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EDITORIAL

One of the missions of the Ethiopian Public Health Institute is to generate evidence-based information and dissemination of this information to improve the health of the public. This mission can be achieved by creating a communication network and medium of exchange. With this in mind, we are launching the Ethiopian Journal of Public Health and Nutrition (EPHNJ), to be published twice per year.

The EJPHN, an official organ of EPHI, is a multidisciplinary peer-reviewed journal for publication of original research and theoretical or methodological papers within the area of public health and allied sciences.

The journal will play an essential role in educating the public on health and nutrition matters by providing up-to-date, reliable and authoritative scientific information.

Our goal is to provide evidence based information and analysis to the public and the professional community to make the right choices and decisions concerning their health and health services provided to ensure better health for all.

The Ethiopian Journal of Public Health and Nutrition (EJPHN) considers submissions on the following disciplines:

- All aspects of public health,
- Traditional and modern medicines
- Human nutrition, food science and technology
- Biochemistry, biotechnology, physiology and toxicology
- Communicable diseases (microbiology, medical parasitology, virology, TB, HIV/AIDS, malaria, etc.)
- Non-communicable diseases (diabetes, hypertension, oncology)
- Molecular biology (genetics, immunology, metabolism, molecular and cell biology, neurobiology)
- Epidemiology of diseases; hygiene and environmental health
- Integrated Disease Surveillance (IDSR) and Public Health Emergency Management (PHEM).

However, this list may not be exhaustive.
The emphasis of this new journal will be the promotion of good health through public health services, nutrition and the primary prevention of diet related illness in the population. It would serve as an important continuing education role for local health officers nationally and provide them with a communication network and medium of exchange.

The circulation will be worldwide, and the journal should be accessible to all health and allied sciences professionals, regardless of their physical location. It is our hope that access to our journal will increase collaboration among researchers from both developing and established developed countries.

So, please come and join us! Submit your articles in all areas of health and allied sciences. We welcome you to this new Journal. We promise a quick turnaround for submitted articles and publish your article soon after its acceptance.

Ethiopian Public Health Institute

Amha Kebede (PhD)
Director General

Anemia and Associated Factors among Pregnant Women Attending Antenatal Care in Karamara Hospital, Jigjiga Town, Eastern Ethiopia: A Cross-sectional Study

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Abstract
Background: Anemia occurs at all stages of human life cycle. It is more prevalent in pregnant women and young children. The burden of anemia in pregnant women remains unacceptably higher especially in the developing countries. The 2011 Ethiopian Demographic Health Survey reported 22% and 46.8% anemia prevalence among pregnant women for the National and Ethiopian Somali region, respectively.
Objective: The objective of this study was to assess the prevalence of anemia and its associated factors among pregnant women attending antenatal care in Karamara Hospital, Jigjiga, Ethiopia Somali Region.
Methods: Facility based cross sectional study was conducted on 411 pregnant women coming for first ANC service and permanent residents in the study area were included while those who were seriously ill were excluded.
Structured questionnaire was administered. Blood and stool samples were collected and diagnosed for hemoglobin and intestinal parasites, respectively. The data were entered into Epi Info version 3.5.1 then exported to SPSS version 16 and analyzed. The relationships between variables were examined through bivariate and multivariate logistic regression. P value of ≤0.05 was considered as statistically significant.
Result: The age of the study participants was ranging from 15 to 45 years. Hemoglobin value ranges from 11.49g/ dl to 42.81g/dl with the median and inter quartile range of 10.60 and 3.83, respectively. Of the participants, 44.2% had mild, 26.6% moderate, 27.9% severe and 1.3% very severe anemia. Age, third trimester, drinking tea immediately after meal and consumption of iron rich foods in the previous 24 hours were significantly associated with anemia during pregnancy.
Conclusion: The prevalence of anemia is high (55.8%). Age, third trimester, drinking tea after meal and organic iron rich foods consumption in the previous 24 hours are factors associated with anemia in pregnant women.
Key words: Anemia, Pregnant Women, Antenatal Care

Introduction
Anemia is a global public health problem, which affects the health as well as socio-economic development of people. Although, anemia occurs at all stages of human life cycle, it is more prevalent in pregnant women and young children. The burden of anemia in pregnant women remains unacceptably higher especially in developing countries. The currently estimated prevalence of anemia among pregnant women in the years 1993-2005 in the world was 41.8% with higher rates in African (61.3%) and Southeast Asian (52.5 %) countries (Panda *et al.* 2015). Multiple nutritional and environmental factors influence the prevalence and severity of anemia. In Sub-Saharan Africa, the causes of anemia during pregnancy include an iron and folate deficient diet and infections such as malaria, hookworm and increasingly human immunodeficiency virus (Shaw and Friedman 2011).

In Ethiopia, the proportion of anemia among pregnant women is 22%, which is the highest when compared to the proportion among breastfeeding women (19%) and neither pregnant nor breastfeeding women (15%). Although, the 2011 Ethiopian Demographic Health Survey (EDHS) showed higher rate of anemia (46.8%) among pregnant women in the Somali region (CSA 2012), studies conducted on the prevalence and associated factors with anemia in the study area are limited. Studies that assess the prevalence and associated factors with anemia are important to take appropriate intervention and reduce maternal and child mortality attributed to malnutrition due to anemia.

Evidences indicate that if anemia is not addressed in early pregnancy, it would lead to pregnancy related maternal deaths, preterm delivery, low birth weight and possibly inferior neonatal health. This study was therefore designed to determine the prevalence of anemia among antenatal care (ANC) attending women in Karamara Hospital and to identify factors associated with the anemia.

Materials and methods
The study was conducted in Karamara Hospital, Jigjiga town of Ethiopian, Somali region. It is 1669m above sea level. The dominant ethnic group living in the town is Somali and rice is the major staple food (Somali Region Health Bureau 2012). Facility based cross sectional study was conducted among pregnant women attending antenatal care in Karamara Hospital

from March 11 to May 18, 2013. All pregnant women registered and attending their ANC in Karamara Hospital fulfilling the inclusion criteria and available during the data collection period were the study population. Pregnant women who were coming for first ANC service and permanent residents in the study area were included whereas pregnant women who were seriously ill during data collection were excluded.

Sample size for this study was calculated by using single population proportion formula. According to 2011 EDHS survey report; prevalence rate of anemia among pregnant women in Ethiopian Somali Region was 46.8% (CSA 2012). By using the absolute precision of 5% with 95% confidence interval; the sample size was determined by the following formula:

$$n = \frac{z^2 p(1-p)}{d^2}$$

Where d = margin of error between the sample and the population, n = sample size, z =95% confidence interval and P = prevalence rate of 47%.

$n = \frac{1.96^2\,(0.47)(0.53)}{0.05^2}$ = 383. By adding 10% for non-response rate, the sample size was determined to be 421.

A structured questionnaire adopted from Food and Nutrition Technical Assistant (FANTA) for Dietary Diversity Questionnaire and Food Frequency Questionnaire (FAO Nutrition and Consumer Protection Division 2008) as well as tools from different literature sources for anemia assessment were adapted to local context. The questionnaire originally was developed in English and translated into Somali language. The questionnaire was pre-tested on 21 pregnant women in Kebribeyah health center. The questionnaire contained information on socio-demographic, socioeconomic, present and past medical history in pregnant women, ANC and current iron rich foods related questions.

Two trained midwifery nurses who can speak and read the local language (Somali) fluently administered the questionnaire. Furthermore, two senior laboratory technologists took 50 micro liter of blood from their fingertips using capillary tube. Gestational age was assessed by midwifery nurses and principal investigator using fundal height (if measurable) and the last menstrual period, and occasionally by immunological pregnancy tests.

Clean, dry, disinfectant-free wide-necked container and clean applicator sticks were used to collect fresh stool specimen. Stool smear was prepared using a drop of saline and a piece of stick was used to mix the specimen with each drop; then, covered with a cover glass (Shimizu 2007). Direct smear was examined by 10X (10 times) and 40X (40 times) microscopic magnifications. A blood-sample was obtained by finger-prick after disinfection with alcohol, drying of the skin and removal of the first drop of blood. Giemsa stained of thick and thin smears were prepared to determine the presence or absence of malaria parasite (Shimizu 2007). Hemoglobin was used as screening test for anemia (ITCA WIC Training Program 2012).

To assure the data quality the questionnaire was translated to Somali language by a translator and retranslated in to English by another person who was blind to the original questionnaire. After an intensive two days training of data collectors, pre-testing of the questionnaire was undertaken. The final version of the questionnaire was used for the data collection. The principal investigator designed proper data collection materials and closely supervised the overall activity. All completed questionnaires were examined for completeness and consistency every day. Data was cleaned and coded before entering into Epi Info 3.5.1.and then exported to a SPSS version 16 and analyzed. Errors identified during the cleaning up of the data were corrected.

The associations between variables were examined through bivariate analysis; by computing odds ratio (OR) at 95% confidence level as well as multivariate logistic regression at 95% CI. A P value $\leq$0.05 was considered as statistically significant. Permission to undertake the study was obtained from the Ethical Review Committee of College of Health Sciences, Mekele University. Written informed consent was obtained from all study participants. All pregnant women who were found to be anemic were referred to health facility for free treatment.

Results

The age of the study participants was ranging from 15 to 45 years, with mean age of 24.9 (±5.5) years. Three hundred eighty nine (94.6%) of the study participants were married, 84.9% were Muslims and 284 (69.1%) were the Somali ethnic group.

Nearly half of the study participants were 186 (45.3%) illiterate. More than three fourth (78.6%) were house wives and less than 10% were employed. The median monthly income of respondents was 900 birr with interquartile range (IQR) of 1500 birr (Table 1).

Table1: Back ground characteristics of study participants attaining ANC service in Karamara Hospital (n=411)

Variables	Number	Percent
Age(years)		
15-24	192	46.7
25-34	186	45.3
>35	33	8
Marital status		
Married	389	94.6
Non-married *	22	5.4
Religion		
Muslim	349	84.7
Orthodox	48	11.7
Protestant	10	2.4
Catholic	4	1
Ethnicity		
Somali	284	69.1
Oromo	65	15.8
Amhara	34	8.3
Gurage	14	3.4
Tigray	10	2.4
Silte	4	1
Educational status of respondent		
No education	186	45.3
Elementary(1-8)	165	40.1
high school(9-12)	32	7.8
Collage and above	28	6.8
Occupational status		
House wife	323	78.6
Daily laborer	30	7.3
Government or private employee	27	6.6
Merchant	24	5.8
Student	7	1.7
Monthly income (Birr)		
<500	124	30.2
501-1000	112	27.3
>1000	175	42.6

**single, divorced or widowed,*

Prevalence of Anemia: The hemoglobin value of study participants was ranging from 3.43g/dl to14.27g/dl with the median and IQR of 10.60 and 3.83, respectively. Prevalence of anemia among pregnant women who come for first routine ANC visit in the current study after adjusted for altitude and trimester was 55.7%. Among the women, 44.2% have mild, 26.6% moderate 27.9% severe and 1.3% very severe Anemia. Table 2 shows differences in status of anemia among study participants.

Table 2: Prevalence of anemia in pregnant women attending ANC service in Karamara Hospital (n=411)

Methods used to classify	Status	
	Anemic	**Non anemic**
WHO classification	129(31.4%)	282 (68.6%)
Hemoglobin Adjusted for altitude	143 (34.8%)	268(65.2%)
Hemoglobin Adjusted for trimester	195(47.4%)	215(52.6%)
Hemoglobin Adjusted for altitude and trimester	233(55.8%)	178(44.2%)

About three quarter of the participants (74.7%) have habit of drinking tea immediately after meal. Dietary diversity scores revealed that the most consumed food groups by study participants were Vitamin A rich fruits and vegetables (41%), other fruits and vegetables (19.7%) and meat (24.7%) among non-anemic group of pregnant women as compared to anemic group in 24 hours preceding the survey. Among the 411 study participants, 243 (59.1%) pregnant women, consumed heam-iron rich foods prior to 24 hours of the survey and 168 (40.9%) didn't consume. Among those 168 pregnant women who didn't consume heam-iron rich foods, 36.3% were non-anemic and 63.7% were anemic.

The food consumption frequency study showed that, milk (76.4%), vegetables (69.7%), fish (14.6%), pulses (55.1%), meat (71.9%) and egg (26.4%) were the most consumed food groups seven days prior to the survey by the non-anemic group of pregnant women as compared to anemic groups (Table 3).

Table 3: Food groups consumed during 24 hours and 7 days prior to survey (n=411)

		Anemia status			
		Anemic (N=178)		**Non Anemic** (N=233)	
Time	**Food groups**	Yes N (%)	No N (%)	Yes N (%)	No N (%)
Previous 24 hours	Starch staples	223(95.7)	10(4.3)	164(92.1)	14(7.9)
	Milk and milk products	182(78.1)	21.9	137(77)	41 (23)
	Green vegetables	160(68.7)	73(31.3)	115(64.6)	63(35.4)
	Vitamin A rich fruits and vegetables	85 (36.5)	148 (63.5)	73(41)	105(58)
	Other fruits and vegetables	39(19.7)	194(83.3)	35(19.7)	143(80.3)
	Meat and fish	132(56.7)	101(43.3)	89(50)	89(50)
	Legumes ,nuts and seed	63(27)	170(73)	45(25.3)	133(74.7)
	Organ meat	31(13.3)	202(86.7)	44(24.7)	134(75.3)
	Eggs	37(15.9)	196(84.1)	28(15.7)	150(84.3)
Before seven days	Cereals	225(96.6)	8(3.4)	168(94.4)	10(5.6)
	Milk and milk products?	166(71.2)	67(28.8)	136(76.4)	42(23.6)
	Green vegetables	162(69.5)	71(30.5)	124(69.7)	54(30.3)
	Meat and fish	28(12)	205(88)	26(14.6)	152(85.4)
	Pulse and legumes	115(49.4)	118(50.6)	98(55.1)	80(44.9)
	Fruits	73(31.3)	160(68.7)	52(29.2)	126(70.8)
	Meat	153(65.7)	80(34.3)	129(72.5)	49(27.5)
	Eggs	58(24.9)	175(75.1)	47(26.4)	131(73.6)
	Oils and fats	109(46.8)	124(53.2)	72(40.4)	106(59.6)

The presence of anemia was assessed based on different pregnancy and nutritional variables. Maternal age, trimester, drinking tea immediately after meal and consumption of iron rich foods in the previous 24 hours were significantly associated with anemia during pregnancy. Pregnant women aged

higher than or equal to 35 years were 3.27 times more likely to be exposed to anemia as compared to

pregnant women in the age of 15 to 24 years (AOR 3.27 [1.21, 8.83]). Pregnant women who were in third stage of trimester (gestational age ≥28 weeks) were 2.59 times more likely to be exposed to anemia as compared to pregnant women in their first trimester (gestational age ≤12 weeks) (AOR=2.59 [1.46, 4.58]). Consumption of iron rich foods in the previous 24 hours can help to prevent anemia (38%) in pregnant women than those who didn't consume (AOR=0.62 [95% CI=0.62[0.41, 0.95]). Pregnant women who drink tea immediately after meal were 1.71 times more likely to be anemic than those women who didn't drink tea (AOR=1.71 [95% CI= [1.07, 2.73]) (Table.4).

