the quality of the service they got from the modern health

care system as good and (35.2%) very good (Table 5).

3.5 Factors Associated With Utilization of Traditional Medicine

A bivariate logistic regression analysis was used to determine them. The factors considered and statistically associated with parental TM use for children at p -value < 0.05 (95% C.I.) are; monthly income of household, residence, utilization of TM for the previous child, ease of accessibility of TM, cultural influence, religious influence, parental TM use and, duration of illness (Table 6). This was followed by a further multivariate analysis of variables that showed significance (p -value < 0.2) bivariate associations. After adjusting for potential confounders in multivariate logistic regression analysis: parents who had medium monthly income (500-850) were less likely to use traditional medicine when compared to low-income those who had low income (less than 500) [AOR: 0.25 (0.08, 0.78)]. Parents who perceived TM due to Cultural belief were 3.01 times to use TM [AOR=3.01 (1.16, 7.83)] for children than their counterparts. Parents who utilize TM for children because of religious influence were 3.17 times when compared to those who were not influenced by religious belief [AOR=3.17 (1.26, 7.93)]. Similarly, those have an acute duration of illness (< 30 days) were 3.11 times to use TM for their children when compared to those have a chronic duration of illness (≥ 30 days) [AOR=3.11 (1.07, 9.02)].

4. Discussions

4.1. Prevalence of traditional medicine for children

In this community-based cross-sectional study we assessed the status of traditional medicine utilization among parents for children and its determinants. The prevalence of parental traditional medicine use for children was 212(79.4%). This is in line with findings from other studies in Ethiopia, up to 80% of the

population uses traditional medicine (1). However, this is lower than WHO declaration at Beijing in China that stated about 85% of people worldwide seek traditional health practitioners as their first choice (18). A community-based study done in the Amhara region Kachisi town indicated that 88.2% had used at least one form of traditional medicine for their children in the last 12 months (4). However, the findings from this study were higher than findings from global and western studies. For example, the global rate of using CAM in children varies between 9–73% (17). A National Health Interview Survey, 2007-2012 report in the United States showed that the use of complementary health approaches among children did not change significantly since 2007 (from 12.0% in 2007 to 11.6% in 2012) (19, 20). In Netherlands, 37.6% of gastrointestinal patients are turning to CAM for their child and even more, will do so when their child is not helped adequately by conventional medicine (21). Similarly, a study in Korea on university hospitals indicated that parents or caregivers 51.5% have ever received CAM for their children, and the current CAM utilization rate was 19.0% (22). A study in Ajman, United Arab Emirates (UAE), also showed that about 73 (53.6%) of parents reported the utilization of CAM among their children (23).

The difference might be from methodological and cultural differences between the studies. The previous studies were conducted in a health care setting while this study is at the community level.

4.2. Type of Traditional medicines used for their children

About 73 (34.4%), 55 (25.9%), and 25 (11.8%) of respondents used herbal medicine, massage, and religious/prayer therapy for children respectively. Traditional & CAM practices vary widely from country to country (24). For example in Netherlands herbal remedies (46.0%), food supplements (36.0%), manual therapies (23.7%), and homeopathy (21.9%) were the most commonly used CAM modalities among

pediatrics patients (21). Among cerebral palsy pediatrics patients in the USA, the most common forms of CAM were massage therapy (25%), aqua therapy (25%), and hippo therapy (17%) (25). A study in Ajman, UAE, shows that nearly 80% of the parents reported giving their child herbal medicine among the various forms of CAM therapies. This was followed by dietary supplements, prayer, homeopathy, and massage therapy (23). Traditional healers extract healing ingredients from wild plants, animals, and rare minerals (26). According to a study done in Kachisi town, northern Ethiopia, traditional medicine use for their children reveal herbal medicine 66.9% followed by religious/prayer practice 52.8%, massage 22.8%, bone settler 21.8%, tooth extractors 10.8% and 4.2% have mentioned as they used other forms of traditional medicine named *ye hareg ressa*, *Salehu dress* and *yebuda medhanit* in their culture (4).

Concerning the source of traditional medicine, 77 (50.7%) who practiced T & CAM got these services from the nearby healer. This is in line with findings from West Shewa that showed herbalists are an important national health care resource and they are potentially valuable partners in the delivery of health care (27). In Ethiopia, the traditional health practitioners are categorized into herbalists, bonesetters, traditional birth attendants, spiritual healers, diviners, and magicians (28). Traditional healers such as herbalists, midwives, and spiritual healers constitute the main source of assistance for at least 80-90% of the rural population with health problems in developing countries (29).

In this study less than one-half of parents, 118 (44.2%) preferred both traditional medicine and modern medicine. Only 8 (3%) of parents preferred traditional medicine. This is lower than findings from a community-based cross-sectional study conducted among 271 residents of Jimma town to determine preference and practice of Traditional Medicine and associated factors showed that 25 (9.2%) of the participants preferred to use traditional medicine. Affordability

25 (100%), religious affiliation 21 (84)), and distance from home (20 (80%)) were some reasons for preference (30). The variation could be due to differences in the study population. Our study was conducted to evaluate parental traditional medicine use practice for children, while the Jimma study was conducted to evaluate the preference of the general community. This is because parents are more concerned with the safety of medicines given to their children than themselves. Since the majority of traditional medicine practices have no adequate information on safety and side effects. Only, biofeedback, guided imagery, hypnosis, mindfulness, and yoga are some of the complementary health approaches that have the best evidence of being effective for children for various symptoms (such as anxiety and stress) and are low-risk.

The popular reasons to practice traditional medicine for their children in this study were religious belief 121(57.1%) and ease of access 118 (55.7%) followed by cultural belief 114 (53.8%) and low cost 114 (53.8%). This is supported by other studies that showed high use of herbal medicines may be due to accessibility, affordability, availability, and acceptability of traditional herbal medicines by the majority of the population in developing countries (31). In Ethiopia, traditional medicine is used due to the cultural acceptability of healers and local pharmacopeias, the relatively low cost of traditional medicine, and difficulty in accessing modern health facilities (32). The widespread use of Chinese TM is due to the belief that works and it is part of the history, culture, and politics of the country (32). According to the largest pediatric CAM utilization studies in Canada, parents' reasons for seeking care for their children from CAM providers included, word of mouth, particular treatment was considered effective, fear of drug adverse effects, dissatisfaction with conventional medicine, and the need for more personal attention (33). Similarly, Netherlands parents use CAM for their children due to severe

adverse effects of modern medicine (OR 3.5(1.5-8.2)95%CI) and low perceived effect of modern medicine OR(2.2(1.2-4.1) 95%CI) (22).

The majority of parents 182 (86%) used traditional medicine to treat illness and relief symptoms [gastrointestinal (27.1%), headache (20.1%), and fever (13.7%)]. This is supported by a study conducted in China that showed Traditional Chinese Medicine is used for the treatment of infants, toddlers and children revolve around acute conditions. The most common conditions for infants are colic, fever, cough, and vomiting (34). However, this is against evidence from National Center for Complementary and Integrative Health (NCCIH), for children report, complementary health approaches were most often used for back or neck pain, head or chest colds, anxiety, or stress, other musculoskeletal problems, attention-deficit hyperactivity disorder, and insomnia. In children with other chronic conditions, CAM use is 44% in those with epilepsy 54% with sickle cell disease, 59.6% with diabetes mellitus, 64% with rheumatoid arthritis, 67.6% with attention deficit and hyperactivity, and 64% with other special health care needs (12). The variation could be explained by differences in culture, understanding, and accessibility of conventional medicine (24).

4.3. Factors Associated with Parental Traditional Medicine Use for Children

Parents having a medium monthly income (500-850 Ethiopian birr) were less likely to use traditional medicine when compared to low-income those who had low income (less than 500) [AOR: 0.25 (0.08, 0.78)]. Similarly, a study done in Abuna District, Ethiopia stated that local poor people who had little access and couldn't afford the cost of modern medications had used traditional herbal medicine in their primary health care systems (52). Again study done in Kachisi town, Ethiopia indicated that the reason to use traditional medicine for their children are, 34.4% cheap in price, 31.5% having a low income (4). Studies done in Africa indicated that the use of

traditional medicine was related to low economic status. For instance, Cameroonians still use traditional medicine because they cannot afford pharmaceuticals or conventional medical care. Nearly twice as many people from poor households rely on TM as do people from rich households (6). However, a National Health Interview Survey was conducted in Taiwan among 5,971 children and adolescents showed that a high prevalence of traditional and complementary medicine visits was associated with higher socio economic status (HSES) (35).

Eighty-eight (41.5%) and 65 (30.7%) of the parents reported that their children became good and very good after treatment, respectively. This indicates 153 (72. 2%) of children who received have improved treatment outcomes. However, in UAE, Ajman, from those who used CAM for their children about 47% of parents reported good results and 30% excellent results with CAM in their children (22). The difference could be explained by variations in socioeconomic and cultural factors. In addition to this, it is important to conduct further research on claimed use of traditional medicines practiced in the area to improve treatment outcomes. Traditional medicine should be alternative for a cure not for trial in the community that has incomplete coverage of the modern medical system, shortage of pharmaceuticals, and unaffordable prices of modern drugs (56).

In this study, the cultural belief of parents was significantly associated with parental TM use. Among the total respondents, 114 (53.7%) perceived TM due to it being accepted in the community. This is highly greater than the previous study done in Kenya that some (14.9%) of the respondents believe that herbal medicine is well accepted culturally by the community (36). Similarly, a study done in the Jimma zone of Ethiopia revealed that the local people have been seeking traditional herbal medicine even in preference to modern medications due to some sort of connection with the community's belief (37). This difference indicates there was strong

cultural acceptability of traditional medicine in that community for some diseases. Concerning cultural influence, parents who perceived TM due to cultural belief were 3.01 times to use TM[AOR=3.01(1.16,7.83)]for children than their counterparts. According to one `pediatric CAM utilization studies in Canada showed that many cultural groups may use CAM because of cultural values and beliefs (38). Traditional medicines have the benefit of substantial prior clinical use as well as stronger cultural associations (14). Also, healers understand the social problems and cultural experiences of their communities (7).The socio-cultural environment of the family and the communities were another pushing factors to TM practice (19).

Religious belief is also found to be a key factor that influences parental use of traditional medicine. Parents who have used traditional medicine for their children due to religious beliefs were 3.11 times more likely when compared to those who have no religious view[AOR=3.17, 95% CI: 1.26, 7.93]]. Similarly, in Nigeria, a study indicated that about 23.0% used traditional medicine for their children due to religious beliefs (39). The reasons include strong religious belief in this community with their daily activities and health or the belief that diseases are the manifestation of God's punishment for humanity.

Parents of children with acute illness (< 30 days) were 3.11 times to use TM for their children when compared to those who have a chronic duration of illness AOR=3.11(1.07, 9.02)]. However, The use of CAM and dietary supplements were found to be fairly common in children and adolescents, especially those needing frequent medical care and hospitalizations for chronic conditions (40).Another study medical center in Ann Arbor, USA indicated that children with quadriplegic Cerebral Palsy are more commonly exposed to CAM (OR of 2.5) (1). Similarly study conducted in Korea among patients who visited the pediatric rehabilitation clinic with longer disease duration (≥ 48 months) used CAM when compared with the group with shorter disease duration was OR,

3.36(95%CI1.71–6.59, $p<0.0001$)(22). A study in Ajman, UAE, showed that the common clinical indications reported by the parents for the use of CAM therapy were gastrointestinal disorders and respiratory disorders followed by fever, and dermatological conditions (23). A study conducted in Northern Tanzania showed that the most common reasons for using TM were daily symptomatic ailments and chronic diseases (39).

Strengths and Limitations

The strength of this study relies on its methodology (i.e. being a community based and addressing pediatrics populations who are vulnerable to medication adverse effects). However, the findings of this study should be applied in light of its limitations including addressing only parental characteristics, and due to the cross-sectional nature of the study, it is not possible to identify whether TM practice affects the associated factors and whether there is an association or effect between variables.

Conclusion

There was high parental traditional medicine practice for children in this study 212(79.4%). The reported overall improvement in treatment outcome was about seven out of ten. There was a huge difference between parent preference and the use of traditional medicine. The monthly income of households, cultural influence, religious influence, and, duration of illness were significantly related to parental TM use for their children. Therefore, since there was a high prevalence of TM use in the community, the integration of traditional medicine as part of modern medicine with due consideration of the efficacy of claimed interventions by relevant stakeholders is important. To reduce the gap between preference and practice of traditional medicines, improving access and affordability of modern medicines, and improving healthcare distribution and insurance are critical to getting desired benefits from traditional medicine. Finally, subsequent studies should be done by

accounting limitations of this study for a better understanding of traditional medication practice in children.

Abbreviations

AOR	Adjusted Odd Ratio
ASD	Autism Spectrum Disorder
CAM	Complementary and Alternative Medicine
CDM	Consumer Decision Making Model
CI	Confidence Interval
DD	Developmental Delay
ENV'T	Environment
HEW	Health Extension Worker
HSES	High Socioeconomic Status
HTTP	Harmful Traditional Practice
LSES	Low Socioeconomic Status
NCCIH	National Center for Complementary and Integrative Health
OMPs	Orthodox Medical Practitioners
OR	Odd Ratio
SBM	Socio-behavioral Model
SES	Socioeconomic Status
SPSS	Statistical Package for Social Science
TBA	Traditional Birth Attendant
TCM	Traditional Chinese Medicine
TM	Traditional Medicine
TMPs	Traditional Medicine Practitioner
UAE	United Arab Emirate
USA	United State of America
WHO	World Health Organization

REFERENCES

1. Kebede Deribe, Kebede DK, Alemayehu Amberbir, Binyam Getachew. A Historical Overview of Traditional Medicine Practices and Policy in Ethiopia. *Ethiopian Journal of Health Development*. 2006;20(2):127-34.
2. Rukangira E. Medicinal plants and traditional medicine in Africa: constraints and challenges. *Sustainable Development International*. 2001;4:179-84.
3. Organization WH. WHO country cooperation strategy: India 2012-2017. 2012.
4. Melesse TG, Ayalew Y, Getie GA, Mitiku HZ, Tsegaye G. Prevalence and factors associated with parental traditional medicine use for children in Motta Town, Amhara Regional State, Ethiopia, 2014. *Altern Integr Med*. 2015;4(179):2.
5. Raphael EC. Traditional medicine in Nigeria: current status and the future. *Research journal of pharmacology*. 2011;5(6):90-4.
6. Abdullahi AA. Trends and challenges of traditional medicine in Africa. *African journal of traditional, complementary and alternative medicines*. 2011;8(5S).
7. Hillenbrand E. Improving traditional-conventional medicine collaboration: perspectives from Cameroonian traditional practitioners. *Nordic Journal of African Studies*. 2006;15(1).
8. Anselm A. Modern African remedies of herbal medicine and community development in Nigeria. Policy Voice Series, United Kingdom London: African Research Institute. 2015.
9. Enawgaw M, Zergabachew A. The manual of Ethiopian medical history. *People to People*. 2013;7:12.
10. Solomon T. Assessment of Possible Intellectual Property Protection Options of Traditional Knowledge Systems in Ethiopia: Special Reference in Herbal Medicine for Livestock. 2012.
11. Chandrodyam S. Studies on traditional medicinal plants in Ambagiorgis area of Wogera District, Amhara Regional State, Ethiopia. *International Journal of Pure & Applied Bioscience*. 2016;4:38-45.
12. Gibbard WB. The use of complementary and alternative medicine by children and adolescents with autistic spectrum disorders: *Medicine*; 2005.
13. Adams D, Dagenais S, Clifford T, Baydala L, King WJ, Hervas-Malo M, et al. Complementary and alternative medicine use by pediatric specialty outpatients. *Pediatrics*. 2013;131(2):225-32.
14. Abyot E, Zewdu B, Tefera A, Mohammedberhan A, Mulugeta F. Capacity buildings of traditional medicine practitioners' as a primary health Care Workers in Gondar Town, Northwest Ethiopia. *J Homeop Ayurv Med*. 2014;3(1000151):2167-1206.
15. Organization WH. Technical Consultation on Medium-term Agenda on Traditional Medicine for Universal Health Coverage in the Western Pacific Region, Manila, Philippines, 28-29 June 2018: meeting report. 2018.
16. Abbott R. Documenting traditional medical knowledge. Ryan Abbott, Documenting Traditional Medical Knowledge, World Intellectual Property Organization (March, 2014). 2014.