Table 4. Factors affecting ANC service attendance in Karamara Hospital (n=411)

Variables	Anemia status		COR [95% CI]	AOR [95% CI]
	Anemic N (%)	**Non-anemic** N (%)		
Age (years)				
15-24	96(41.2)	96(53.9)	1.00	1.00
25-34	110(47.2)	76(42.7)	1.45 [0.96,2.17]	1.42[0.89,2.25]
≥35	27(11.6)	6(3.4)	4.50[1.78,11.39]**	3.27[1.21,8.83]*
Trimester (weeks)				
First(<12)	38(16.3)	49(27.5)	1.00	1.00
Second (13-27)	94(40.3)	78(43.8)	1.55[0.92,2.61]	1.78[1.04,3.06]
Third(≥28)	101(43.3)	51(28.7)	2.55 [1.49,4.39]**	2.59[1.46,4.58]**
History of abortion				
No	174(74.7)	153(86.0)	1.00	1.00
Yes	59(25.3)	25(14.0)	2.08[1.24,3.48]*	1.72[0.96,3.10]
Drink tea/coffee				
No	48(20.6)	58(31.5)	1.00	1.00
Yes	185(79.4)	112(68.5)	1.77 [1.13,2.77]*	1.71[1.07,2.73]*
Consumption of iron rich foods(24h)				
No	107(45.9)	61(34.3)	1.00	1.00
Yes	126(54.1)	117(65.7)	0.61 [0.41,0.92]*	0.62[0.41,0.95]*
Gravidity				
Primigravida	80(34.3)	79(44.4)	1.00	1.00
Mulitigravida	153	99(55.6)	1.53 [1.02,2.78]*	0.93[0.56,1.53]

*****P<0.05, ******P<0.01, COR (Crude odds ratio), AOR (Adjusted odds ratio), CI (Confidence interval)

Discussion

In this study, the prevalence of anemia among pregnant women who seek ANC service was 55.8%. It is consistent with studies done among pregnant women in Malaysia (Rosmawati *et al*. 2012) and Gligel Gibe dam area (Getachew *et al*. 2012) with prevalence rates of 57.4% and 53.9 %, respectively, but higher than pregnant women in Sri Lanka (Chathurani *et al.* 2012), Cape-Verde (Okeke 2011), Tanzania (Msuya *et al*. 2011) and Nigeria (Jeremiah 2016) with prevalence of 34.4%, 38.8%, 47.4% and 23.2%, respectively. The finding of the current study is also higher than those reported in different parts of Ethiopia with prevalence of 21.6 % in Azezo town, North Gondar Zone of Amhara Region, 36.6% in Shalla and 46.8% in Ethiopian Somali Region (Alem *et al*. 2013; (Nigusie *et al.* 2013; CSA 2012).

The highest varation in anemia prevalence in this study might be attributed to the use of different cutoff points. The previous studies used WHO cutoff points to define anemia, which is 11g/dl. In the current study, the values are adjusted for gestational age and altitude of the study area. But the prevalence of anemia in the current study is lower as compared to study findings from other parts of Ethiopia, Uganda with the prevalence of 63.1% (Mbule *et al*. 2013) and Kenya with 70% (Ouma *et al*. 2007). The variation may be due to study population and study area. They included rural community and malaria endemic is whereas the present study was done in malaria free urban population. The finding indicated that pregnant mothers who are in third trimester, drink tea immediately after meal and whose age category is ≥35 years were more likely to be anemic than their counterparts.

Consumption of heme-iron rich foods in the previous 24hrs helps to prevent anemia, and increased maternal age (age ≥35 years) is found to be risk factor for anemia.

Similar results were reported from studies in Azezo (North Gondar of Amhara Region, Ethiopia) and Malaysia ; Rosmawati *et al*. 2012). Higher fertility rate and less pregnancy spacing may have exposed them to be anemic (Shaw and Friedman 2011; Okwu and Ukoha 2008).

Prevalence of anemia significantly increased as gestational ages increased. This might be due to increasing demand of micronutrients and depletion of iron in the pregnant women during the third trimester. Pregnant women who consumed organic iron rich foods in the previous 24 hours (AOR=0.62[95% CI= [0.41, 0.95]) prevent anemia better than non-consumers. This might be due to consumption of iron from animal sources, which are known to be bioavailable iron sources. The current study further showed that pregnant women who drink tea immediately after meal are 1.71 times more likely to be exposed to anemia than those women who didn't drink tea immediately after meal (AOR=1.71 [95% CI= [1.07,2.73]). This might be due to presence of higher level of tannin in tea, which is known to reduce absorption of bivalent minerals, especially iron from plant food sources.

Conclusion

The prevalence of anemia among antenatal care (ANC) attending women in Karamara Hospital was 55.8 %, showing its public health significant in the area higher than the WHO criteria(>40%).

Third trimester, maternal age (≥35 years), drinking tea immediately after meal and consumption of heme iron rich foods are factors that are associated with anemia in pregnant women.

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Conflict of interest

There are no personal, financial and/or non-financial competing interests among the authors of this paper.

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Field Residual Efficacy of PermaNet®2.0 against *gambiae* s.l in different rural communities of Ethiopia

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Abstract
Background: Long lasting insecticide treated mosquito nets (LLINs) could retain their effective biological activity for at least twenty WHO standard washes under laboratory conditions and three years effective life under field conditions. However, the dynamics of insecticide loss could vary with users' practices in handling and wearing of LLINs. Therefore, bio-efficacy test is recommended to detect net status before it gets lost its lethal dose.
Method: Long lasting treated mosquito nets were sampled randomly from three different localities found in different districts and the performances of these nets were evaluated using WHO cone test procedures. Efficacy was measured in terms of mortality and knockdown effects on susceptible *Anopheles gambiae* s.l.
Results: Except two nets all LLINs showed high level of mortality and knockdown rates implying that the nets could retain their biological activity under field conditions as recommended.
Conclusion: The present study showed both success and failures showing that LLINs could retain the lethal effect for three years as recommended if used properly while failures suggest the need to conduct a follow-up efficacy tests in order to identify the limitations that could contribute for net failure or shrink the effective life of the nets.
Key words: residual efficacy, long lasting mosquito nets, *An. gambiae* s.l, Ethiopia

Introduction
Insecticide treated mosquito nets are one of the key components of the existing malaria vector control strategies both at national and international level. Insecticide treated mosquito nets could be either conventionally treated mosquito nets (ITNs) or long lasting insecticidal mosquito nets (LLINs). Conventional treated nets are mosquito nets that have been treated by dipping in a WHO-recommended insecticide that requires re-treatment after three washes, or at least once a year for its continual insecticidal effect while LLIN are factory-treated mosquito nets made with netting material that has insecticide incorporated within or bound around the fibers. Long lasting insecticidal mosquito net does not require retreatment because LLINs could retain effective biological activity for at least twenty WHO standard washes under laboratory conditions and three years effective life under field conditions. Thus, LLINs are preferred to conventional treated nets because LLINs avoid the problems associated with re-treatment of conventional nets. PermaNet® 2.0 is one of LLINs approved by World Health Organization Pesticide Evaluation Scheme (WHOPES) which could provide 80% mortality for up to twenty laboratory washes (WHO, 2005).

The effective life of LLINs could be influenced by misuse of nets such as spreading LLINs under mattress to kill household pests like bedbugs, fleas, lice, as bed sheets or bedspreads, boundary marker for pit latrines, washing and drying them in direct sun light and staining, could cause loss of residual insecticide more quickly and compromise LLINs efficacy (Laura and Douglas 2011). Development of insecticide resistance by the vector could also reduce the efficacy of LLINs. Therefore, a follow-up efficacy assessment is recommended along with the distribution of these nets to identify the factors that could contribute for loss of nets efficacy and take corrective measures before the occurrence of any control failure.

The use of ITNs was initiated in Ethiopia in 1997/98 and the country has had great success in rapidly scaling up the national ITNs distribution and coverage since then although a slight decrease has been observed in 2011 (ENMIS 2011). The national ITNs coverage in 2000, 2005, 2007 and 2011 was 0.2%, 6.5%, 68.9%, and 55.2%, respectively, (Central Statistics Authority 2001 and 2006; Jima *et al*. 2010; ENMIS 2011). According to Ethiopia national malaria indicator survey (ENMIS 2011), the use of ITN among malaria vulnerable groups was different, i.e., ITNs usage among children under five years of age increased from 60.2% in 2007 to 64.5% in 2011 while the statistics was the same among pregnant women. According to Jima *et al*. (2010) and

ENMIS (2011) 95% of the nets that have been distributed and used by households in all parts of the country were/are LLINs. Field efficacy trials on PermaNet® showed that this LLIN was effective at least for three years in different countries (Grham *et al.* 2005 and Kroger *et al*. 2004). A similar study from Ethiopia showed below 80% mortality within three years of use in real life condition (Fetene *et al*. 2009) which is less than the cut of pint recommended by WHO. Valerie and his colleagues also (2012) reported 73% mortality from field used insecticide treated mosquito nets in Burkina Faso. The differences observed from these studies demonstrate that effective life of insecticide treated mosquito nets could vary with eco-cultural and eco-epidemiological conditions. Therefore, the performances of these frontline malaria preventive intervention vector control tools should be determined under local ecological settings.

Although Ethiopia is scaling up ITNs distribution and usage rapidly, LLINs efficacy field data were/are limited or not available so that this study was initiated to generate evidence based information for policy makers and malaria control programmers. Thus, the purpose of this study was to measure the effective life of LLIN against the major malaria vector in different rural communities (communities having different cultures and environmental conditions) of Ethiopia.

Materials and methods

Descriptions of the study area: The study was conducted in three different communities found in three different districts of three different regions (Amhara, SNNP, Afar) having different local ecological settings between November 2006 and January 2007 (Table 1).

Study design: The three districts were selected based on history and intensity of malaria transmission, LLINs distribution, difference in attitudes and practices of communities because all are anticipated to influence the effective life of mosquito nets. A study site in each district was also selected based on history and intensity of malaria transmission, LLINs distributions in consultation with district health offices and accessibility and proximity to the district capital. LLINs were also sampled randomly from houses having close proximity to mosquito breeding sites where the probability of mosquito biting would be high so that people could make use of their mosquito nets intensively to protect themselves from mosquito biting and other nuisance insects.

Table 1: Description of the study sites, distribution and survey of period of LLINs assessed (December 2006 - January 2007)

Locality/district	Major economic activities	Altitude(in meters)	Coordinates	Period of LLNs distribution	Study period (Month &year)
Andassa/ Bahirdar zuria	Irrigated & rain fed farms Farms, animal keeping	1698	N11^{0}37'.954" E37^{0}29'.274"	July,2005	December,2006
Sabure/Awash-Fentale	State cotton & orange plantation, pastoralist	802	N 09^{0}07'.879" E 039^{0}57'.677"	August,2005	January,2007
Ado/Wondogenet	Small scale irrigated & rain fed farms, cattle keeping	1178	N06^{0}5'9.789" E038^{0}34'.738"	July,2005	December,2006

Coding of LLINs: All the nets used for the test were PermaNet® 2.0 treated with delthamethrin. A total of 12 LLINs (four nets/study site) were sampled at random and coded as for Andassa AB, K, G and Y, for Sabure C, ES, D and P, and for Ado EW, W, AW and T nets (Table 2). Mosquito nets were in use for about eighteen months and received between 0 and 4 washes during this time (Table 2).

WHO test tube insecticide susceptibility test: Indoor resting blood fed female *An. gambiae* s.l that had been used for cone bio-efficacy test were collected

early in the morning manually using mouth aspirators and identified using morphological keys developed by Veron (1962) and Gilles and Coetzee (1987). Susceptibility of female *An. gambiae* s.l against 0.05% delthamethrin was confirmed using WHO test procedures (WHO, 1998) before using them for the cone tests. A minimum of four treatments and two controls replicates (25 female *An. gambiae* s.l / test tube) were used at each study site. Knockdown scored after 60 minutes and mortality after 24 hours holding period. All the experiments were undertaken under field conditions but within insecticide free premises maintained at 62% relative humidity and temperature of 26^0C.

WHO cone bio-efficacy test: Five susceptible blood fed female *An. gambiae* s.l were exposed to LLIN nettings for 3 minutes under WHO plastic cones (WHO, 2005). Mortality and knockdown rates (kdr) were measured after 24 hours and 60 minutes, respectively. Knock down (collapsing and falling down of mosquitoes at the bottom of the test tube) rates are indicative of susceptibility status of *Anopheline* under examination.

Table 2: Locality/ district, code given to LLINs and number of washes each LLIN received

Locality/ district	Code	Number of washes
Andassa/ Bahirdar zuria	AB	2X
	K	2X
	G	0
	Y	1X
Sabure/Awash-Fenetale	C	2X
	ES	2X
	D	4X
	P	4X
Ado/Wondogenet	EW	2X
	W	2X
	AW	2X
	T	0

Card boards were used as control for the cone tests because obtaining untreated mosquito nets was impossible during the study period. A total of 50 mosquitoes/net and 200 mosquitoes/4 LLINs were used at each study sites. All the experiments were carried out under field conditions but within insecticide free premises maintained at 62% relative humidity and temperature of 26 degree centigrade.

Data analyses: Abbots (1925) formula was used to correct percent mortality when control mortality rates were between 5% and 20%.

Results

WHO test tube insecticide susceptibility test: Insecticide susceptibility status of *An. gambiae* s.l to the diagnostic dose of delthamethrin is given (Table 3). The percentage knock down rates after 60 minutes were 97.6%, 100% and 97.6% while mortality rates after 24 holding hours were 98%, 100% and 98% in Ado, Sabure and Andass, respectively. Both percent mortality and knockdown rates indicated that the vector was susceptible to delthamethrin at all study sites.