17. Zerabruk S, Yirga G. Traditional knowledge of medicinal plants in Gindeberet district, Western Ethiopia. *South African Journal of Botany*. 2012;78:165-9.
18. Antwi-Baffour SS, Bello AI, Adjei DN, Mahmood SA, Ayeh-Kumi PF. The place of traditional medicine in the African society: the science, acceptance and support. *American Journal of Health Research*. 2014;2(2):49-54.
19. Black LI, Clarke TC, Barnes PM, Stussman BJ, Nahin RL. Use of complementary health approaches among children aged 4-17 years in the United States: National Health Interview Survey, 2007-2012. *National health statistics reports*. 2015(78):1-19.
20. Black LI, Clarke TC, Barnes PM, Stussman BJ, Nahin RL. Use of complementary health approaches among children aged 4–17 years in the United States: National Health Interview Survey, 2007–2012. *National health statistics reports*. 2015(78):1.
21. Vlieger AM, Blink M, Tromp E, Benninga MA. Use of complementary and alternative medicine by pediatric patients with functional and organic gastrointestinal diseases: results from a multicenter survey. *Pediatrics*. 2008;122(2):e446-e51.
22. Jeong M-J, Lee H-Y, Lim J-H, Yun Y-J. Current utilization and influencing factors of complementary and alternative medicine among children with neuropsychiatric disease: a cross-sectional survey in Korea. *BMC complementary and alternative medicine*. 2016;16(1):1-8.
23. Lisha Jenny J, Jayakumary M, Jayadevan S, Shatha AS, Mohamed A, Maryamossadat M, et al. Utilization of complementary and alternative medicine by parents for their children: a cross sectional study in Ajman, UAE. 2013.
24. Lhamo N, Nebel S. Perceptions and attitudes of Bhutanese people on Sowa Rigpa, traditional Bhutanese medicine: a preliminary study from Thimphu. *Journal of Ethnobiology and Ethnomedicine*. 2011;7(1):1-9.
25. Hurvitz EA, Leonard C, Ayyangar R, Nelson VS. Complementary and alternative medicine use in families of children with cerebral palsy. *Developmental medicine and child neurology*. 2003;45(6):364-70.
26. Fulas. F. Ethiopian Traditional Medicine in Scientific Perspective: Herbal Medicine.2010.
27. Kubukeli PS. Traditional healing practice using medicinal herbs. *The lancet*. 1999;354:SIV24.
28. Mangue P, Nacala M. Data Collection and Analysis for Forest Management in ACP Countries; Linking National and International Efforts, Country Report on Woodfuels Review and Assessment. Ministry of Agriculture and Rural Development 60pp. 2000.
29. Zhang X, Organization WH. Traditional medicine strategy 2002 2005. 2002.
30. Chali BU, Hasho A, Koricha NB. Preference and Practice of Traditional Medicine and Associated Factors in Jimma Town, Southwest Ethiopia. *Evidence-Based Complementary and Alternative Medicine*. 2021;2021:9962892.
31. Tamuno I. Traditional medicine for HIV infected patients in antiretroviral therapy in a tertiary hospital in Kano, Northwest Nigeria. *Asian Pacific journal of tropical medicine*. 2011;4(2):152-5.
32. Goss PE, Strasser-Weippl K, Lee-Bychkovsky BL, Fan L, Li J, Chavarri-Guerra Y, et al. Challenges to effective cancer control in China, India, and Russia. *The lancet oncology*. 2014;15(5):489-538.

33. Italia S, Brand H, Heinrich J, Berdel D, von Berg A, Wolfenstetter SB. Utilization of complementary and alternative medicine (CAM) among children from a German birth cohort (GINIplus): patterns, costs, and trends of use. *BMC complementary and alternative medicine*. 2015;15(1):1-10.
34. JAKE PAUL FRATKIN O, LAC. Treating Infants and Small Children With Chinese Herbal Medicine. *Acupuncture Today*. 2007;08(09).
35. Shih C-C, Liao C-C, Su Y-C, Yeh TF, Lin J-G. The association between socioeconomic status and traditional chinese medicine use among children in Taiwan. *BMC Health Serv Res* [Internet]. 2012 2012/02//; 12:[27 p.]. Available from: <http://europepmc.org/abstract/MED/22293135>
<https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/22293135/pdf/?tool=EBI>
<https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/22293135/?tool=EBI>
<https://doi.org/10.1186/1472-6963-12-27>
<https://europepmc.org/articles/PMC3359156>
<https://europepmc.org/articles/PMC3359156?pdf=render>.
36. Ondicho JM. Factors associated with use of herbal medicine among the residents of Gucha sub-county, Kenya: JKUAT; 2015.
37. Yineger H, Yewhalaw D. Traditional medicinal plant knowledge and use by local healers in Sekoru District, Jimma Zone, Southwestern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*. 2007;3(1):1-7.
38. Kemper KJ, Vohra S, Walls R. The use of complementary and alternative medicine in pediatrics. *Pediatrics*. 2008;122(6):1374-86.
39. Rumun AJ. The socio-cultural factors influencing the utilization of maternal and child healthcare services in kwande local government area of Benue state, Nigeria. *Int J Humanit Soc Sci Invent*. 2013;2(7):19-20.
40. Ventola CL. Current issues regarding complementary and alternative medicine (CAM) in the United States: part 1: the widespread use of CAM and the need for better-informed health care professionals to provide patient counseling. *Pharmacy and Therapeutics*. 2010;35(8):461.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) STABILITY INDICATING METHOD FOR THE DETERMINATION OF BROMAZEPAM VIA ITS COPPER (II) CHELATES

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Abstract

A stability-indicating HPLC method was developed and validated for determination of bromazepam by chelation with copper (II). The chromatographic separation of the drug by chelation with copper (II) and from its degradation products obtained during acid hydrolysis was achieved isocratically on octyl C-8 column (Beckman) with 250 x 4.6 mm internal diameter and 5 μ m particle size using a mobile phase containing methanol, acetonitrile and buffer adjusted to pH 6.2 with glacial acetic acid (26.5:21.5:52 v/v%) at a flow rate of 1.5ml/min and UV detection wavelength at 309nm. The buffer was composed of potassium dihydrogen phosphate (0.005M) and ammonium acetate (0.1M). The method was validated for linearity, range, precision, and accuracy, limit of detection and limit of quantitation. Linear regression analysis data for the calibration plot showed there was a good linear relationship between response and concentration in the range 20 - 50 μ g/ml with a correlation coefficient of 0.9989. The limit of detection and quantitation were determined to be 3.36 and 11.21 μ g/ml, respectively. The slope (\pm SD) and intercept (\pm SD) were 1080.41 \pm 33.27 and -1186.15 \pm 1211.13, respectively. The RSD values for intra and inter day precision were <2.15 and <2.65%, respectively. The recovery of the drug from a mixture of spiked samples ranged between

94.28 and 99.7%. The method was successfully applied for determination of the drug in commercial tablet dosage form.

1. INTRODUCTION

Anxiolytics are drugs used to relieve anxiety or prevent anxiety attack which is broadly defined as a state of unwarranted or inappropriate worry, often accompanied by restlessness, tension, destruction, irritability, and sleep disturbances [1]. Benzodiazepines are among the most frequently used psychotropic drugs because of their efficiency, rapid effect and low toxicity. Since the introduction of the first benzodiazepine, chlordiazepoxide, in 1960, more than 50 benzodiazepine drugs has been made available for the treatment of anxiety.

Bromazepam ($C_{14}H_{10}BrN_3O$), chemically known as 7-bromo-1, 3-dihydro-5(2-pyridyl)-2H-1, 4-benzodiazepin-2-one, is a member of the 1, 4-benzodiazepine drug that was first synthesized by Fryer, in 1964 [2, 3]. Bromazepam, like most of the benzodiazepine derivatives, is hydrolyzed in acidic aqueous solution leading to a series of degradation products [2]. The rate of acidic hydrolysis of bromazepam is believed to be dependent on the state of protonation of the pyridyl and azomethine nitrogen atoms [4, 5]. It is hydrolyzed in acidic condition via intermediate product to give the final product of 2(2-amino-5-bromobenzoyl) pyridine (II) and glycine [6, 7].

Different methods such as non-aqueous titration [8] flow injection [9], sequential injection [10], voltammetric [4, 11], spectrophotometric [12, 13], chromatographic [14 - 16], capillary electrophoresis [17], and ion selective sensor methods [18] have been reported for the determination of bromazepam in pharmaceutical preparations, blood and plasma. Though BP recommends non-aqueous titration and spectrophotometric methods for the analysis of bromazepam in raw materials [8], there is no official method for its analysis in tablets. In addition to determination of the active components in a specific drug, stability test is important in pharmaceutical industry to provide evidence on how the quality of an active

substance or pharmaceutical product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light.

Although different chromatographic methods such as thin layer chromatography (TLC) [19, 20], HPTLC [21, 22], and gas chromatography (GC) [23, 24] have been reported for stability indicating assay, their application is limited; for example, the variability and non-quantitative nature of TLC, expensiveness of HPTLC, and thermally instability of the drug in case of GC can be taken as limitations.

Alternatively, HPLC has been very widely employed. It has been used in stability studies due to its high resolution capacity, sensitivity and specificity. Non-volatile, thermally unstable or polar/ionic compounds can also be analyzed by this technique. Therefore, most of the stability indicating assay methods have been established using HPLC [25, 26]. Therefore, in this study, we developed a simple analytical and stability indicating method for the determination of bromazepam based on bromazepam-copper (II) complex formation using the $-N=C-C=N-$ moiety, that enables bromazepam to form complex with some divalent metal ions such as copper (II), cobalt (II), nickel (II) and iron (II) [11].

2. EXPERIMENTAL

2.1. Chemicals and Solvents

Bromazepam (of 99.3% purity) working standard was obtained from Remedica Pharmaceuticals Ltd. (Remedica, Cyprus). HPLC grade methanol and acetonitrile, methanol absolute acetone free, copper (II) nitrate, ammonium acetate, potassium dihydrogen phosphate, glacial acetic acid, sodium hydroxide, chloroform and acetone were purchased from Fisher Scientific (UK). Sulfuric acid was obtained from BDH (England). Bromazepam tablets (Lexotanil®, Roche, Switzerland) were obtained from local pharmacy

in Addis Ababa, Ethiopia. Deionized water was used throughout the experimental works.

2.2. Instruments and Equipment

Spectronic unicam Ultraviolet-visible double beam spectrophotometer (model number-UVA 083430 type Helison alpha) using 1 cm quartz cells was used to obtain UV-visible spectra by scanning from 200 to 500 nm wavelength pumping system (LC-20AT), auto sampler unit (SIL-20A), column oven (CTO-20AC), Ultraviolet-visible detector (SPD-20AV), communication bus module (CBM-20A), personal computer installed with class VP software for data integration and analysis was used for HPLC analysis.

2.3. Preparations of Solutions

2.3.1. Preparation of Bromazepam-Copper (II) Complex

Initially 500 µg/mL methanolic standard bromazepam solution was prepared and the working solutions were prepared from the standard solution just before the experiments. Similarly standard copper (II) solution was prepared by dissolving 60.5 mg of copper (II) nitrate in 50 mL volumetric flask using methanol as the solvent. From these solutions bromazepam- Cu (II) complex was prepared.

2.3.2. Preparation of Degraded Bromazepam Solution for Kinetic Study

Forced degradation of bromazepam was performed under acid hydrolysis using 10 N sulphuric acid. Laboratory degraded bromazepam solution (200 µg/mL) was prepared by taking 50 mg of pure bromazepam in a quick fit flask and then adding 10 mL of 10 N sulfuric acid. This solution was refluxed in boiling water bath for 3 hours and then neutralized with 10 N sodium hydroxide. The mixture was then quantitatively transferred into a separatory funnel and extracted with 15 mL diethyl ether three times by shaking with magnetic shaker for 10 minutes. The ether extracts (upper layers) were collected in an evaporating dish and evaporated to dryness in a hot water bath (approximately 50°C) and dried in a vacuum desiccator over

sulfuric acid for 30 minutes. Finally, the residue was transferred to a 250 mL volumetric flask with methanol. The degraded was tested for its complete degradation by TLC using silica gel 60 F254 plates and a mobile phase of chloroform: acetone (4:1, v/v), and chloroform: methanol (2:1, v/v). The developed plate was visualized under UV light at 254 nm.

2.3.3. Sample Preparation Using the Dosage Form

Twenty tablets were taken and their mean weight was determined. They were powdered with mortar and pestle and mixed to homogeneity. The powdered tablets equivalent to 25 mg of bromazepam was weighed and transferred into a 250 mL beaker. The drug from the powder was extracted three times with 80 mL of methanol at a time. To ensure complete extraction of the drugs, the mixture was sonicated for about 20 minutes before separation of the two phases. Whatman filter paper wetted with methanol was used to filter the resulting methanol extract and diluted in 250 mL volumetric flask using methanol.

2.4. Chromatographic Condition

Chromatographic separation of bromazepam chelated with copper (II) was performed using reversed phase octyl C-8 column (Beckman) (250 x 4.6 mm internal diameter and 5 µm particle size). Separation was achieved under isocratic conditions using a mobile phase consisting of a mixture of HPLC grade methanol and acetonitrile, and buffer (26.5:21.5:52). The buffer was composed of 0.005 M potassium dihydrogen phosphate and 0.1 M ammonium acetate adjusted to pH 6.2 with 0.1 M glacial acetic acid. The mobile phase was filtered through a 0.45 µm Millipore filter and degassed in ultrasonic bath prior to use. HPLC measurements were done by setting the conditions as follows: the flow rate of the mobile phase was 1.5 mL/min; injection volume was 10 µL, column temperature was 30°C with the detector wavelength of 309 nm. Then, the condition of chromatographic system assumed to give good separation efficiency such as good resolution

between peaks, minimum tailing or fronting and retention time repeatability were optimized.

2.5. UV-Visible Spectrophotometry and HPLC Measurements

2.5.1. UV-Visible Spectrophotometric Measurement of Pure Bromazepam Solution

The maximum absorbance of bromazepam solution was determined from working solutions of bromazepam of concentration 10, 15, 20, 30, 40 and 50 µg/mL. Similarly, for the degraded bromazepam solution, three solutions of concentrations 16, 20 and 50 µg/mL were taken and scanned with the same wavelength range.

2.5.2. UV-Visible Spectrophotometric Measurement Bromazepam-Copper (II) Complex

A range of bromazepam solution (10 to 50 µg/mL) were prepared and transferred to 50 mL volumetric flasks followed by addition of 2.5 mL of copper (II) nitrate stock solution and completed to volume with methanol and then scanned. Similarly, for the complex of degraded bromazepam with copper (II), degraded bromazepam solution (15, 20 and 50 µg/mL) were prepared and transferred to a 50 mL volumetric flasks followed by addition of 2.5 mL of copper (II) nitrate stock solution and completed to volume with methanol. The resulting solutions were sonicated and absorbance measurements were done.

2.5.3. HPLC Determination of Pure and Degraded Bromazepam

For HPLC determination of pure bromazepam 10, 15 and 25 mL were taken from the working solution and transferred to 50 mL volumetric flask and completed to volume with methanol. Then the solutions were sonicated for about 20 minutes and filtered through a 0.45 µm Millipore filter and injected into the HPLC column. Similarly, for degraded bromazepam 5, 7.5 and 12.5 mL were taken from the stock solution and transferred to 50 mL volumetric flask and completed to volume with methanol. Then the solutions were sonicated for about 20 minutes

and filtered through a 0.45 µm Millipore filter and injected into the HPLC column.

2.5.4. HPLC Determination of Bromazepam-Copper (II) Complex

In order to obtain the calibration curve for bromazepam- copper (II) complex, seven working solutions of concentrations 20, 25, 30, 35, 40, 45 and 50 µg/mL were prepared and transferred to a 50 mL volumetric flasks followed by addition of 2.5 mL of copper (II) nitrate stock solution and completed to volume with methanol. The resulting solutions sonicated for about 20 minutes and filtered through a 0.45 µm Millipore filter and injected into the HPLC column.

2.5.5. HPLC Determination of the Dosage Form

From the stock sample solution of the dosage form 10 mL was taken and transferred to a 50 mL volumetric flask followed by addition of 2.5 mL of copper (II) nitrate stock solution and completed to volume with methanol. Then the resulting sample solution was sonicated for about 20 minutes and filtered through a 0.45 µm Millipore filter and injected in triplicate into the HPLC column.

3. RESULTS AND DISCUSSION

3.1. Spectrophotometric Study

Initially, spectrophotometric measurements were performed to study the absorption features of bromazepam, degraded bromazepam, copper (II) solution, bromazepam and degraded bromazepam in the presence of copper (II) solution in wavelength range of 200 to 500 nm.

As shown in the UV-visible spectrum of bromazepam in Fig. (1a), two maximum absorption bands were observed at 234 and 325 nm from which 234 nm was selected for HPLC determination of bromazepam for it is more intense.

Similarly two maximum absorption bands at 237 and 396 nm were observed for the degradation product of bromazepam as demonstrated in Fig.

(1b). Only one maximum absorbance was available for copper (II) solution around 214 nm (spectrum not shown).

Complex formation of copper (II) with bromazepam with copper (II) resulted in appearance of four bands at wavelengths of 214, 241 and 284 nm and a small hump at 357 nm Fig. (1c) and the maximum absorption band at 284 was taken for HPLC study of bromazepam-copper (II) complex for 214 and 241 nm could be

highly dependent on the concentration of copper (II) so that band overlaps would be a problem. The two bands that have been observed for copper (II) and the degraded bromazepam are observed for their mixture UV-visible absorbance spectrum. The formation of no new bands in the mixture as shown on Fig. (1d) confirms that degraded bromazepam cannot form complex with copper (II).