Table 3: WHO test tube bioassay test results with percentage mortality, total number of mosquitoes tested (n) and percent knock down of *An. gambiae* s.l used for cone bioassay

Insecticide Locality	Locality	Mean % Mortality(n)	Mean % knock down	Resistance status
Delthamethrin (0.05**%)**	Ado	98 (125)	97.6	Susceptible
	Sabure	100 (100)	100	Susceptible
	Andassa	98 (122)	97.6	Susceptible

WHO cone bio efficacy test: The cut of points for LLINs efficacy are ≥ 80% mortality and knock down ≥ 95% (WHO, 2005). Mortality and knock down results of cone tests for all LLINs are shown on Table 4. Except P and D mosquito nets from Sabure, all LLINs showed > 80% mortality and > 95%

knockdown demonstrating that the nets were effective against susceptible *An. gambiae* s.l after being used about eighteen months. Mortality and knockdown rates for P and D nets were ≤ 4% and ≤ 6%, respectively, indicating that the performances of these nets were declined before their recommended three years effective life.

Table 4: WHO cone bioassay test results of percent mortality with the total number of mosquitoes tested (n) and percent knockdown

Locality/ district	Code of LLIN	% mortality(n) after 24 hours	% knock down at 60 minutes
Ado/Wondogenet	EW	100 (50)	100
	W	100 (50)	100
	Aw	100 (50)	100
	T	100 (50)	100
Sabure/Awash- Fentale	C	88 (50)	100
	ES	96 (50)	100
	D	4 (50)	6
	P	2 (50)	2
Andassa/Bahirdar zuria	AB	100 (50)	100
	K	100 (50)	100
	G	100 (50)	100
	Y	100 (50)	100

Discussion

The main purpose of this study was to assess whether the efficacy of LLINs that have been used in real life condition under local ecological settings of Ethiopia meets the criteria (> 80% mortality and 95 % knock down) set by WHO. Based on these criteria 83.3% of the LLINs tested demonstrated high level of mortality and knock down rates while 16.7 % of them (D and P LLINs nets) showed < 4% and 6% rate of mortality and knockdown, respectively. D and P nets were washed 4 times while other nets were washed ≤ 2 times so that this would be the potential reason for the differences observed in bio-efficacy results of the nets tested. Other malpractices due to knowledge gaps in handling of LLINs while using and wearing them may also diminish nets' effective life (Laura and Douglas 2011). Some mosquito net users use LLINs as bed sheets or bed spreads to kill household pests such as lice, bed bug, fleas , to collect animal feed, those who are living in warmer areas use nets as refuges and barrier for mosquito bites out of but nearby their houses to stay away from hotter room temperatures, others wash and expose nets to direct sun light.

Similarly, Fetene and his colleagues (2010) reported 72.15 % mortality and 94% knockdown and 67% mortality and 100% knockdown rates from PermaNet® LLINs that have been used for three years under two different ecological settings. In a multi-county efficacy trial PermaNet®2.0 indicated that the nets retained lethal dose of the insecticides that showed 80% mortality in *Anopheles* mosquitoes after 20 washes (Graham *et al*. 2005; Kroegere *et al.* 2004).

The differences observed in bio-efficacy results between the present and the previous studies could be justified by time differences that LLINs were in use; i.e. the present study did the test on LLINs that have been used about eighteen months while the previous studies were done on LLINs that have been used for about three years. Furthermore, the role of eco-cultural and eco-epidemiological factors should be taken into consideration.

Conclusion

The present study revealed that most of LLINs show high level of efficacy in terms of killing and knocking down mosquitoes after being used for about eighteen months in real life condition while two of them lost their efficacy before the expected three years of effective life. This signifies that a follow-up

assessment on field performances of LLINs under different ecological settings is vital to determine when LLINs should be replaced and plan should be in place for how best to prepare for net failure.

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Conflict of interest
The authors declare that they do not have any conflict of interest.

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Adverse Drug Reactions of Highly Active Antiretroviral Therapy on Adult Patients at Debre Markos Referral Hospital, Ethiopia: A Retrospective study

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Abstract
Background: Highly Active Antiretroviral Therapy could suppress viral replication and stop progression of the disease. Data on adverse drug reactions related to antiretroviral uses are few. This study aimed at assessing antiretroviral adverse reactions and associated factors.
Objective: To assess major antiretroviral drug adverse reactions and associated factors among adult patients initiated highly active antiretroviral therapy from September 2005 to August 2010, at Debre Markos Hospital.
Methodology: Retrospective data was collected at Debre Markos Hospital. A total of 930 adult subjects' clinical records were selected using systematic random sampling technique. Chi-square and logistic regression were utilized to quantify magnitude and identify independently associated risk factors for adverse drug reactions of highly active antiretroviral therapy.
Results: Five hundred twelve (55.1%) of the participants were females. The median age was 34(IQR=28-40) years. The median CD4 T cell count was 116 (IQR, 59 -167) cells/µl. Three hundred fifty one (37.1%) patients were taking 3TC-d4t-NVP and 272(29.3%) were on 3TC-AZT-NVP. Two hundred fourteen (23.0%) patients had reported one or more adverse drug reactions. The most frequently reported adverse drug reactions were anemia, 65(25.0%), fat change, 45(17.2%), and skin rash, 42(16.1%). Patients with CD4 T cell count of 50-99 cells/µl (AOR=1.9, 95%CI [1.115-3.364]), 3TC-d4t-NVP (AOR=5.8, 95% CI [1.692-9.591]), 3TC- AZT-NVP (AOR=4.2, 95% CI [1.227-10.673]) were associated risk factors for adverse drug reactions.
Conclusion: Sever forms of adverse reactions were reported by the participants. Proper drug counseling to report on the adverse reaction should be implemented. Prospective cohort study is recommended to illustrate the impact of adverse drug reactions on the outcomes of Highly Active Antiretroviral Therapy (HAART) HAART.
Key words: HAART, adverse drug reactions, zidovudine based regimen, stavudine-based regimen

Introduction
Antiretroviral therapy has improved the quality of life of HIV-infected individuals. It decreases the HIV viral burden in the body, thus leading to improved immunity and clinical wellbeing. However, anti-retroviral therapy has potential life threatening adverse drug reactions (ADRs).

Like most chronically administered drugs, antiretroviral drugs (ARVs) have documented toxicities and ADRs range from mild to life threatening with short and long term effects (Hogg *et al*. 1999). The spectrum of adverse effects associated with ARVs may vary between developed and developing countries (Subbaraman *et al*. 2007). Studies in developing and developed countries showed that the incidence of ADR is between 11%–35.9% (Bonfanti *et al*. 2000, Severe *et al*. 2010). Incidence of severe ADR has been reported to be as high as 10% (Severe *et al*. 2010) with a study observing an incidence rate of 8 per 100 person years (Duval *et al*. 2004). The long term effects of highly active anti-retroviral treatments (HAARTs) are largely unknown but ongoing research provides insights into some ADRs of ARV (WHO 2007). These include peripheral neuropathy and lipodystrophy associated with stavudine (Duval *et al*. 2004; Palella *et al*. 1998), anemia associated with zidovudine (AZT) (Laurent *et al*. 2008; Pollard 1998), nevirapine (NVP) based hepatotoxicity and rash(Baylor and Johann-Liang 2004; De Maat *et al*. 2003; Dieterich *et al*. 2004; Martínez *et al*. 2003; Montessor *et al*. 2004; Phanuphak *et al*. 2007; Stern *et al*. 2003). Incidence of hepatotoxicity was observed to be 16% and 8% for patients on NVP and effavirenze (EFV); respectively (Sulkowski *et al*. 2003) while incidence of anemia ranged from 3–12% among patients on zidovudine in developing countries (Subbaraman *et al*. 2007).

There are limited or no studies to the best of the authors' knowledge that provides reliable information on the adverse drug reactions to HAART in Ethiopia. The purpose of this study was to assess the prevalence of ADRs and to determine risk factors associated with ADRs among HIV positive patients on ARVs.

Materials and Methods

Study Design, Area and Period: A retrospective cohort study was conducted at Debere Markos Referral Hospital (300 Km Northwest of Addis Ababa) from December, 2011 to May, 2012.

Inclusion / Exclusion criteria: All adult patients who started HAART from September, 2005 to August, 2010 were included in the study. Excluded were medically incomplete records of WHO clinical stage or CD4 cell count and transferred in patients from other health facilities.

Sample Size Determination: The sample size was estimated by using Cox proportional hazards model (Sstpower cox) in STATA 11 menu (Stata Corp, College Station, Texas USA). Calculation was based on the assumption that α = 5%, power =80%. The significant predictors assessed from the different study were used to calculate the largest sample size. Data incompleteness was anticipated to be 10%, and then the total sample size was 930.

Data Collection and Data Source: The data was collected by advanced ART nurses from ART data base, ART intake form and ART follow-up form by using data extraction form prepared for this purpose. Anemia classification was modified as non-anemic if haemoglobin level >=10 g/dL, and anemic if haemoglobin level <10 g/dL) (WHO 2001).

Study variable: The occurrence of ADRs was the outcome variable. The independent variables were age, sex, educational level, marital status, residence, WHO clinical stage, anemia, drug regimen, CD4 cell count, cotrimoxazole prophylaxis and TB co-infection.

Data Quality Assurance: Training was given to the data collectors on how to collect data by using the data extraction format for two consecutive days. The principal investigator closely supervised the data collector on a daily basis to ensure data accuracy and completeness. Every incomplete questionnaire had been sent back to the corresponding data collector for checkup under supervision. Ten percent of the collected data was randomly selected and re-entered and compared with the already entered to check the correct entry of the data.

Statistical analysis: Data analysis was conducted by STATA version 11.0 (Stata Corp, USA). Pearson chi-square was used to determine the association of the independent variable with the outcome variable. Multicollinearity was excluded using Spear-man's correlation coefficient with a cutoff at 0.5. Baseline variables significant at $p< 0.2$ level in bivariate analysis were included in the multivariable logistic regression model. All tests were two-sided and level of significance was set at $P < 0.05$.

Ethical Considerations: The study was approved by the Health Research Ethics Review Committee of Mekele University, College of Health Sciences.

Results

Cohort Basic Characteristics at the initiation of HAART: Table 1 describes the characteristics of the study participants. Of the 930 patients, five hundred twelve (55.1%) were females. Half of the study participants were below age of 35 years and the median age was 34 (IQR=28 - 40) years. Two hundred ninety (31.2%) of the patients were illiterate and 41.6% of the patients were married.

Six hundred ninety nine (75%) of the participants were on WHO stage III. About 90 % of the study participants had CD4 count less than 200 cells/µl and the median CD4 count was 116 (IQR, 59 -167) cells/µl. The median hemoglobin value was 12.4 (IQR, 10.6-14.0)g/dl. Five hundred ninety three (63.8%) and 381 (41%) of patients had fever (> 1 month, unexplained) and recurrent upper respiratory tract infection, respectively. One hundred eighty two (19.6%) of study participants had TB co-infection. One hundred ninety one (20.5%) of the clients showed poor adherence. Three hundred fifty one (37.7%) of the patients were taking d4t-3TC-NVP and 272 (29.2) were taking AZT-3TC-NVP and 66 (7.1%) were taking TDF-based treatment (Table 1).

Table 1: Baseline characteristics and associated adverse drug reactions among 930 HIV-infected patients starting ART at Debre Markos Hospital, 2012

		N (%)	Adverse effects (%)	Pearson X^2(p)
Sex of participants	Male	418(44.9)	93 (43.5)	0.25(0.618)
	Female	512(55.1)	121 (56.5)	
Regimen	3TC- d4t-NVP	351(37.7)	92(43.0)	10.73(0.030)*
	3TC -d4t-EFV	165(17.7)	31(14.5)	
	3TC -AZT-NVP	272(29.2)	69(32.2)	
	3TC- AZT-EFV	76(8.2)	15(7.0)	
	TDF-3TC- EFV	66(7.1)	7(3.3)	
Marital Status	Unmarried	118(12.7)	29 (13.6)	
	Married	387(41.6)	96 (44.9)	1.89(0.388)
	Separated	425(45.7)	89 (41.6)	
Education	Illiterate	290(31.5)	48 (22.5)	
	Primary	309(33.6)	71 (33.3)	13.72(0.001)*
	Secondary $^{+}$	322(35.0)	94 (44.1)	
Residence	Urban	673(73.6)	163 (76.9)	1.58(0.209)
	Rural	242(26.4)	49 (23.1)	
Past Cotrimoxazole	Yes	554(66.6)	130 (67.0)	0.02(0.886)
	No	278(33.4)	64 (33.0)	
Anemia	No	431(51.7)	114 (58.8)	4.5(0.025)*
	yes	402(48.3)	80 (41.2)	
Adherence	Good	648(77.2)	156 (78.4)	0.20(0.656)
	Poor	191(22.8)	43 (21.6)	
WHO stage	Stage I-III	815(87.6)	196 (91.6)	4.01(0.045)*
	Stage IV	115(12.4)	18 (8.4)	
CD4 Cells/μl	≥200	95(10.2)	17 (7.9)	13.02(0.005)*
	100-199	428(46.0)	121 (56.5)	
	50-99	212(22.8)	43 (20.1)	
	<50	195(21.0)	33 (15.4)	
Age (Years)	15-24	94(10.1)	16(7.5)	3.42(0.332)
	25-34	375(40.3)	89(41.6)	
	35-44	304(32.7)	67(31.3)	
	45^{+}	157(16.9)	42(19.6)	

*Note: *=Significant, 3TC-d4T–NVP=Lamivudine, stavudine, and nevi-rapine; 3TC-AZT–NVP=lamivudine, zidovudine, and nevirapine; 3TC-d4T-EFV=lamivudine, stavudine, and efavirenz; 3TC-AZT-EFV= lamivudine, zidovudine, and efavirenz.*

Adverse Drug Reactions: After initiation of HAART, 214 patients (23.0%) of the 930 study populations developed at least one adverse event during the follow-up periods. One hundred twenty three (57.8%) of those patients taking AZT 84 (39.3%) of those taking stavudine and 2.9% of those taking TDF based regimen showed ADRs.

Among the 261episodes of HAART adverse drug reactions, the most commonly observed adverse drug reactions by the patients were anemia 65(25.0%), fat change 45(17.2%), skin rash 42(16.1%), peripheral neuropathy 32 (12.3%) and abdominal pain 26(10%) (Table 2).

Peripheral neuropathy was common ADR among patients on stavudine-based regimen while anemia and fat change were common with patients on AZT based regimen (Table 2). Of the 214 patients who experienced at least one drug related adverse event, 52 (24.3%) substituted their regimens due to drug toxicity and skin rash.