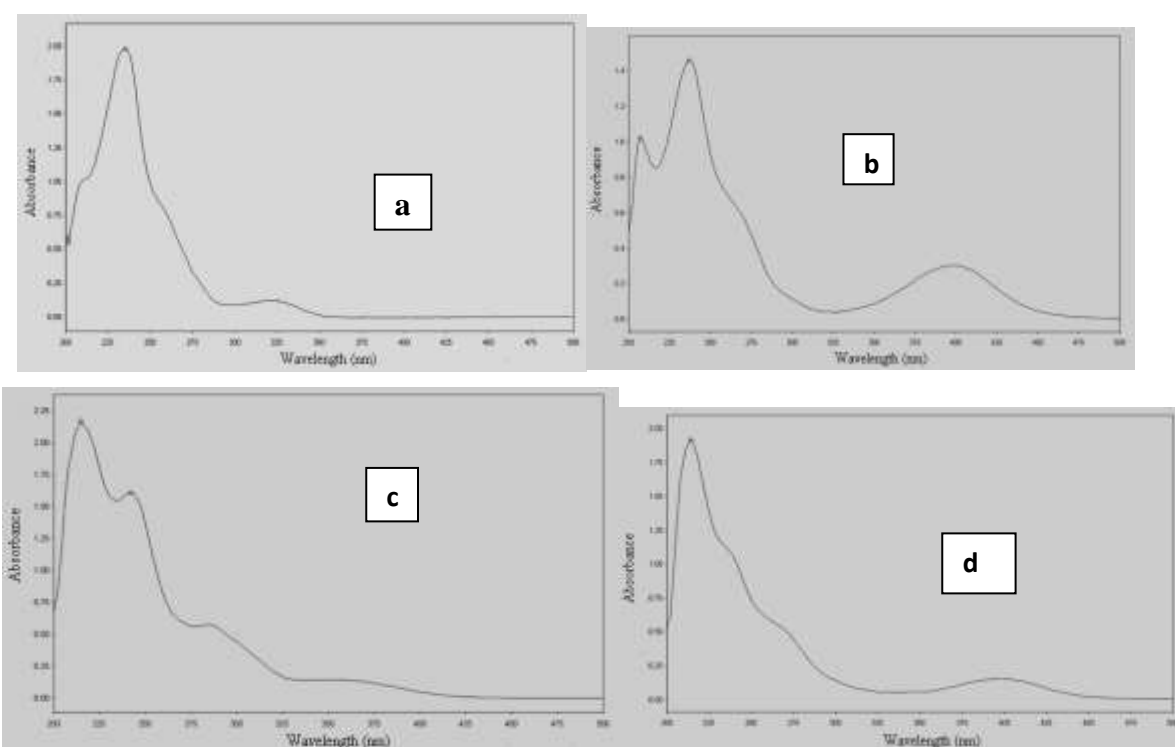


Fig. 1. UV-visible spectra of methanolic solution of 20 µg/mL of (a) intact bromazepam (b) degraded bromazepam (c) intact bromazepam in the presence of 0.0605 mg/mL of copper (II) and (d) degraded bromazepam in the presence of 0.0605 mg/mL of copper (II).

3.2. Forced Degradation Study

Forced decomposition studies were conducted to generate degradation products of the drug for the development of stability indicating assay method.

These studies are employed under different conditions such as hydrolytic, photolytic and oxidative decomposition. The hydrolytic degradation of a drug can be studied by refluxing the drug in acidic and alkaline conditions. In a similar manner, degradation under neutral condition can be studied by refluxing the drug in water. To test for oxidation, it is suggested to use hydrogen peroxide in the concentration range of 3-30% and the photolytic studies should be carried out by exposure to light [25, 27].

In this investigation acid hydrolysis using 10N H₂SO₄ was carried out for the degradation study of bromazepam. The acid hydrolysis of bromazepam resulted in the formation of 2-amino-5-bromobenzoyl pyridine and glycine [7, 9]. The 2-amino-5-bromobenzoyl pyridine was separated from glycine by extracting with ether to avoid glycine interference.

The degraded was tested by TLC using silica gel 60 F254 plates, and the mobile phases chloroform: acetone (4:1, v/v) [7] and Chloroform: methanol (2:1, v/v) [28] for the presence of any undegraded bromazepam. About 20 µL from the stock solution of bromazepam, its degradation product obtained by acid hydrolysis and a mixture containing both were applied in form of a band on a silica gel plate. Then it was developed for about 45 minutes. The developed plates were inspected under UV light at 254 nm and R_f values for observed spots were calculated.

In chloroform: acetone (4:1, v/v), bromazepam gave a blue spot under UV light at 254 nm at the base line, which was initially colorless solution, where as its degradation product gave a yellow spot with an R_f value of 0.72. The mixture gave two spots, blue at the point of application and the other yellow having an R_f value of 0.72. In chloroform: methanol (2:1, v/v), bromazepam gave a blue spot under UV light at 254 nm, at an R_f of 0.55, where as its degradation product gave a yellow spot at an R_f of 0.84. The mixture gave spots with the same color and R_f value as that of bromazepam and its degradation product. The above TLC results of degradation product showed the absence of any undegraded bromazepam which indicates that the degradation was completed.

Chelation of degraded bromazepam with copper (II) was carried out and did not show any complexation Fig. (1d) or change of color through the adjacent nitrogen and oxygen atoms under the experimental conditions. This is probably because oxygen is more electronegative with a

lower electron-donating capability than nitrogen [10]. Hence this renders the stability-indicating assay method more useful for the assay of bromazepam and for checking the extent of its degradation in pharmaceutical preparations.

3.3. Development and Optimization of the Stability-Indicating HPLC Method

An isocratic high performance liquid chromatographic method was developed for the determination of bromazepam in the presence of its degradation products by chelation with copper (II). To optimize the HPLC assay parameters the type of column and its dimension, mobile phase condition and choice of wavelength of detection from spectrophotometric measurements were investigated.

Different types of columns were used, such as ODS-2 C-18 column (Inertsil), ODS C-18 column (Beckman) and octyl C-8 column (Beckman) with 250 x 4.6 mm internal diameter and 5 µm particle size.

Different mobile phases with different composition were used, such as acetonitrile and methanol (3:1, v/v); acetonitrile, methanol and 0.1 M acetic acid (3:1:1, v/v/v); methanol, acetonitrile and a mixture of potassium dihydrogen phosphate and ammonium acetate in the ratio 2:1:1, 1:2:1 and 26.5:21.5:52 (v/v/v) by adjusting the pH with glacial acetic acid. The injection volume was varied from 5 to 20 µL, and the flow rate varied from 0.8 to 2 mL/min. The temperature was varied from 25 to 35°C and that of run time was also varied from 10 to 25 minutes.

We followed Panderi *et al.* [2] procedure to optimize the conditions for the chromatographic system and resulted in good separation efficiency such as good resolution between peaks, minimum tailing or fronting and retention time repeatability.

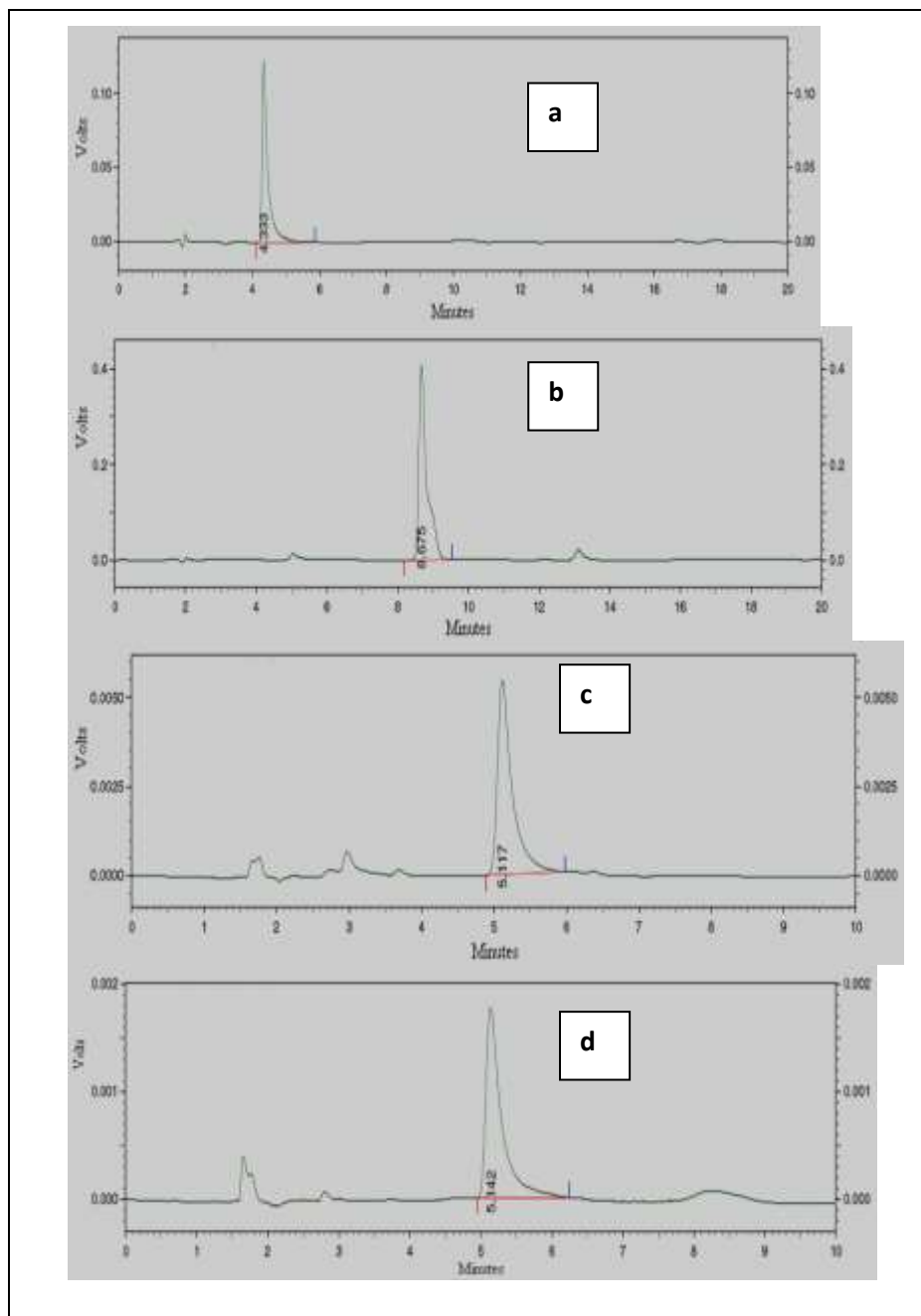


Fig. 2. Typical chromatogram of bromazepam (a), degraded bromazepam (b), and bromazepam chelated with copper (II) at 284 nm (c) and at 309 nm (d).

3.4. HPLC Determination

Based on the UV-visible spectrophotometric results, bromazepam, its degradation product and bromazepam chelated with copper (II) were determined using the developed mobile phase

with flow rate of 1.5 mL/min. The detector was set at 234 nm for the determination of bromazepam and its degradation product, and wavelengths of 284 and 309 nm were used for the determination of bromazepam chelated with copper (II) as shown in Fig. (2). Good separation with sharp peak, minimum tailing and retention time repeatability was obtained. Retention time for bromazepam and for its degradation was found to be 4.333 and 8.675 min respectively. Bromazepam chelated with copper (II) had

almost the same retention time of 5.117 and 5.142 min at wavelengths of 284 and 309 nm respectively.

3.5. Hydrolysis of Bromazepam Under Acidic Condition

Benzodiazepines are unstable substances, because they easily hydrolyze in acidic solution and also decompose in UV light. Hydrolysis in acidic solution leads generally to 2-minobenzophenone derivatives, through the splitting of the N1-C2 bond of the diazepine ring [29]. Bromazepam like most of the benzodiazepine derivatives is hydrolyzed in acidic aqueous solutions leading to a series of degradation products. The mechanism of acid hydrolysis of bromazepam proceeds through the intermediate N-(4-bromo-2(2-pyridylcarbonyl) phenyl)-2-aminoacetamide leading to the final products, 2-amino-5-bromobenzoyl pyridine and glycine. As it was reported, formation of the intermediate is very fast while the formation of the final product is rather slow [2].

Kinetic study of bromazepam degradation under acid hydrolysis (10 N H₂SO₄) was carried out at different time points (30, 60, 90, 120 and 150 minutes). Degradation was performed by refluxing in boiling water bath for the specified time points separately. After refluxing the solution was neutralized with 10 N NaOH. Then it was extracted with diethyl ether and evaporated to dryness in a hot water bath and dried over sulfuric acid. Finally the residue was taken up with methanol and determined at a wavelength of 309 nm and a flow rate of 1 mL/min. The corresponding HPLC chromatograms of the degradation product after acid hydrolysis are shown in Fig. (3) and the percent peak area of the chromatograms at different time points are recorded Table 1. From the chromatogram it was observed that the retention time of intact bromazepam was 7.265 ± 0.290 and that of the degradation product was 2.787 ± 0.196 .

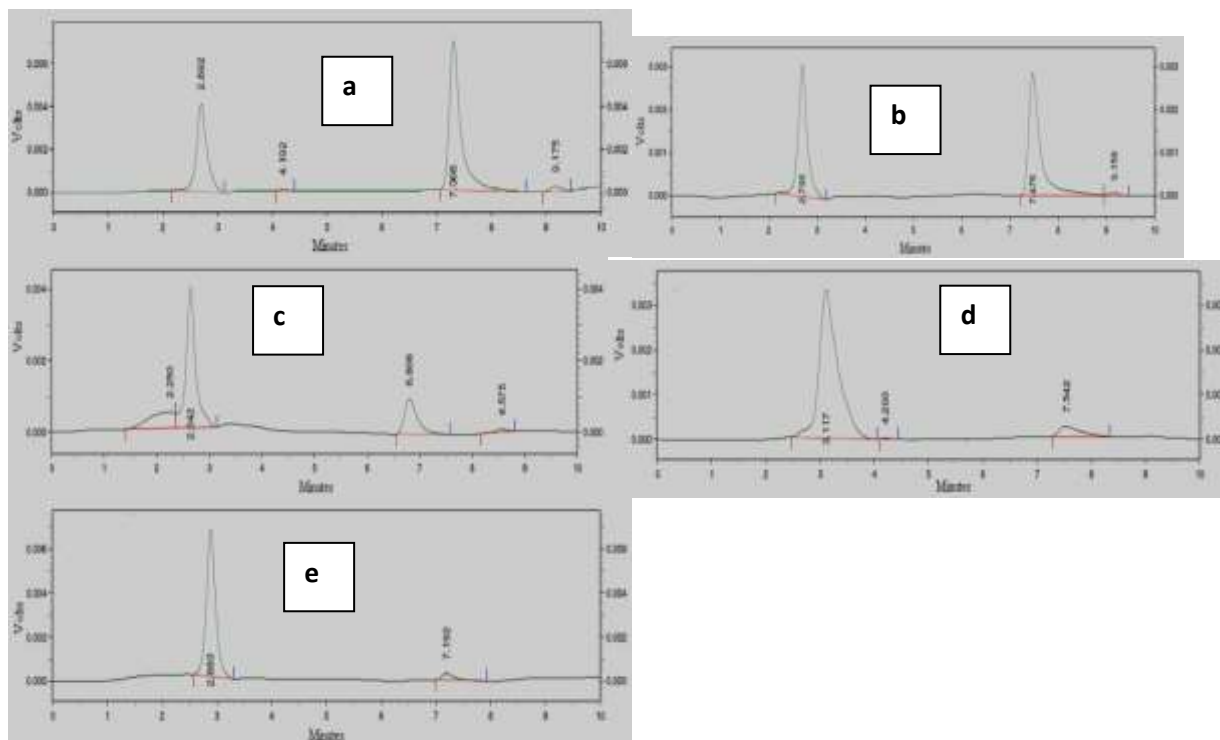


Fig. 3. Chromatogram of degradation products of bromazepam obtained during the kinetic study of

acid hydrolysis after 30 (a), 60 (b), 90 (c), 120 (d), and 150 (e) minutes.

For kinetic calculations, time zero was considered as the time before degradation and the peak area obtained at zero time was regarded as 100%. Appearance of degradation products and disappearance of the parent compound were analyzed on the basis of percent areas. As observed from Table 1, the HPLC chromatogram percent area versus time profile, after the reaction proceeds for 30 minutes about 60% of bromazepam and after 60 minutes about 40% of bromazepam was present in the reaction

medium. Thus, within the first 30 minutes, bromazepam decreased rapidly to its degradation product, while after 60 minutes the degradation product increased rapidly and most of bromazepam was actually degraded.

Table 1- Percentage peak area of bromazepam and its degradation product in the chromatogram after degradation at different time points

Time (min)	Bromazepam area (%)	Degradation product area (%)
0	100	0
30	63.719	33.854
60	40.247	52.844
90	19.936	60.359
120	9.279	87.293
150	5.863	94.137

The rate order, rate constant and half-life of degradation were also determined and it was observed that the degradation reaction follows first order kinetics. The rate constant and half-life were calculated from the first order equation and found to be 0.015 and 46.2 minutes respectively.

3.6. Analysis of Dosage Form

The proposed method was applied to the determination of bromazepam in marketed formulation (Lexotanil® tablets). The corresponding chromatogram obtained following

the assay of bromazepam tablets is shown in Fig. (4). From the experimental results the amount of bromazepam in replicate analysis (n = 3) was found to be 98.63% of the label claim for the tablets and its retention time was found to be 5.003 ± 0.134 . The retention time found was in a good agreement as that was obtained for bromazepam (Fig. 2d). The results of the assay indicate that the method is selective for bromazepam in pharmaceutical dosage forms since it was in good agreement with the label claim without any interference from the excipients which are normally present in tablets.

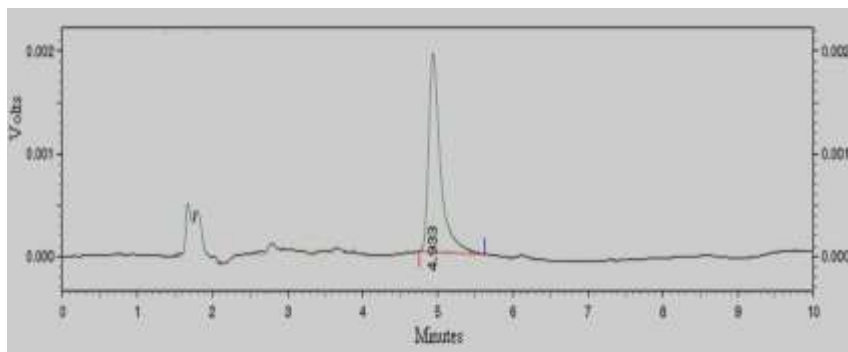


Fig. 4. Chromatogram of bromazepam obtained from analysis of tablet dosage forms.

3.7. Method Validation

One of the most critical factors in developing pharmaceutical drug substances and products is ensuring that the analytical test methods used to analyze fine chemicals and products generate valid and meaningful data in terms of reliability, accuracy and precision, regardless of whether it is intended for acceptance, release, stability or pharmacokinetic studies [30]. Therefore, in this work validation parameters such as linearity, precision, accuracy, limit of detection and limit of quantitation were used in developing this method and the results are given in supplementary data.