Table 2: Adverse effects of HAART during the follow up periods of September, 2005 up to August, 2011 at Debre Markos Hospital, 2012

	AZT-based	d4t-based	TDF -based	Total (%)
	N (%)	N (%)	N (%)	
Abdominal pain	25(9.58)	-	1(0.38)	26(10.0)
Anemia	35(13.41)	26(10.0)	4(1.53)	65(25.0)
Diarrhoea	2(0.77)	3(1.15)	-	5(1.9)
Dizzy, anxiety, night mare	5(1.92)	1(0.38)	-	6(2.3)
Fat change	27(10.34)	17(6.51)	1(0.38)	45(17.2)
Fatigue	3(1.15)	1(0.38)	-	4(1.5)
Headache	1(0.38)	-	1(0.38)	2(0.8)
Jaundice	2(0.77)	14(5.36)	-	16(6.13)
Nausea	10(3.83)	4(1.53)	-	14(5.4)
Numbness/tingling	4(1.53)	-	-	4(1.5)
Peripheral neuropathy	12(4.60)	20(7.66)	-	32(12.3)
Skin rash	23(8.81)	18(6.90)	1(0.38)	42(16.1)
Total occurrence of adverse effects				261

Note: d4T = Stavudine-based; AZT = Zidovudine-based TDF-based = TDF-3TC- EFV

Factors associated with the occurrence of HAART Adverse drug reactions: Pearson chi square measures of association showed that the occurrences of ADRs were associated with regimen type, base line hemoglobin level, WHO clinical stage, baseline CD4 count and educational level (Pearson chi square, $p<0.05$) (Table 1).

In the bivariate logistic regression analysis, sex, HAART regimen, marital status, occupation, residence, cotrimoxazole prophylaxis and adherence were not associated with the occurrence of adverse drug reactions (Table 3). In multivariable analysis with CD4 $\geq$ 200 cells/µl as the reference, patients on CD4 50-99 cells/µl (AOR=1.9, 95%CI [1.115- 3.364]) were more likely to develop ADR. With TDF based regimen as the reference, patients on d4t-3TC-NVP (AOR=5.8, 95% CI [1.692-9.591]) and AZT-3TC-NVP (AOR= 4.2, 95% CI [1.227-10.673]) were more likely to develop an ADR.

Discussion

The occurrence of adverse drug reactions of ARVs among patients on HAART in this study is 23% which is in agreement with other studies which reported that the incidence of side effect ranges between 11.0%–35.9% (Asfaw 2010; Bonfanti *et al*. 2000, Severe *et* al. 2010). Only 24.3% of patients where substituted and no change their regimen due to the adverse effects of HAART. This suggests good tolerance level to ARVs in general.

The prevalence of anemia in this study was 25.0% (65). Among these, 13.4% (35) of anemia occurred in those patients taking zidovudine based regimen. This finding is in agreement with previous studies from Nigeria, Cote d'Ivoire, Haiti, and India found that a rate of zidovudine-related anemia is 3%–12% (Idoko *et al*. 2002; Kumarasam *et al*. 2008; Moh *et al*. 2005; Severe *et al*. 2005; Subbaraman *et al*. 2007). In this cohort, the incidence of fat change was 17.2%.

Table 3: Factors associated with the occurrence of adverse drug reactions of patients on HAART at Debre Markos Hospital, 2012

Covariates		COR	[95% CI]		*p*	AOR	[95% CI]		*p*
Sex	Female vs. male	1.081	0.795	1.471	0.618	0.918	0.626	1.347	0.662
Hemoglobin	<10g/dl	1.447	1.046	2.004	0.026	1.359	0.915	2.02	0.129
Adherence	Good vs. Poor	1.091	0.743	1.603	0.656	0.936	0.589	1.488	0.779
WHO clinical stage	I-III vs. IV	1.706	1.006	2.893	0.047	1.427	0.737	2.763	0.291
CD4 cells/μl	CD4 ≥ 200	1.00				1.00			
	CD4 100-199	1.07	0.562	2.038	0.837	1.285	0.575	2.871	0.542
	CD4 50-99	1.935	1.259	2.973	0.03	1.936	1.115	3.364	0.019
	CD4 <50	1.249	0.756	2.064	0.385	1.33	0.715	2.475	0.368
Educational level	Illiterate	1.00				1.00			
	Primary	0.481	0.325	0.712	< 0.001	0.449	0.276	0.73	0.001
	Secondary +	0.724	0.506	1.035	0.076	0.761	0.492	1.175	0.218
Residence	Urban vs. Rural	1.259	0.879	1.804	0.210	0.85	0.551	1.311	0.463
Cotrimoxazole	Yes vs. No	1.025	0.729	1.442	0.886	1.05	0.712	1.548	0.805
HAART Regimen	TDF-3TC- EFV	1.00				1.00			
	d4t-3TC-NVP	2.994	1.32	6.79	0.036	5.758	1.692	9.591	0.042
	d4t-3TC-EFV	1.95	0.812	4.68	0.875	3.291	0.906	11.95	0.490
	AZT-3TC-NVP	2.865	1.25	6.568	0.032	4.242	1.227	10.673	0.036
	AZT-3TC-EFV	2.073	0.789	5.445	0.752	3.327	0.857	8.909	0.489

COR=Crude Odds Ratio; AOR=Adjusted Odds Ratio, 3TC - d4T – NVP=Lamivudine, stavudine, and nevi-rapine; 3TC-AZT–NVP= lamivudine, zidovudine, and nevirapine; 3TC-d4T-EFV= lamivudine, stavudine, and efavirenz; 3TC-AZT-EFV= zidovudine, lamivudine, and efavirenz

This is comparable to the study report in Southeast Asian cohorts (16.8%) and Thai cohort (17%); but lower than the report from Rwandan cohort (24.8%) (Puttawong *et al*. 2004; Tin *et al*. 2005; Van-Griensven *et al*. 2010). The prevalence of peripheral neuropathy was 12.3% which is higher than the Indian cohort (9%) and Rwandan cohort (8 %) (Kumarasam *et al*. 2008; Van-Griensven *et al*. 2010); but lower compared to the study in South Africa 17 % (Menezes *et al*. 2011). The occurrences of ADRs are the reason for poor adherence to treatment, counseling the patients on the expected ADRs are crucial to maximize the benefit of the HAART.

In this study patients with CD4 50-99 cells/μl (AOR=1.9, 95%CI [1.115- 3.364]), d4t-3TC-NVP (AOR=5.8, 95% CI [1.692-9.591]), AZT-3TC-NVP (AOR= 4.2, 95% CI [1.227-10.673]) are more likely to develop the ADRs. Other studies show the association of low CD4 count and adverse drug reactions (Subbaraman *et al*. 2007). Eluwa *et al*. (2012) reported that patients on stavudine and zidovudine were less likely to develop ADR with TDF based regimen as the reference may be due to the different sample size and small patients on TDF based regimen (1.7%).

Our study showed no difference in adverse drug reactions with sex, age, adherence, hemoglobin and WHO clinical stage. Eluwa *et al*. (2012) also showed that age, gender and CD4 count were not significantly associated with developing ADRs. However, Bonfati *et al*. (2000) observed that women experienced significantly greater number of adverse effects compared to men and could be due to the large proportion of women in the study compared to our study. Knowledge of the risk factors of ADRs of the ARV regime can help focus scarce resources to managing ADRs in a resource limited countries. The study has the following strengths: First it is a six year cohort study and including large sample size, it reflects the reality in the ART follow up study thus may be applicable to other similar settings. The study has also several limitations: the data were collected

retrospectively from patient files in a context of routine care and hence there might be a certain degree of under reporting of events. The study was confined to report on known adverse drug reactions only. Thus the specific ADRs in this study are most likely under reported. Finally, the reported adverse drug reactions do not have their complete details and graded.

To conclude, adverse drug reactions are common with HAART in HIV-infected patients and thus regular screening for side effect is required. Prospective cohort and randomized clinical trials will be required to confirm our findings and studies on the relationship of duration of therapy and ADRs should be conducted in the future to manage ADRs.

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Conflict of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

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A Comparison of total phenolic content, free radical scavenging potential and anti-hyperglycemic condition from leaves extract of *Moringa stenopetala* and *Moringa oliefera*

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Abstract
Background: In Ethiopia *Moringa stenopetala* exists in the arid and semi-arid regions in the southern Rift valley and it is widely grown for its edible leaves and used traditionally for treating *Diabetes mellitus.*
Objective: The purpose of this study was to compare the total phenolic content, DPPH scavenging potential and anti-hyperglycemic effect in diabetic mice form leaves extracts of *M. stenopetala* and *M. oliefera.*
Method: Phenolic content in each Moringa sample was measured using the Folin Ciocalteu's reagent at an absorbance of 752 nm, whereas free radical scavenging potential was measured by *in vitro* by the DPPH assay. The anti-hyperglycemic effect of Fasting Blood Glucose level was assessed by administration of 300mg/kg of methanol water (80:20) extract and ethyl acetate fraction of the two Moringa leaves in Streptozotocin induced mice.
Results: The ethyl acetate fraction of *M. stenopetala* showed highest total phenolic content and strong free radical scavenging potential. The ant-hyperglycemic effect result showed that ethyl acetate fraction of *M. stenopetala* treatment resulted in significant reduction of fasting blood glucose level initially was 190 ± 3.4351 mg/dl and it reduced to 129 ± 1.8708 mg/dl and 109 ± 4.6368 mg/dl respectively after 14 and 21 days administration.
Conclusion: The results support the widely claimed use of *M. stenopetala* as potential antioxidant and anti-hyperglycemic effect to treat diabetes mellitus.
Key words: Total Phenolic Content, antioxidant, ant-hyperglycemic, *Moringa stenopetala* and *M. oliefera*

Introduction
The Family Moringaceae consists of a single genus with about 14 species indigenous to several countries. Among these *Moringa oliefera* is native to Northern India, Pakistan and Nepal. This tree also known as horseradish tree, drum stick tree, benzolive tree, *kelor*, *marango, mlonge*, *moonga*, *mulangay*, *nebeda*y or *ben oil tree*. The other species *M. stenopetala* is native to South Ethiopia, Northern Kenya and Eastern Somalia. This tree is often named as African Moringa (Beyene 2005). In Ethiopia it exists in the arid and semi-arid regions in the Southern Rift valley. *M. stenopetala* widely grown for its edible leaves and used traditionally for treating diabetes mellitus and had already been explored (Makonnen 1997). It is known as *Haleko* and *Shelaqta* in Gamo and Konso (Mekonnen *et.al*. 1998).

All parts of the plant are utilized by human. It is rich in nutrients and has pharmacological properties as recognized by popular use and corroborated by the scientific community. Aqueous extract of *M. oliefera* leaves has significant hypoglycemic and anti-diabetic potential in a rat model (Dolly 2009). Dehydrated Drumstick leaves have positive impact on the lipid profile of hyperlipidemias (Vanisha 2009). It is also reported as anti-inflammatory, antimicrobial, antioxidant, anticancer, cardiovascular, anti-ulcer, hepatoprotective, diuretic, antiurolithiatic, and anthelmintic (Fozia 2012).

Different morphology of *M. oliefera* extracts such as leaf, pod and fruit has a potential in preventing Newcastle Disease virus, effective chemotherapy in renal carcinogenesis and strong reducing power and free radical scavenging capacity respectively (Didacus 2012; Paliwal 2011; Suaib 2011). *Moringa oliefera* is a significant source of β-caroeine, ascorbic acid (Vit.C), $\propto$-tocopherol (vitamin E), iron (Ray-Yu Yang, 2006), potassium, calcium and protein (Suchada 2010). In *M. stenopetala* leaves, vitamins and minerals exist are present in significant concentration (Abuye *et.al*. 2003).

Increased intake of antioxidants may protect against chronic diseases including cancers, cardiovascular and cerebrovascular diseases (Prior 2002). A high content of γ-tocopherol has been found in practically the whole plant, ranging from 5.7μg/g to 27.8μg/g of dry mass and xanthins (neoxanthin 219mg/kg, violaxanthin 76.5mg/kg, zeaxanthin 19.4mg/kg) are also found (Paulo Michel Pinheiro 2008). Major polyphenols in *M. oliefera* leaf powder are quercetin glucosides, rutin, kaempferol glycosies and chlorogenic acids (Ndong 2007).

Inhibition of α-amylase and α-glucosidases involved in the digestion and absorption of carbohydrates can decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet. Flavonols and quercetin had the highest maltase, glucoamylase, and isomaltase inhibitory activities (Jo 2010). Chlorogenic acid is an important intermediate in lignin biosynthesis. This compound, long known as an antioxidant, also slows the release of glucose into the bloodstream after a meal (Johnston et.al. 2003). It has also been demonstrated that flavonoids can act *per se* as insulin secretagogues or insulin mimetic (Brahmachari 2011). Thus, the present study was compare total phenolic content and free radical scavenging potential, blood glucose lowering effect of the hydroalcohol extract and ethyl acetate fraction of *M. stenopetala* and *M. oliefera* leaves.

Materials and methods

Collection of Plant material: Fresh leaves of *M. stenopetala* was collected from Melkasa Agricultural Research Center 150km east of Addis Ababa and *M. oliefera* was collected from Debrezeyet Agricultural Research Center 47km east of Addis Ababa. Leave samples were identified and authenticated by a taxonomist in Ethiopia Agricultural Research Institute. The collected samples were washed well distilled water, dried under shade, crushed and powdered for extraction.

Standard drugs: Streptozotocin (STZ) (Sigma, USA) for induction of hyperglycemia and Glibenclamid (Glitisol, Cyprus) was used as a standard hypoglycemic drug.

Instrumentation: Rota vapor (Buchi R-500, Switzerland), lyophilizer (Labconco, USA), UV/Visible Spectrophotometer (Evolution 220, Thermo Scientific Germany) Sensocard glucometer (77 Electronic kit, Hungary) and GLAB active glucose test strip (77 Electronic kit, Hungary) were used in this study.

Chemicals and solvents: All the chemicals, reagents, and solvents used in the assay protocols were analytical grade methanol (Riedel-de Haen, Germany), petroleum ether (40^0C-60^0C, Fluka, Germany), ethyl acetate (Park Scientific, Nottingham UK),1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Sigma, USA), sodium hydroxide (Riedel-de Haen, Germany), ascorbic acid (Merck, Germany), Folin Ciocalteu's reagent (Sigma, USA) and sodium bicarbonate (Na_2CO_3) (Park Scientific, Nottingham UK).

Preparation of total hydroalcohol extracts of M. stenopetala and M. oliefera: Air dried powdered leaves of *M. Stenopetala and M. oliefera* (400g) were soaked in (80:20) methanol: water (Arnnok, 2012) for three days with occasional shaking. The extraction was repeated three times and filtered with Whatman No.1 filter paper and the filtrates combined. The combined filtrates were concentrated using a Rota Vapor. The aqueous residues were lyophilized and kept in desiccators for future use. The methanol-water extract yielded 20.415 % (81.66g) for *M. stenopetala* and 18.753% (75.021g) for *M. oliefera*.