The linearity of the proposed method was determined by measuring the peak area at different concentration levels. The calibration curve was constructed by plotting mean peak area versus concentration of bromazepam and linear relation for the concentration range of 20 to 50 $\mu\text{g/mL}$ was obtained with correlation coefficient of 0.9976. The precision of the proposed method was determined as repeatability (intra-day precision) and intermediate precision (inter-day) and expressed in terms of percentage relative standard deviation (%RSD) of the peak area. The analyses were performed using concentrations at three levels (25, 35 and 45 $\mu\text{g/mL}$). Each concentration was analyzed in five replicates ($n = 5$) for intra-day precision and inter-day precision was analyzed in triplicate ($n = 3$) on three separate days. The results showed that the RSD values for intra-day and inter-day precision were found to be less than 2.15%

and 2.65%, respectively which is within acceptable range.

For determination of accuracy in this method, standard addition method was employed. Thus, equal amount of the dosage form were spiked with three different amounts of the reference standard of the drug product. Later on copper (II) solution was added and subsequently analyzed by the proposed method. Then, the response for both spiked and unspiked preparations have been measured. Accuracy of the method was calculated as percentage recovery from the regression equation and satisfactory recoveries (94.28 – 99.74%) of the spiked drug were obtained at each added concentration level.

Finally, limit of detection and quantitation were determined for bromazepam chelated with copper (II) by the proposed method based on the standard deviation of the response (SD) and the slope of the calibration curve (S) and the limit of detection and quantitation were found to be 3.36 and 11.21 $\mu\text{g/mL}$ respectively.

CONCLUSION

HPLC stability indicating method was developed for the determination of bromazepam in the tablet dosage forms. Its property to form complex with copper (II) is the essential property for developing this method. Chromatographic separation of bromazepam chelated with copper (II) was best achieved using octyl C-8 column. Determination of kinetic order for degradation of bromazepam was found to be simple. This method can be further used in drug

manufacturing quality control or regulatory analysis laboratory for determination of bromazepam in pharmaceutical dosage forms.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

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REFERENCES

- [1] Sandford JJ, Argyropoulos SV, Nutt DJ. The psychobiology of anxiolytic drugs. Part 1: Basic neurobiology. *Pharmacol Ther* 2000; 88(3):197-212.[[http://dx.doi.org/10.1016/S0163-7258\(00\)00082-6](http://dx.doi.org/10.1016/S0163-7258(00)00082-6)] [PMID: 11337025]
- [2] Panderi I, Archontaki H, Gikas E, Parissi-Poulou M. Acidic hydrolysis of bromazepam studied by high performance liquid chromatography. Isolation and identification of its degradation products. *J Pharm Biomed Anal* 1998; 17(2): 327-35. [[http://dx.doi.org/10.1016/S0731-7085\(97\)00201-X](http://dx.doi.org/10.1016/S0731-7085(97)00201-X)] [PMID: 9638586]
- [3] Nevado JJ, Penalvo GC, Bernardo FJ. A contribution on the determination of bromazepam by its ferrous complex formation. *Anal Lett* 1997;30:2515-25. [<http://dx.doi.org/10.1080/00032719708001762>]
- [4] Valdeon MJ, Escribano TS, Hernandez L. Determination of bromazepam and its urinary metabolites, with a previous hydrolysis reaction, by voltametric and spectrophotometric techniques. *Analyst (Lond)* 1987; 112: 1365-8. [<http://dx.doi.org/10.1039/AN9871201365>]
- [5] Smyth MR, Beng TS, Smyth WF. A spectral and polarographic study of the acidbase and complexing behaviour of bromazepam. *Anal Chim Acta* 1977; 92(1): 129-38. [[http://dx.doi.org/10.1016/S0003-2670\(01\)84579-7](http://dx.doi.org/10.1016/S0003-2670(01)84579-7)] [PMID: 18952]
- [6] Tache F, Mutihac L, Medvedovici A, *et al.* Considerations on MS/MS detection of bromazepam after liquid chromatographic separation from plasma samples: Application to a bioequivalence study. *J Liq Chromatogr Tech* 2007; 30: 2699-715. [<http://dx.doi.org/10.1080/10826070701560603>]
- [7] Amer MM, Abdel Razek SA. Stability indicating method for the determination of bromazepam via its mercury (ii), silver (i) and zirconium(iv) chelates. *Anal Lett* 1998; 31: 631-49.[<http://dx.doi.org/10.1080/00032719808001867>]
- [8] British pharmacopoeia. London: Her Majesty's Stationary Office Publication 2004.;

- [9] Sultan SM, Suliman FE. Use of sequential injection technique for mechanistic studies and kinetic determination of bromazepam complexed with iron (ii) in hydrochloric acid. *Analyst (Lond)* 1996; 121: 617-21. [http://dx.doi.org/10.1039/an9962100617]
- [10] Sultan SM. Flow injection stopped-flow kinetic determination of the anxiolytic sedative bromazepam in dosage forms. *Analyst (Lond)* 1992 117: 773-6. [http://dx.doi.org/10.1039/an9921700773]
- [11] Correia Dos Santos MM, Famila V, Simoes GM. A voltametric study of the complexation of copper by the psychoactive compounds 1,4-benzodiazepines. *Electroanalysis* 2000; 12: 216-22. [http://dx.doi.org/10.1002/(SICI)1521-4109(200002)12:3<216::AID-ELAN216>3.0.CO;2-Q]
- [12] Pencheva I, Obreshkova D. Comparative analytical study of bromazepam in pharmaceutical dosage forms. *Acta Pharmaceutica Turcica* 2002;44: 52-5.
- [13] Salem AA, Barsoum BN, Izake EL. Spectrophotometric and fluorimetric determination of diazepam, bromazepam and clonazepam in pharmaceutical and urine samples. *Spectrochim Acta A Mol Biomol Spectrosc* 2004; 60(4): 771-80. [http://dx.doi.org/10.1016/S1386-1425(03)00300-7] [PMID: 15036087]
- [14] Andraus MH, Wong A, Silva OA, *et al.* Determination of bromazepam in human plasma by high-performance liquid chromatography with electrospray ionization tandem mass spectrometric detection: application to a bioequivalence study. *J Mass Spectrom* 2004; 39(11): 1348-55. [http://dx.doi.org/10.1002/jms.667] [PMID: 15532067]
- [15] Zhang XX, Kudo K, Imamura T, Jitsufuchi N, Nagata T. Sensitive determination of bromazepam in human tissues using capillary gas chromatography-mass spectrometry. *J Chromatogr B Biomed Appl* 1996; 677(1): 111-6. [http://dx.doi.org/10.1016/0378-4347(95)00427-0] [PMID: 8925082]
- [16] Chèze M, Villain M, Pépin G. Determination of bromazepam, clonazepam and metabolites after a single intake in urine and hair by LCMS/MS. Application to forensic cases of drug facilitated crimes. *Forensic Sci Int* 2004; 145(2-3): 123-30. [http://dx.doi.org/10.1016/j.forsciint.2004.04.066] [PMID: 15451083]
- [17] Hansen SH, Sheribah ZA. Comparison of CZE, MEKC, MEEKC and non-aqueous capillary electrophoresis for the determination of impurities in bromazepam. *J Pharm Biomed Anal* 2005; 39(1-2): 322-7. [http://dx.doi.org/10.1016/j.jpba.2005.03.017] [PMID: 15935599]
- [18] Al Attas AS. Construction and analytical application of ion selective bromazepam sensor. *Int J Electrochem Sci* 2009; 4: 20-9.
- [19] Fabre H, Hussam-Eddine N. Separation and quantitative determination of dipyrone and its

- degradation products by thin-layer chromatography. J Pharm Pharmacol 1982; 34: 425-8. [http://dx.doi.org/10.1111/j.2042-7158.1982.tb04749.x] [PMID: 6126537]
- [20] Baveja SK, Singh S. Thin-layer chromatographic examination of the degradation of centbucridine in aqueous solutions. J Chromatogr A 1987;396: 337-44. [http://dx.doi.org/10.1016/S0021-9673(01)94071-3] [PMID: 3624379]
- [21] Puthli SP, Vavia PR. Stability indicating HPTLC determination of piroxicam. J Pharm Biomed Anal 2000; 22(4): 673-7. [http://dx.doi.org/10.1016/S0731-7085(99)00300-3] [PMID: 10768357]
- [22] Salo JP, Salomies H. High performance thin layer chromatographic analysis of hydrolyzed tinidazole solutions. I. Development and validation method. J Pharm Biomed Anal 1996; 14(8-10): 1261-6. [http://dx.doi.org/10.1016/S0731-7085(96)01769-4] [PMID: 8818043]
- [23] Bergh JJ, Lotter AP. A stability-indicating gas-liquid chromatographic method for the determination of acetaminophen and aspirin in suppositories. Drug Dev Ind Pharm 1984; 10: 127-36. [http://dx.doi.org/10.3109/03639048409038297]
- [24] Stavchansky S, Wu P, Wallace JE. Gas liquid chromatographic determination of promethazine hydrochloride in cocoa butter-white wax suppositories. Drug Dev Ind Pharm 1983; 9: 989-98. [http://dx.doi.org/10.3109/03639048309042837]
- [25] Bakshi M, Singh S. Development of validated stability-indicating assay methods critical review. J Pharm Biomed Anal 2002; 28(6): 1011-40. [http://dx.doi.org/10.1016/S0731-7085(02)00047-X] [PMID: 12049968]
- [26] Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs-A review. J Pharm Anal 2014; 4: 159-65. [http://dx.doi.org/10.1016/j.jpha.2013.09.003]
- [27] Singh S, Bakshi SM. Guidance on the conduct of stress tests to determine inherent stability of drugs. Pharm Technol 2000; 24: 1-14.
- [28] Fukumoto M, Cornning KK. Spectrophotometric determination of bromazepam. Chem Pharm Bull (Tokyo) 1980; 28: 3678-81. [http://dx.doi.org/10.1248/cpb.28.3678]
- [29] Hancu G, Gáspár A, Gyéresi A. Separation of 1,4-benzodiazepines by micellar electrokinetic capillary chromatography. J Biochem Biophys Methods 2007; 69(3): 251-9. [http://dx.doi.org/10.1016/j.jbbm.2006.02.003] [PMID: 16563516]