Solvent-solvent partitioning of the hydroalcoholic extracts: The procedure for solvent-solvent separation was adopted from (Samsam-sharjat 1992).Ten grams of the lyophilized hydroalcoholic extracts of the plant materials were dissolved in 100 ml of methanol and distilled and deionized water (80:20). The dissolved extracts were separated in a separator funnel with ethyl acetate (40-60^0c), successively until the extracting solvents became colorless. In all cases of separation, 150 ml of solvents were used. After completing the separation process, the solvents were recovered by Rota Vapor. The separates were kept in the refrigerator for the next experiments.

Total Phenolic content determination: Phenolic content in each Moringa leaves powder sample was measured using the Folin Ciocalteu's reagent at an absorbance of 752 nm (Anderessa *et al*. 2013). The results are expressed as g Gallic Acid Equivalent. First, 2 mL Folin Ciocalteu's Reagent in 20 mL of water was prepared to form a stock solution of Folin Ciocalteu's reagent solution. Saturated solution of Na_2CO_3 (15%) was prepared by dissolving 7.5 g of Na_2CO_3 in 50 mL of water. About 100 mg of the powdered *M. stenopetala* and *M. oliefera* was placed in a 25 mL volumetric flask and 20 mL of 80% methanol in water (v/v) was added and sonicated for 25 minutes. After sonication, the flasks were filled to volume with water and 40 µL of the extract was transferred to a centrifuge tube with 900 µL of Folin Ciocalteu's reagent solution and set aside for five minutes. 400 µL of 15% Na_2CO_3 was added to the mixture, allowed to react for 45 minutes and measured at 752 nm. To develop the calibration curve, 6.0 mg of gallic acid was added into 25 mL of 60% methanol solution to provide the standard solutions. Six dilutions of concentrations ranging from 0.25 mg/mL to 0.0078 mg/mL and a blank were prepared.

Y = 5.4135X – 0.007 (r^2 = 0.999). 40 µL of each dilution was also used for the calibration curve.

DPPH (Diphenylpicrylhydrazine) assay: Free radical scavenging activity of methanol-water extract and the ethyl acetate fractionate of each sample was measured by the modified DPPH method (Manish 2011). DPPH scavenging activity was measured by the spectrophotometric method. Briefly: To 1 ml of various concentrations of extracts, 1 ml solution of DPPH (0.1 mM) was added. An equal amount of methanol and DPPH served as control. After 20 min of incubation in the dark, absorbance was recorded at 517 nm. The experiment was performed in triplicate and the percentage inhibition calculated by using the formula.

Scavenging %= [(Abs. of Blank - Abs. of Sample) /Abs. of Blank]/x100

Pharmacological Evaluation Laboratory Animals: Laboratory bred Swiss albino mice, 6-8 weeks, weighing 25-30g were obtained from Ethiopian Health and Nutritional Research Institute, Addis Ababa. All animal procedures were in accordance with the standards set forth in guidelines for the care and use of experimental animals by committee for purpose of supervision of experiments on animals. All mice were fasted for 24 hour before diabetes was induced with STZ. The animals were allowed to acclimatize for 2 weeks before the experiment. The animals were housed in polypropylene cages inside a well-ventilated room. Each cage consists of not more than 5 rats. They were maintained under standard laboratory conditions and 12 hour light/dark cycle. They were fed a standard commercial pellet diet and water *ad libitum*. The diet consists of 71% carbohydrate, 18% protein, 7% fat, 4% salt mixture and adequate minerals and vitamins.

Induction of Diabetes Mellitus: Streptozotocin (STZ) was dissolved in cold 0.01 M citrate buffer, pH 4.5 and prepared freshly for immediate use. STZ injections were given intraperitoneally at 50mg/kg body weight. Blood glucose concentration was measured week from the day of STZ injection. The blood samples were collected from the tail vein once a week and blood glucose measured by glucose oxidase method.

Determination of anti-hyperglycemic effect of crude and fractionated extracts M. stenopetala and M. oliefera in STZ induced mice: STZ-induced diabetic mice fasted for 16 hours were selected and divided into six groups of five mice per group. Group 5 and 6 respectively served as positive and negative controls and received glibenclamide (0.66 mg/kg body weight) and distilled water (10 ml/kg body weight) daily for 21 days by oral by gavage. Group 1 to 4 were administered with 300 mg/kg body weight of hydroalcoholic and fractionated extracts of *M. stenopetala* and *M. oliefera* respectively, daily for 21 days by oral gavage.

In all cases of pharmacologic evaluations, the extracts were dissolved in distilled. The blood samples were collected from the tail vein once a week at 0, 7, 14 and 21 day and the obtained blood glucose concentration determined by the glucose oxidase method.

Statistical analysis: The results are expressed as mean ± S.D. and all statistical difference between the treatment and the control were tested by one-way analysis of variance (ANOVA) followed by Duncan multiple test comparisons using SPSS version 16. The difference in the mean values showing a p level of 0.05 or lower was considered to be statistically significant.

Results

Total phenolic content: The quantitative analysis of total phenolic content in crude extract and fraction of the leaves of *M. stenopetala* and *M. oliefera* revealed that the ethyl acetate fraction of *M. stenopetala* leaves containing highest amount of total phenolic content (283.8µg GAE/ml) followed by crude methanol-water extract of *M. stenopetala* leaves (211.9 µg GAE/ml) whereas moderate amounts recorded in ethyl acetate fraction of *M. oliefera* leaves (93.56 µg GAE/ml) followed by methanol-water crude extract of *M. oliefera* leaves (58.7 µg GAE/ml) (Figure 1).

DPPH (Diphenylpicrylhydrazine) scavenging activities: By reduction of the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine, the scavenging activity of the DPPH radical was tested. According to the experiment, *M. stenopetala* ethyl acetate fraction showed maximum activity of 96.22% followed by *M. stenopetala* methanol-water crude extract 94.14% whereas *M. oleifera* methanol-water crude extract showed 90.03% scavenging activity followed by *M. oleifera* ethyl acetate fraction 86.09% (Figure 2).

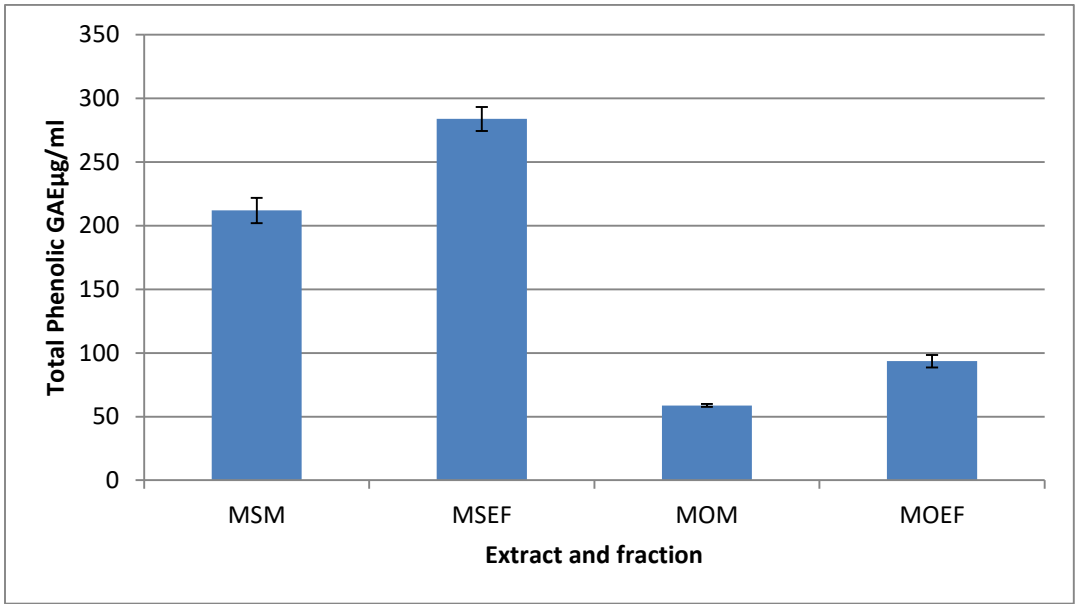

Figure 1: Total phenolic content GAEµg/ml

MSM-Moringa stenopetala Methanol-Water Extract, MSEF-Moringa stenopetala Ethyl Acetate Fraction
MOM-Moringa oliefera Methanol-Water Extract, MOEF- Moringa oliefera Ethyl Acetate Fraction
SD-Standard Drug, DC-Diabetic Control

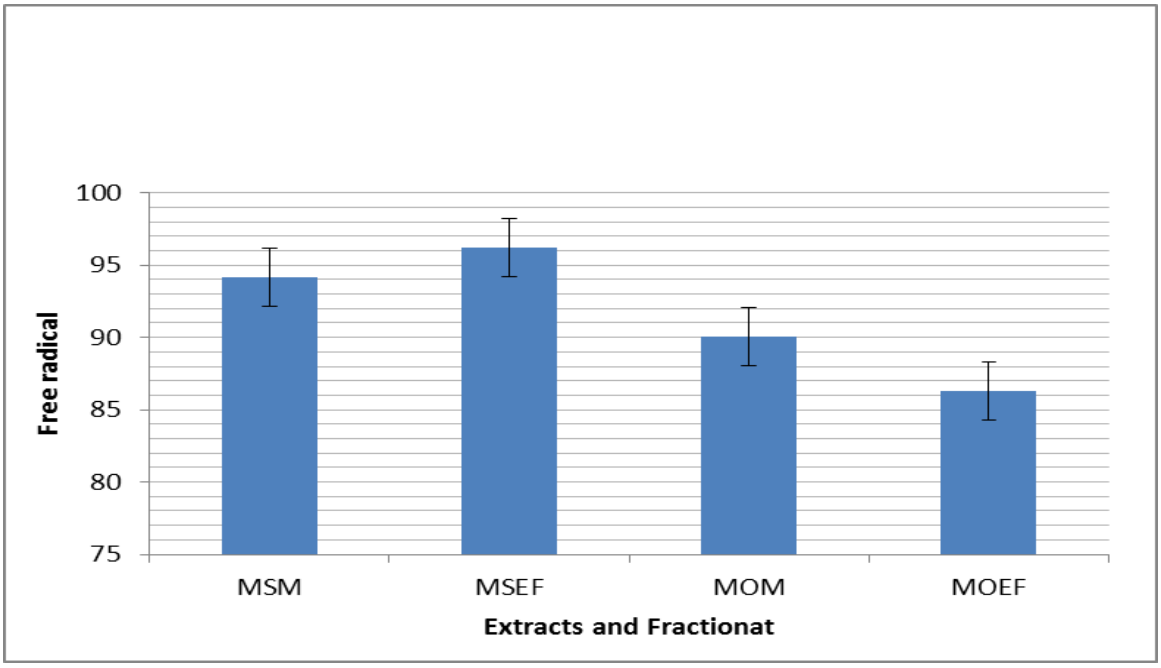

Figure 2: Free radical scavanging potential %

Ant-hyperglycemic effect of hydroalcoholic and fractionated leaf extracts of M. stenopetala and M. oliefera on fasting blood glucose level in STZ-induced mice: During the three weeks of extract treatment, fasting blood glucose level was measured once weekly. The results are summarized in Table 1 and Figure 3. Before induction of diabetes, there was no significant difference in fasting blood glucose (FBG) level among the treatment groups. FBG levels in the treatment groups showed no significant differences at 0 day of the administration ($p<0.05$). After repeated oral administration of *M. stenopetala* ethyl acetate fraction and standard drug showed significant reduction in FBG level at the 7th day ($p<0.05$). *M. stenopetala* methanol-water extract and *M. oliefera* ethyl acetate fraction showed similar reduction in FBG level at the 7th day ($p<0.05$). *Moringa stenopetala* ethyl acetate fraction and *M. stenopetala* methanol-water crude extract showed significant reduction in FBG level at 14th and 21th day ($p<0.05$). *Moringa oliefera* ethyl acetate fraction and *M. oliefera* methanol-water crude extract showed similar reduction in FBG level at 14th day ($p<0.05$).

Table 1: Fasting Blood Glucose level (mg/dl)

Group	Treatment	0 day	7 day	14 day	21 day
I	MS M	198.6±6.3087	174.4±3.8471	147±5.1478**	117.2±10.7331***
II	MS F	190.6±3.4351	160.4±12.7397**	129±1.8708***	109±4.6368***
III	MO M	199.8±6.3796	182.6±8.1731	160.6±4.7223	129.8±10.3779***
IV	MO F	189.2±7.4297	173.4±6.2689	156.8±12.9884**	135±4.6368***
V	SD	200.8±6.8337	124.6±4.615***	135.8±8.6718***	115.6±7.4699***
VI	DC	183.8±5.4037	192.2±5.5857	217.2±4.037	253.2±1.304

***=p<0.01, ***= p<0.001 as compared to the control, Results are means ±SD of n=5*
Dose of crude extracts and fraction = 300mg/kg, Dose of glibenclamide = 0.66mg/kg

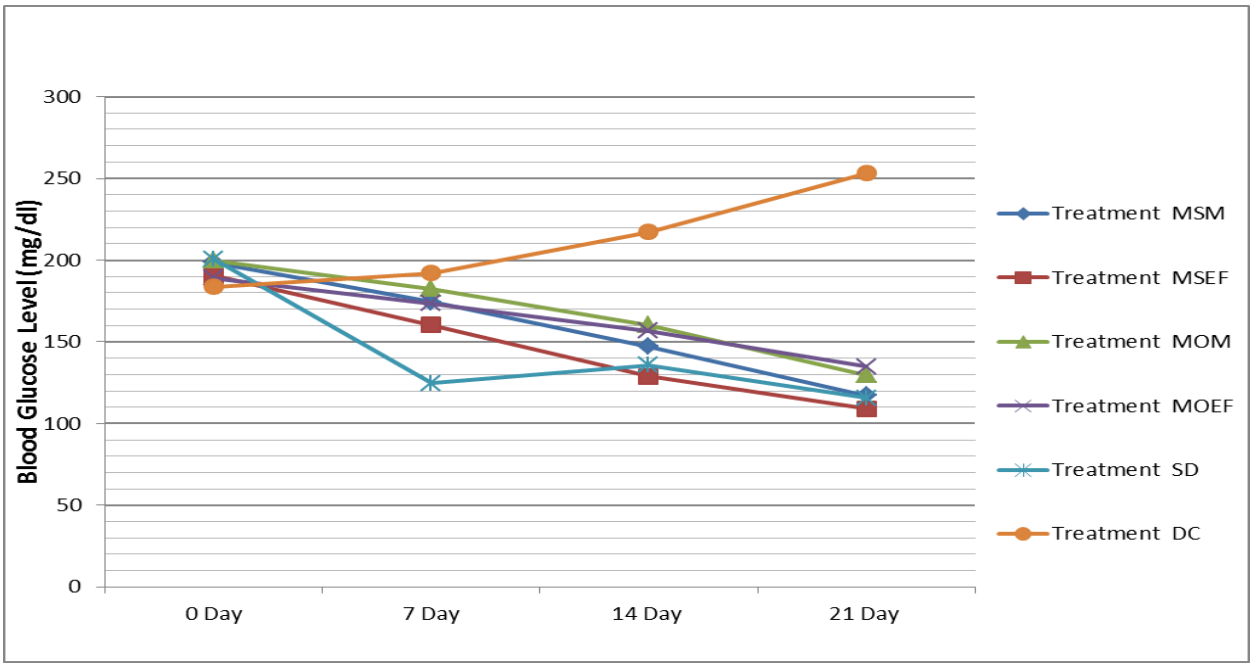

Figure 3: Antihyperglycemic effect produced by repeated dose oral administration of crude extracts and fraction of *M. stenopetala* and *M. oliefera*

MSM-Moringa stenopetala Methanol-Water Extract, MSEF-Moringa stenopetala Ethyl Acetate Fraction
MOM-Moringa oliefera Methanol-Water Extract, MOEF- Moringa oliefera Ethyl Acetate Fraction
SD-Standard Drug, DC-Diabetic Control

Discussion

Diabetes is a defect in the body's ability to convert glucose (sugar) to energy. It develops when the pancreas fails to produce sufficient quantities of insulin -Type 1 diabetes or the insulin produced is defective and cannot move glucose into the cells-Type 2 diabetes. This metabolic disorder is characterized by hyperglycemia (fasting blood glucose level greater than 126 mg/dl taken on at least two separate occasions) and disturbances of carbohydrate, protein and fat metabolisms. The effects of *Diabetes mellitus* include long term damage, dysfunction and failure of various organs (WHO 1999).