- [30] Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J Chromatogr A* 2003; 987(1-2): 57-66. [[http://dx.doi.org/10.1016/S0021-9673\(02\)01536-4](http://dx.doi.org/10.1016/S0021-9673(02)01536-4)] [PMID: 12613797]

ABOUT THE PRESENTERS

Michael Pou Machar

Michael Pou Machar is a South Sudanese student who is currently studying Medicine at Africa Medical College. He has Masters Degrees in Global Health and MPH. He is also a Medical Physiology candidate at Addis Ababa University. He has two articles published on academic journals.

Mr Assefa Takele

Mr Assefa Takele is a Senior Expert of Quality Audit and Enhancement at Education and Training Authority. He received his MSc. Degree in Pharmaceutical Analysis and Quality Assurance through European Joint Master's Degree in Quality in Analytical Laboratories; Department of Chemistry and Pharmacy; Faculty of Science and Technology, University of Algarve, Faro, Portugal.

Dr Esubalew Tesfahun

Dr Esubalew Tesfahun received his PhD in Public Health (Epidemiology specialty) from Addis Ababa University in 2015 G.C. (2007 E.C.) and Master's Degree in Public Health from Addis Ababa University in 2007 G.C. (1999 E.C.). He is a professional with proven record of accomplishment of producing profound and quantifiable contributions for organizations, and communities spanning from rural villages to leading national organizations. He has more than 20 research articles published on various academic journals to his credit.

Mr Adane Tesfaye

Mr. Adane Tesfaye is a Ph.D. Candidate at Addis Ababa University in Human Nutrition. He received his MPH Degree in Public Health Nutrition from Haramaya University.

Mr Adane served as the Head of Human Nutrition Department at Dilla University Health and Medical Science College and participated as an Early Child Development Data Collector for EPHA, (Early Child Development Project). He was also a Lecturer of Human Nutrition for Masters and undergraduate level students, research advisor and supervisor of CBTP/ TTP at Dilla University. Mr. Adane has published more than 20 research articles on various local and international scientific journals.

Dr Takele Achalu

Dr Takele Achalu is a lecturer at Africa Medical College. He has two PhDs in Epidemiology and Business Administration, and an MSc in Epidemiology. He published more than 7 books in English, Afan Oromo and Amharic and has published more than 25 articles on various local and international scientific journals.

APPENDICES

Appendix 1- Annual national conference on the theme “Evidence for Health-Related Sustainable Development Goal”

February 17, 2023.

Addis Ababa


Day		Time	Activities	Moderator/Facilitator
FEBRUARY 10	MORNING	8.30-9.00	Registration AMC Staff	
		9.00-9.20	Opening Speech <u>Dr Mekonen Belay, Vice President of Africa Medical College</u>	Dr Kassahun Kebede, Research and Community Service Office Head
		9.20-9.45	Keynote Speech <u>Dr Tesfaye Teshome DVP</u> President of Unity University	Dr Kassahun Kebede
		9.45-10.15	Health Break	
		10.15-10.40	Presentation One Seroprevalence and Associated Risk Factors for Hepatitis B Virus Infections among Apparently Healthy Pregnant Mothers Attending Anc in Rubkona Primary Health Care Centre in Rubkona County, Unity State, South Sudan. <u>Mr Michael Pou Machar</u>	Dr Tekebash
		10.40-11.05	Presentation Two Under-nutrition among Pregnant Adolescent, A scoping Review <u>Mr Adane Tesfaye</u>	Dr Tekebash
		11.05-12.30	Question and Discussion	
	AFTERNOON	12.30-2.00	Lunch Break	
		2.00-2.25	Presentation Three Assessment of Traditional Medicine Utilization and Its Determinants among Parents of Children, in Gindeberet Oromia, Ethiopia <u>Dr Takele Achalu</u>	Dr Hadush
		2.25-2.50	Presentation Four High Performance Liquid Chromatography (HPLC)	Dr Hadush

Africa Medical College

			Stability Indicating Method for the Determination of Bromazepam Via its Copper (II) Chelates <u>Mr Assefa Takle</u>	
		2.50-3.30	Question and Discussion	
		3.30-3.45	Health Brea	
		3.45-4.30	Closing Speech <u>Dr Mekonen Belay, VP of Africa Medical College /</u> <u>Dr Alem Abrha Dean of Africa Medical College</u>	Dr Kassahun Kebede
		4.30-5.00	Handover of certificates	Dr Kassahun Kebede

Appendix-2 Call for Paper

CALL FOR PAPERS



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Africa Medical College

Africa Medical College has planned to hold a national conference on the theme “Evidence for Health-Related Sustainable Development Goal” on February 3, 2023. The conference will be a platform to all stakeholders to share their experience and set a way forward to improve the medical and health practices in the country. Thus, we cordially invite researchers and scholars to present their recent research output (findings) on the conference.

Please make sure that your work has not been presented elsewhere, nor is it currently under consideration for presentation elsewhere.

THE PAPERS TO BE PRESENTED ON THE CONFERENCE COVER THE FOLLOWING SPECIFIC AREAS:

- Health promotion and disease prevention
- Mental health and substance abuse
- Non communicable diseases

IMPORTANT DATES

- Deadline for the Abstract Submission: December 15, 2022
- Submission of Full Paper: January 16-25, 2023

PLEASE INCLUDE THE FOLLOWING IN YOUR SUBMISSION:

- Name and updated CV of the author
- Title of paper
- Affiliation
- Bio Data (not more than 100 words)
- Abstract (Not more than 300 words)
- Email address, Mobile phone

Please send your abstract to Dr Kassahun Kebede at:
kassahunkd@yahoo.com

Mobile Phone: +251 91 153 1142

N.B.

The College will pay modest honorarium to researchers whose papers will be selected and presented at the Conference.

Appendix – 3 Conference Organizing Committee Members


Ref.No. Yrmb/11/00A17/15
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 Date. 16/05/2015
 ቀን፡

ለዶ/ር ካሳሁን ከበደ
 ለአቶ ደስታ በርሄ
 ለአቶ ተክለኃይማኖት መዝገብ
 ለአቶ ብዙአየሁ ደስታ
 ለአቶ ኤፍሬም ጥላሁን
 ለአቶ ደሳለኝ ተፈራ
አጤኮ

ጉዳዩ፡- የኮሌጁ ብሔራዊ የጥናትና ምርምር ኮንፈረንስ አስተባባሪ ኮሚቴ አባል ሆነው
ስለመመደብዎ

የአፍሪካ የጤና ኮሌጅ ጥር 26 ቀን 2015 ዓ/ም "Evidence for Health-related Sustainable Development Goal" በሚል ርዕስ ብሔራዊ የጥናትና ምርምር ኮንፈረንስ የሚያዘጋጅ ሲሆን በኮንፈረንሱ ላይ ለጤናው ዘርፍ ከፍተኛ አስተዋጽኦ የሚያደርጉ የምርምር ጽሑፎች ተርበው ውይይት የሚደረግባቸው ሲሆን ከመንግስትና ከግል ተቋማት የሚጋበዙ ምሁራንና እንግዶች ይገኛሉ ተብሎ ይጠበቃል፡፡


ስለዚህ ኮንፈረንሱ በስኬት እንዲከናወን ኮሌጁ ባዋቀረው የኮንፈረንስ አስተባባሪ ኮሚቴ አባል እንዲሆኑ የተመረጡ ሲሆን የተጣለብዎትን ታላቅ አደራ በብቃት እንዲወጡ ከአደራ ጋር አሳስባለሁ፡፡



7ልባዊ

- ለዲን ጽ/ቤት
- ለጥናትና ምርምርና ማህበረሰብ አገልግሎት ክፍል

አጤኮ

ከሰላምታ ጋር

16/05/15

In replying, please quote our Ref. No.
 እባክዎን ለደብዳቤዎቻችን መልስ ሲልጡ የደብዳቤ ቁጥር ይጥቀሱ