For hundreds of years, traditional healers have prescribed different parts of Moringa for treatment of skin diseases, respiratory illnesses, ear and dental infection, hypertension, diabetes, cancer treatment, and water purification, and they have promoted its use as a nutrient dense food source (Fozia 2012; Makonnen 1997; Suchada 2010). The previous works on hypoglycemic activity evaluation of the crude aqueous extract of *M. stenopetala* leaves (Makonnen 1997), hypoglycemic effect evaluation of the cured aqueous extract and n-butanol as well as chloroform fractions of *M. stenopetala* leaves (Mussa 2008), effect of *M. oliefera* leaves aqueous extract therapy on hyperglycemic rats (Dolly *et al*. 2009) and anti-diabetic property of drumstick (*M. oliefera*) leaf tablets (Giridhari *et al.* 2011) confirmed the traditional claim. A comparative *in vivo* and *in vitro* analysis of anti-hyperglycemic effect and anti-oxidant activities for hydroalcoholic and fractionated extracts of *M. stenopetala* and *M. oliefera* leaves in the present study.

In this study, the anti-hyperglycemic effect was carried out in Streptozotocin induced diabetic mice. Streptozotocin (STZ, N-nitro derivative of glucosamine) is a naturally occurring, broad-spectrum antibiotic and cytotoxic chemical that is particularly toxic to the pancreatic, insulin producing beta cells in mammals. It has been widely used to induce DM in experimental animal models, allowing the investigation of hypoglycemic agents in treatment of diabetes. Streptozotocin injection consistently produces symptoms of DM including hyperglycemia, decreased insulin levels, polyuria, and weight loss. The dose of STZ required for inducing diabetes depends on the animal species, route of administration and nutritional status. In mice, doses vary between 50-60 mg/kg by intraperitoneal route; clinical symptoms

of diabetes are clearly seen within 2-4 days. (Abdu *et.al*. 2009).
The diabetic mice used in the present study were treated for 21 days with methanol-water crude extract and ethyl acetate fraction of *M. stenopetala* and *M. oliefera* leaves. The result showed that the ethyl acetate fraction of *M. stenopetala* administration produced significant hypoglycemia effect starting from the 14^{th} day as compared with the standard drug. Hydroalcoholic crude extract of *M. stenopetala* and the standard drug caused similar significant reduction in blood glucose level at 21^{th} day of administration. Hydroalcoholic and fractionated extracts of *M. oliefera* did not cause any significant change in blood sugar level as compared to the control.

Total phenolic content of the hydroalcoholic and fractionated extracts were performed using Folin-Ciocalteu's method that indicated *M. stenopetala* ethyl acetate fraction containing the highest amount followed by methanol-water crude extract. The exact antidiabetogenic mechanisms of actions of Moringa are unknown. However, it may be speculated that hydroalcoholic and fractionated extracts of Moring leaf acts in a similar way as the standard antidiabetic drugs like glibenclamide. Glibenclamide is a second generation sulfonylurea derivative which acts by stimulating release of insulin from pancreatic-cells and by increasing sensitivity of peripheral tissue to insulin. Glibenclamide is not effective in pancreas ectomized animals or patient with no endogenous insulin (Habibuddin *et.al*. 2008).

Plants can have properties similar to known drugs like glibenclamide, which reduce blood sugar level in normoglycemic and hyperglycemic animals. Some other plants do not reduce sugar level in normal states of the animal like metformin (Stumvoll *et.al*. 1995). As the extracts of Moringa were observed to reduce sugar level in Streptozotocin induced diabetic mice similar to glibenclamide, the extracts perhaps have a mechanism of action similar to insulin secret gouges like glibenclamide.

In *in-vitro* antioxidant test, hydroalcoholic and fractionated extracts showed the presence of antioxidant components as visualized by DPPH reagent. Diabetogenic effect of Streptozotocin is due to generation of free radicals that can affect the normal functioning of β-cells of pancreas (Szudelske 2001). Hence, there might be relationship between the existing antioxidant and antihyperglycemic effect of extracts. As the antioxidants can normalize the β-cells of the pancreas and might renew cells to secrete efficient amount of insulin. However, this must be supported by further studies.

Conclusion

Based on the observation, the ethyl acetate fraction of *M. stenopetala* leaves maintain the fasting blood glucose level in Streptozotocin induced mice. The results support the potential antioxidant activity of *M. stenopetala* leaves followed by *M. oliefera* which adds one more positive attribute to its known pharmacological properties and hence its use in traditional system of medicine.

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Prevalence of urethral pathogens and HIV co-infection among men presenting with urethral discharge

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Abstract
Background: Sexually transmitted infections are major public health challenges currently, due to the high frequency of infections accompanied by a declining of treatment options. Especially in developing nations, the spread of STIs/HIV infection continues to affect millions of young and productive population. This study was aimed to determine the prevalence of STI agents among male patients presenting with urethral discharge syndrome.
Methods: An institutional based cross sectional study was conducted at health centers in Addis Ababa from August 2013-August 2014.Urethral discharge specimens were cultured on Modified Thayer Marthin media and suspected *gonococcal* colonies were confirmed using Oxidase, Superoxol tests followed by API-NH^R. In addition, urine samples were tested against STI agents (*Neisseria gonorrhoeae, Chlamydia trachomatis, Trchomonas vaginalis* and *Mycoplasma genitalum*) by Real Time PCR. Serologic Syphilis testing was done using standard tests. HIV test was done by rapid test (KHB^R, Statpack^R and Unigold^R) at the spot.
Results: Five hundred ninety nine urethral discharge specimens were collected and out of these 415 were microbiology culture positive for gonococcus. The Real Time PCR result showed that 321(53.6%), 79(13.2%), 40(6.7%) and 21(3.5%) were found to be positive for *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis* and *Mycoplasma genitalium* respectively. Out of the 274 patients who got tested for HIV, 19 of them (7%) were found to be HIV positive. The prevalence of syphilis was 1.5% (9/599).
Conclusion: There is a higher burden of urethral discharge syndrome and HIV co-infection among the young men with risky behavior. *Gonococcal* infection is the leading urethral discharge syndrome followed by *chlamydial* infection.
Key words: Sexually transmitted infection, Urethral discharge syndrome.

Introduction
Sexually transmitted infections (STIs) are one of the important public health problems in both developed and developing countries. Sexually transmitted diseases (STDs) are among the first top ten causes of morbidity in young adult males in developing countries and the second in young adult women **(**Workowski *et al*. 2008**)**. The most frequent curable STI infections are caused by bacteria or protozoa *(Treponema palladium, Neisseria gonorrhoeae, Chlamydia trachomatis, and Trichomoniasis vaginalis*). If STI is left untreated, it has serious adverse health consequences including adverse pregnancy outcomes, reproductive morbidity and mortality, and enhanced HIV transmission (WHO2007). In addition, the high prevalence of these infections accompanied by declining of treatment options are the reasons those considered as public health priorities. The high rate of asymptomatic infections and the consequent under- diagnosis explain the widespread problem of under reporting, difficulty in assessing the true incidence and prevalence of STI (Mayaud *et al*. 2004). Quantifying the incidence and burden of these infections is important for planning appropriate interventions and advocating for resources, as necessary (WHO 2007). This study focuses on determination of the prevalence of STI agents among male patients presenting with urethral discharge syndrome in Addis Ababa, Ethiopia.

Materials and Methods
The study was conducted in Addis Ababa City Administration. It is geographically located in the central part of the country. The population of Addis Ababa were estimated to be 3,195,000 consisting of 1,515,000 men and 1,680,000 women (CSA 2014). A cross sectional facility based study was conducted in the health centers of Addis Ababa. The selected health facilities were Arada, Tekalehaimanot, Addis Ketema, Kirkose, Kotebe, Akaki- Kaliti, Shiromeda and Kassanchis health centers. These health facilities were selected purposively based on assessment made

before this study on the flow of STI clients. There were eight study teams, one nurse and one overall study coordinator. Following training on study protocol, procedures, and research ethics, the study team stayed twelve months at the study site collecting samples.

Specimen Collection: Men presenting to the selected health facilities with urethral discharge syndrome, a sterile cotton-tipped swab were used to obtain a metal swab. Then sterile Dacron swabs tipped applicator were used to collect urethral discharges. The swabs were inoculated on in-house prepared Modified Thayer Martin Agar plates made of Gonococcal agar base supplemented with isovitalex (vitox); vancomycin, colistin, nystatin, and trimethoprim (VCNT); and synthetic hemoglobin (Oxoid and BBL).The inoculated plates were incubated on the site using candle jar and transported to the Ethiopian Public Health Institute Clinical Bacteriology and Mycology Reference Laboratory within the same day of collection. Swab was rolled onto a microscopy slide, labeled, heat fixed, placed in a slide box and sent to EPHI for Gram-stain analysis.

In addition to urethral discharge, first-catch urine specimen (approximately 20ml) were also collected to be tested by a Real-Time, multiplex polymerase chain reaction (RT-PCR) assay to determine the presence of *C. trachomatis, N. gonorrhoeae, M. genitalum* and *T. vaginalis*. Five ml whole blood was also drawn using plain tube from the same patients for serological test of syphilis.

Laboratory analysis

a. Culture and identification: In the clinical bacteriology laboratory, inoculated plates were incubated at 35ºC in carbon dioxide enriched environment (5-8 % CO_2) for 72 hours inspecting every day for the growth of small, translucent and non-pigmented colonies. Isolates with convex, glistening, elevated, mucoid and nonhemolytic colony characteristics were taken as probable gonococcus. In addition, a positive reactions for oxidase and supercool (30% H_2O_2) were considered as presumptive of *N. gonorrhoeae*. Confirmatory test was done using API-NH^R.

b. Multiplex Real-Time PCR: Aliquoted urine samples were extracted manually using Qiagen mini kit. The master mix was prepared mixing ready to use multiplex (5-plex) CDC in-house primers, (containing forward and reverse primers of *Chlamydia trachomatis, Trichomonas vaginalis, Neisseria gonorrhoeae,* RNP and *Mycoplasma genitalum*), Roche, nucleotides, $MgCl_2$, Taqman polymerase enzyme were mixed with extracted DNA. Amplification was done by Rotor gene QR machine. To monitor the analysis known positive and negative controls were used along with each run and the result was interpreted using Cycle Threshold (CT) value less than 35 as positive.

***c. Serological tests*:** From the collected blood samples serum was separated and analyzed using rapid plasma reagin (RPR) assays for screening and *Treponema palladium* passive particle agglutination assay (TPPA) for confirmation of syphilis respectively. HIV screening test was done at the spot using rapid test kits recommended by Federal Ministry of Health (KHB^R) followed by StatPack^R and confirmatory Unigold^R.

Ethical clearance

This study was ethically cleared by Scientific and Ethical Review Office (SERO) of Ethiopian Public Health Institute and the IRB of CDC-Atlanta.

At the enrollment visit, all men with urethral discharge (UD) were given written consent diagnosed according to the syndromic treatment guidelines currently approved in Ethiopia. Those who are eligible (> 18 years of age) and willing to participate in the study were asked using structured questionnaire for their demographic and behavioral data. All data were kept confidentially anonymously. Brief counseling on the importance of adherence to STI medications, not having sex while taking medications, HIV/STI prevention, and recommendations to use condoms to reduce STI/HIV acquisition and transmission was given. The newly diagnosed were counseled properly at each facility. The participants were provided condoms and information on use of condoms, partner notification cards. Those clients who had positive syphilis tests, with active syphilis documented by a positive TPPA in the absence of ulcer, were contacted and treated for latent syphilis of unknown duration with Benzanthine penicillin.

Results

Socio-demographic characteristics: A total of 599 patients with urethral discharge syndrome were recruited from eight health centers in Addis Ababa from August 2013 to August 2014. About quarter of the patients was reported from Kazanchis health center and 19% were recruited from Arada Health center (Figure 1).

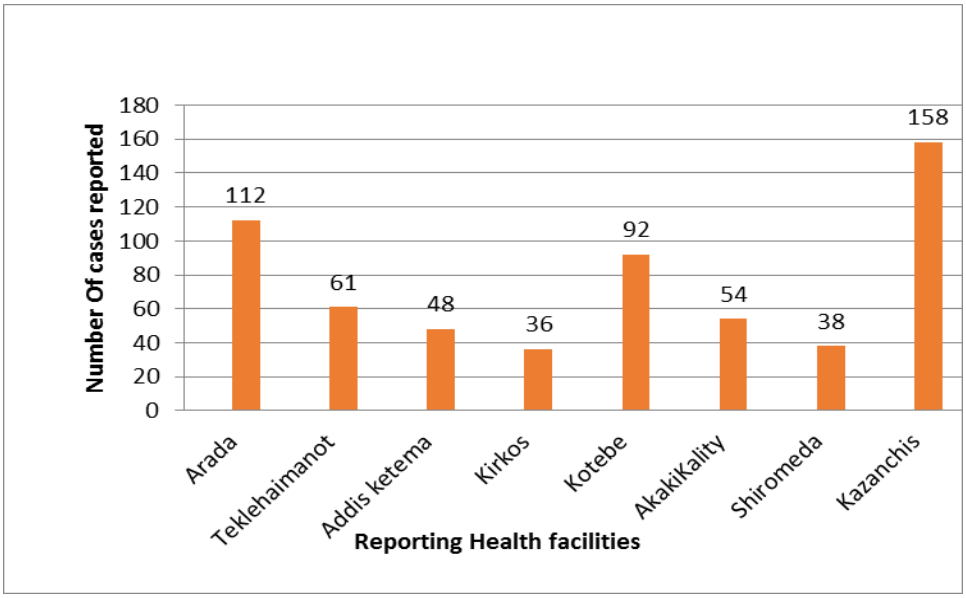

Figure 1: Distribution of study participants with urethral discharge by health facility

Out of 599 participants around 40% patients were in the age group of 25-29 years, and about one third of the patients (33%) were 20-24 years old. A considerable proportion (4.7%) of the patients was 18 or 19 years old (Figure 2).

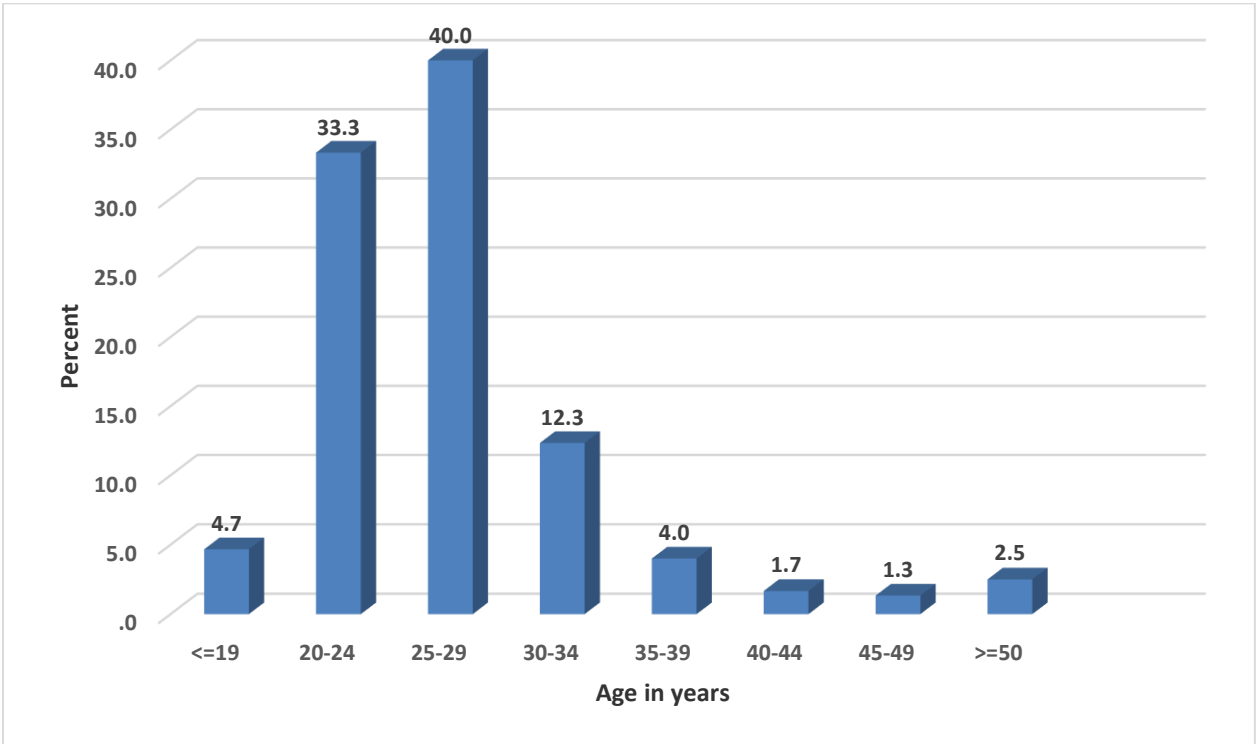

Figure 2: Age distribution of study participants with urethral discharge

Laboratory test results: Gram stain was done for 599 collected specimens and 449 (74.8%) were found to be gram negative diplococcic. GC culture was done for all collected samples 599 specimens and 415 (69.3%) were culture positive and confirmed by API-NH test.

Table 1: Gram stains and culture laboratory results

GC confirmation Method	Result	Frequency	Percent
Gram stain	Positive	449	75%
	Negative	150	25
Culture	Positive	415	69%
	Negative	184	31%
	Total	599	100

HIV status of patients with Urethral discharge and sexual characteristics: Out of the total UD patients enrolled in the study, HIV testing was performed for nearly half (44%) of the patients, 8 patients were known HIV positives and the remaining were not tested since some refused to get tested and others couldn't get the testing at the public health facility due to critical HIV test kit shortage throughout the country during the study period. Out of the 274 patients who got tested for HIV, 19 of them (7%) were found to be HIV positive, 11 of HIV positives were identified on the survey and 8 patients are known HIV positives (Table 2).

Table 2: HIV and Syphilis status co-infection among men presenting with urethral discharge

HIV testing	Response	Frequency	Percent
Offered for HIV testing	Performed	266	44.3
	Known Positive	8	1.3
	Refused/Not Tested	325	54.2
	Total	599	100
HIV test result	Positive	19	6.9
	Negative	255	93.1
	Total	274	100
Syphilis serology- RPR	Reactive	36	6
	Non-Reactive	563	94
	Total	599	100
Syphilis serology- TPPA	Reactive	9	25
	Non-Reactive	27	75
	Total	36	100

The syphilis screening test was done for all the 599 specimens by RPR test and 36 (6%) of them were found to be reactive. The RPR reactive 36 samples were confirmed using TPPA and 9 (25%) of them were confirmed positive for syphilis infection (Table 2).

Concerning sexual characteristics of patients, majority (65%) claimed that they have only one sex partner, and 13% had 3 or more sexual partners in the last three months. Majority of the participants (44%) confessed that they have never used condom in the last three months and only 19% responded consistent use of condom. About 71% of the patients did not use condom during the last sex. Last sex was with their regular partner in 42% of the patients, 28% had last sex with a casual partner and about 15% with a sex worker (Table 3).

Table 3: Sexual behavior, prevalence of urethral pathogens and HIV co-infection among men presenting with urethral discharge

Last sexual Partner	Condom use during last sex	Frequency
Spouse/Live in partner	Yes	10
	No	61
Regular Partner	Yes	40
	No	213
Casual Partner	Yes	59
	No	108
Commercial sex worker	Yes	53
	No	31
Unspecified	Yes	3
	No	15

Of the total 599 UD samples subjected to Multiplex PCR analysis 321(53.6%), 79 (13.2%), 40 (6.7%) and 21(3.5%) were found to be positive for *N.gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and *Mycoplasma genitalum* respectively (Table 4).

Table 4: STI etiologies identified using multiplex PCR from patients treated at health centers in Addis Ababa, August 2013- August 2014

STI etiologies	Multiplex PCR result		
	Positive	Negative	Percent
N. gonorrhoeae	321	278	53.6
C. trachomatis	79	520	13.2
T. vaginalis	40	559	7.0
M. genitalum	21	578	3.5

Discussion

In this study around 40% of urethral discharge patients were in the age group of 25-29 years, and about one third of the patients (33%) were 20-24 years old. Considerable proportions (4.7%) of the patients were 18 or 19 years old. This finding is somehow similar with findings on the EPHI national STI case surveillance report for 2014, where a total of 623 STI cases were reported from eight sentinel surveillance sites located in Amhara, Oromia and Addis Ababa regions, and majority (69%) of the STI patients were in the age group 20-34 and about 7.5% of the patients were below 19 years of age **(**National STI Case Surveillance Report, 2013- 2014). Moreover, these participants were at age group of 18 up to 29 years and have been exercising unsafe sex with their partners or female commercial sex workers (Table 3). Regarding behavioral characteristics of urethral discharge patients, about 28% of the UD patients had their last sex with a casual partner and about 15% with sex workers. Out of 599 participants in the study around 71% of the patients did not use condom during their last sex. The prevalence of risky sexual behavior in this study is found to be worse than the findings on the national STI surveillance where, 40% of them haven't used condom during the last sexual contact with a casual partner**.** This difference could be because the current survey is done only in Addis Ababa, which is purely a major urban town. This implies that there is still a prevailing risky behavior of unprotected sexual intercourse among the younger age group despite the existing behavioral change programs being done in the country.

Around 449 urethral discharges had intracellular or extracellular gram negative diplococci with moderate amount of polymorph nuclear cells. Whereas, 415 (69%) urethral discharges were culture positive. The difference number of detection might be due to prior antibiotic treatment that can inhibit culture growth. Out of 599 urine samples, 321 (54%) were positive for *N. gonorrhoeae*, and 79 (13%), 40(7%) and 21 (3.5%) were positive for *Chlamydia trachomatis*, *Trichomonas vaginalis* and *Mycoplasma genitalum* respectively on PCR test. The pattern of STI etiologies is in line with the study from South Africa, where urine multiplex PCR showed gonococcus led on 85% of Cape Town and 71% Johannesburg urethral discharge patients (Mhlongo *et al*. 2010).

Chlamydial infection was found the second next to gonococcal infection which accounts 13% of the participants. This result is somehow similar to studies conducted in South Africa, where the KZN study found a 16% rate of infection (Sturm *et al* 2004) and another study from Johannesburg reported 19% (Black *et al*. 2008).

This study shows that the prevalence rate of *Trichomonas vaginalis* was around 40 (7%). This finding is higher than the Korean study which was studied by molecular technique that had a prevalence of 4 % (10) and comparable with a study conducted in South Africa which was 6% (Sturm *et al.* 2004).

Since *M. genitalum* takes a very long time to culture, molecular diagnostic methods are better option to diagnose. The pathogen status of *M. genitalum* in this study was around 3.5% which is lower than a study in South Africa, where it was implicated as the causal agent in 18-46% of men with urethritis and 5% in the overall prevalence of South African study (Sturm. *et al*.2004).

In this study around 75 of participants had multiple infections of gonococcal and or non-gonococcal infections. Of the 599 participants, co-infection with *gonorrhoeae* and *chlamydia* was present only in 53 (8.8%), co-infection with *gonorrhoeae* and *trichomonas* only in 12 (2%), co-infection with *gonorrhoeae* and *mycoplasma* only in 6(1%) and *chlamydia* and *trichomonas* only in 4 (0.7 %).

The prevalence of syphilis by RPR test was 36/599 (18.5%) but out of 36 RPR positive only 9 (1.5%) of them were confirmed positive by TPPA which is the more specific test. This result coincides with another study conducted in Gondar (Tessema *et al*.2010).

Out of the 274 patients who got tested for HIV, 19 of them (7%) were found to be HIV positive, 11 of HIV positives were identified on the survey and 8 patients were known HIV positives. The HIV prevalence in this survey is a bit lower than the one reported on the national STI case surveillance, where HIV prevalence among UD patients was 13% (National STI Case Surveillance Report, 2013- 2014). But the HIV prevalence on this survey may not be accurate enough to show the true picture of HIV status among UD patients, because only 44% of patients with UD were tested due to the critical test kit shortage in the country at the time of the survey. In addition, the low rate of HIV positivity in the presence of confirmed bacterial STI might be explained by the longer seroconversion time of HIV than gonococcal infection **(**Incidence Assay Critical Path Working Group 2011)**.**

Conclusion

There is a higher burden of urethral discharge syndrome among the young men less than 30 years of age implying a prevailing risky behavior of unprotected sexual intercourse. There is a high prevalence of risky sexual behaviors, multiple sexual partner contact, unprotected casual sex, low utilization of condom, in the community which pause people at risk of acquiring STIs and a significant co-infection of HIV among patients with urethral discharge. *Gonococcal* infection is the leading cause of urethral discharge syndrome followed by *chlamydial* infection.

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Improving skilled birth attendance: An evidence brief for Ethiopia

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Abstract

Background: Skilled birth attendance in Ethiopia is the lowest in the world (WHO 2007). Little attention has been given to demand-side barriers of healthcare (socio-cultural, geographical and financial) by policy makers or researchers, even though such barriers are particularly important to poor communities. Finding ways to overcome demand-side barriers could help address this problem.

Objective: The objective of this policy brief was to summarize the best available evidence describing the problem and potential solutions for addressing the problem of low level of skilled birth attendance in Ethiopia.

Methods: This policy brief brings together global research evidence (from systematic reviews) and local evidence to inform deliberations about improving skilled birth attendance in Ethiopia. We searched for relevant evidence describing the problem, the impacts of options for addressing the problem, barriers to implementing those options, and implementation strategies to address these barriers.

Results: Policy options include the following:(i) Community mobilization probably increases the proportion of institutional deliveries (ii) Cultural adaptation of birthing places might address one of the reasons why some women do not go to a birth facility, particularly in rural populations. The effects of cultural adaptations of birthing services on increasing skilled birth attendance are uncertain hence need further investigation. (iii) Maternity waiting homes might address the problem of long distances between where people live and birthing facilities for rural populations with limited access to emergency obstetric care. The effects of maternity waiting homes are uncertain hence need further investigation. (iv) Conditional cash transfer programs may increase institutional deliveries. The costs and cost effectiveness of all four options are uncertain. Rigorous evaluation and monitoring of resource use is, therefore, warranted.

Implementation strategies include production of clear guidelines (manuals) for all options, quality improvement programs, establishment of a culturally competent primary healthcare system by developing relevant cultural competence guidelines and mobilization of financial resources.

Conclusions: Socio-cultural, distance and financial barriers are among the major causes for the low level of skilled birth attendance in Ethiopia. A clear strategy addressing these barriers is crucial to improve skilled birth attendance in Ethiopia.

Keywords: skilled birth attendance, institutional delivery, Ethiopia

Introduction

This is a summary of an evidence brief for policy that addresses the need for improving skilled birth attendance in Ethiopia by addressing the root causes of the low level of skilled birth attendance: socio-cultural, distance and financial barriers. The methods used to prepare this policy brief are described in detail elsewhere (Fretheim *et al*. 2010; Lavis *et al*. 2010). This policy brief assesses a health system problem, potential policy options and their impacts, and strategies for implementing those options. The policy brief brings together global research evidence (from systematic reviews) and local evidence to inform deliberations about improving skilled birth attendance. The purpose of this report was to inform deliberations among policy makers and stakeholders, and specifically as a background document to be discussed at meetings (policy dialogues) of those engaged in developing policies for improving skilled birth attendance.

The problem: Skilled birth attendance in Ethiopia is the lowest in the world (WHO 2007). Little attention has been given to demand-side barriers of healthcare (socio-cultural, geographical and financial) by policy makers or researchers, even though such barriers are particularly important to poor communities. Finding ways to overcome demand-side barriers could help to address this problem. The objective of this evidence brief is to summarize the best available evidence describing the low levels of skilled birth attendance in Ethiopia and to outline potential solutions to address the problem.

Size of the problem: Approximately 90% of births in Ethiopia occur at home, without skilled birth attendance (Central Statistical Agency and ICF International 2012). This level is amongst the highest in the world (WHO 2007), and even in developing countries approximately 59% of all mothers receive skilled birth attendance (Stanton 2008). The maternal mortality ratio in Ethiopia is among the highest in the

world with 676 deaths per 100,000 live births (Central Statistical Agency & ICF International 2012), or 19,000 maternal deaths per year (Koblinsky *et al*. 2010) - levels worse than the average maternal mortality for developing countries of 290 deaths per 100,000 births (IRIN 2012). This level also falls far short of the MDG 5 target set for the country, of 350 deaths per 100,000 live births (FMoH 2006). It is estimated that approximately 16%-33% of all maternal deaths could be avoided through the primary or secondary prevention of complications during delivery by skilled attendance (Graham *et al*. 2001) .

Factors underlying the problem: The effects of poverty in Ethiopia – one of the poorest and least developed countries in Africa (UNDP 2011) – cannot be overemphasized, including the effects on maternal healthcare. Studies have noted that additional factors may also impact on the low level of skilled birth attendance in Ethiopia, and these can be classified into four categories, namely: 1. Socio-cultural factors, 2. Economic accessibility factors 3. Physical accessibility factors and 4. Poor health care delivery

Socio-cultural factors: Socio-cultural factors have a direct influence on the decisions of mothers to seek healthcare or not to seek healthcare (Gabrysch and Campbell 2009). According to the latest Demographic and Health Survey of Ethiopia (Central Statistical Agency and ICF International 2012), 61% of women believed that delivery at a health facility was not necessary, while 30% stated that it was not customary. The decision by mothers to seek skilled birth attendance is also influenced by their level of education. Highly educated mothers (i.e. those who have received tertiary education) are most likely to give birth assisted by a skilled provider (74%). The decision to take mothers to a health facility may also be made by a husband or relatives, and requests to go to a health facility by mothers who are experiencing difficulties in labor may be ignored (Bedford *et al.* 2013).

In a qualitative study in Southern Wollo, one of the zones in the Amhara region (Bedford *et al.* 2013), the following beliefs and perceptions were identified as deterrents to mothers delivering at health facilities:

1.The perception that health facilities do not allow relatives or neighbors to accompany mothers into the delivery unit, which is thought to make mothers feel lonely in spite of the presence of care providers during labor.

2.The perception that health facilities prohibit mothers delivering in a kneeling position, and instruct them instead to lie on their back with their legs open. Physical exposure of this kind is considered by mothers to be invasive, especially in front of people unknown to them.

3. The perception that internal physical examinations during delivery are disliked by mothers, as mothers do not want to show their bodies to people they do not know.

Economic accessibility: Family income is also associated with the utilization of skilled birth attendance because of the costs of transportation and care, and the opportunity costs incurred (Gabrysch and Campbell 2009). In the latest Demographic and Health Survey in the country, 46 % of mothers in the highest wealth quintile attended skilled birth compared to the 2% for the lower wealth quintile (Central Statistical Agency and ICF International 2012). Health centers are supposed to provide free maternity services by policy since 2005 (FMoH, 2010). But in reality, among facilities that provide delivery care, 65% charge for some aspect of care including drugs and supplies. Indirect costs such as costs for transport, lodging and food are as much burden as the fees themselves. As a result, as much as 40% of sick people may not seek care due to associated costs (Pearson *et al*. 2011).

Physical accessibility: Ethiopia's road network is not well developed. The majority of rural dwellers (83.9% of the total population) [Government of Ethiopia 2008)] are therefore left isolated and with little or no access to health facilities. Lack of transport to the nearest health facility (71% of respondents) and the distance to a health facility (66% of respondents) are the most important factors hindering the use of skilled birth attendance (Central Statistical Agency and ICF International 2012). Urban residency is closely associated with higher levels of skilled birth attendance: in urban areas,50% of births occur at health facilities, compared to just 4% in rural areas (Central Statistical Agency and ICF International 2012).

***Healthcare delivery problems*:** Although more than 30,000 health extension workers (HEWs) in Ethiopia, contribute to healthcare provision through family planning, antenatal care and HIV testing services, their contribution to the improvement of skilled birth attendance remains insignificant (Medhanyie *et al*. 2012). In addition, health facilities typically face shortages of supplies and equipment for obstetric care due to poor coordination and management (FMoH, 2006). Lack of immediate treatment and onwards referrals, and a lack of the desired skills among care providers, are deterrents to going to health facilities (Bedford *et al*. 2013). Lack of maternity waiting

rooms may also contribute to the low utilization of skilled birth attendance. Mothers who present at health facilities when in the early stages of labour are often turned away and asked to return later. Instances in which, for example, mothers are sent back home on a stretcher because there are no maternal waiting rooms; can give health facilities a bad image (Bedford *et al*. 2013).

***Policy Options*:** Options to increase skilled birth attendance in Ethiopia include: community mobilization, the cultural adaptation of birthing services, the use of maternity waiting homes, and the use of conditional cash transfers (CCT). These four options and their potential impacts on skilled birth attendance are described below.

Policy option1: Community mobilization.
Community mobilization empowers people to organize themselves, recognize opportunities, identify their collective potential, and utilize available resources to realize a shared goal through unified action. Mobilization strategies are diverse, may result in differing intensity levels of engagement and ownership (Rosato *et al*. 2008), and requires an understanding of the social structure of local contexts (Hounton *et al.* 2009). It has also been suggested that community mobilization has substantial potential to change behaviors and enable access to healthcare (Lawn *et al*. 2009), and can potentially increase the number of facility-based births. Different community mobilization strategies have been used in many low-income countries, particularly in Asia, to increase use of maternal and neonatal services (Lee *et al*. 2009).

Impacts of community mobilization: A systematic review evaluated the impact of community mobilization on institutional (facility-based) deliveries (Lee *et al.* 2009). They found that (Steinmann 2010) community mobilization probably increases the proportion of institutional deliveries and its effect depends on the intensity of the mobilization efforts.

Option 2: Cultural adaptation of birthing services.
Child birth in different communities is associated with different practices that are deeply rooted in the cultures and traditions of the community. Some cultural practices in Ethiopia, for example, include eating porridge, putting butter on the head of the mother and conducting a coffee ceremony (FMoH, 2013). Some norms include, giving birth in a sitting position instead of lying down on one's back with open legs, and giving birth among families instead of among strangers (Bedford et al. 2013). Absence of these traditional practices and norms discourages women from going to health facilities for delivery. Hence, availing these traditional practices in health facilities is likely to encourage women to give birth in health facilities.

Impacts of Cultural adaptation of birthing services:
We were not able to find a systematic review of the impacts of cultural adaptation of birthing services. Only one study from Peru has evaluated an intervention to culturally adapt a birthing facility. This study found that cultural adaptation of birthing facilities, such as introducing a rope and bench for a vertical delivery position, allowing family and traditional birth attendants in the delivery process and use of local language at health facilities, increased facility based deliveries from 6% to 83% in nine years (Gabrysch *et al*. 2009). Cultural adaptation of birthing places might address one of the reasons why some women do not go to a birth facility, particularly in rural populations. The effects on increasing skilled birth attendance are uncertain hence the need for further research.

Option 3: Building maternity waiting homes.
Maternity waiting homes are residential facilities, located within easy reach of a health facility, where women defined as "high risk" can await their delivery and be transferred to a nearby medical facility shortly before delivery, or earlier should complications arise. Many consider maternity waiting homes as a key element of a strategy to overcome distance barriers in rural settings to improve access to care for mothers. Besides emphasis is also given to education and counseling regarding pregnancy, delivery and care for the newborn (van Lonkhuijzen *et al*. 2009; WHO 1996) provision of maternity waiting homes has been practiced in many low-income countries: Zimbabwe, Zambia, Tanzania, former Zaire, Ghana, Ethiopia, Nigeria, Liberia, Malawi, Mozambique, Papua New Guinea, Nicaragua, Cuba, Peru, Honduras and Lao (Van Lonkhuijzen *et al*. 2009).

Impacts of maternity waiting homes: There are no systematic reviews on the effect of maternity waiting homes on skilled birth attendance. However there is a systematic review on the effect of maternity waiting homes for improving maternal and neonatal outcome on low-resource settings which found no randomized control trials on the effect of maternity waiting home on perinatal and maternal mortality and morbidity in low resource countries (Van Lonkhuijzen *et al*. 2009). It was found that for rural populations with limited access to emergency obstetric care, maternity waiting homes might address the problem of long distances between where people live and birthing facilities (Dudley 2011). The effects of maternity

waiting homes on increasing skilled birth is uncertain hence the need for further research**.**

Option 4: Conditional cash transfer to mothers giving birth at health facilities. Conditional cash transfer programs (CCT) give money to poor people in return for fulfilling specific behavioral conditions such as children's school attendance, up-to-date vaccinations or regular visits to a health care facility by pregnant women. The purpose is to a make a positive impact on the recipients' health, education or other socio-economic well-being depending on the condition applied. In preventive and primary health care, regular visits to health facilities and timely immunization levels are the most commonly used conditions. CCT started in the late 1990s mostly in Latin America, including in Mexico, Brazil, Colombia, Honduras, Nicaragua and Ecuador. CCT have also been implemented or are being considered in other LMIC, such as Bangladesh, Kenya, Cambodia, Turkey, South Africa, Indonesia and Côte d'Ivoire. There is some encouraging evidence coming mostly from Mexico, where, CCT appear to have successfully reduced infant morbidity and mortality, as well as obesity, hypertension and diabetes in adults. In Honduras and Colombia, a reduced incidence of diarrhea among children (by 3-10%) is attributed to CCT (Doetinchem *et al.* 2008).

Impact of conditional cash transfer: We could not come across a systematic review which dealt with skilled birth attendance as a direct outcome of conditional cash transfer intervention. However a systematic review on impact of conditional cash transfers on care-seeking behavior and immunization coverage (Lagarde *et al.* 2007) has shown favorable result that conditional cash transfer programs can be effective in increasing the use of preventive services and can sometimes improve immunization coverage and health status (Pantoja 2008).

Conditional cash transfer programs could provide incentives for women to go to birthing facilities. Conditional cash transfer programs may increase skilled birth attendance.

Implementation considerations: Community mobilization, cultural adaptation of birthing services, maternity waiting homes, and conditional cash transfers are four potential solutions to improve skilled birth attendance in Ethiopia. Implementing these options requires other changes, including policy changes. Strategies for implementing the options should take advantage of factors that enable their implementation as well as addressing barriers. Enablers of improving skilled birth attendance in Ethiopia include:

1. Strong political commitment from the government for maternal child health in general and skilled birth attendance in particular
2. Rapid economic growth in the country
3. The establishment of the 'Health Development Army' in the country, which can be used for community mobilization
4. More than 30 thousand health extension workers working at the grass root level, who can be used for all four options
5. Major funding opportunities and public-private sector collaboration globally
6. A number of global and local partners and civil society organizations working on maternal health
7. An increasing number of skilled health workers in Ethiopia

Barriers to the four options and implementation strategies that address those barriers are summarized in Tables 1 to 5.

Table 1: Barriers and implementation strategies for all options

Barriers	Descriptions	Implementation strategies
Strategies or guidelines	There are no strategies/and or guidelines in place to implement the options except for community mobilization	Formative research to understand local culture, beliefs and practices and design suitable strategies and manuals (ACCESS 2010).
Financial resources	There may be insufficient financial resources to implement all the options	Pilot study to evaluate costs and cost-effectiveness before full scale implementation Resource mobilization through coordination of governmental and non-governmental organizations. Establish a consortium of stakeholders for maternal health to pool resources and use them for achieving the common goal of increasing the level of skilled birth attendance in Ethiopia Cost sharing with the community (Poovan *et al*. 1990).
Poor quality of care	Poor quality of care could discourage mothers from seeking skilled birth attendance (Kruk *et al*. 2010)	Interventions to improve the quality of care in birthing facilities
Sustainability	Implementation of options may be halted when a decision maker is replaced	Integrating the options into the institutional structure
Inadequate supervision	Since all the options are new and are not institutionalized they may require more supervision	Integrating the options into the institutional structure and providing adequate supervision (ACCESS 2010)
Competing priorities	Options might end up as one time efforts due to competing priorities	Integrating the options into the institutional structure (ACCESS 2010)

Table 2: Barriers and implementation strategies for option 1: Community mobilization

Barriers	Descriptions	Implementation strategies
Cultural barriers	Presence of heterogeneous culture might necessitate culture sensitive community mobilization strategies	Conducting formative research to understand local culture, beliefs and practices and design suitable strategies and manuals (ACCESS 2010)
Sustainability	As activists are volunteers, lack of accountability therefore sustainability could be a challenge	Integration of community mobilization into the institutional structure (ACCESS 2010)
Absence of institutional structure for community mobilization		Integration of community mobilization into the institutional structure (ACCESS 2010)
Burn-out of health extension workers	Health extension workers who could be key players in community mobilization are already overworked	Involving other stakeholders, such as community and religious leaders, social institutions, volunteers, civil societies (Hounton *et al*. 2009). Reducing workload of health extension workers by redesigning the health extension program, introducing motivation packages and increasing the number of health extension workers.

Table 3: Barriers and implementation strategies for option 2: Cultural adaptation of birthing services

Barriers	**Descriptions**	**Implementation strategies**
Absence of strategic plan	There are no strategic plans or manuals in place for cultural adaptation of birthing places	Strategic plans with clear goals, policies and management accountability for cultural competence should be put in place (Anderson *et al*. 2003).
Cultural beliefs, norms and values	There could be various cultural beliefs, norms and values in a certain area where they should all be accommodated in one health facility	Mapping cultural beliefs , values and norms of local communities (Hounton et al. 2009)and adapting birthing services to the various cultural beliefs, values and norms prevalent in a community
Inappropriate norms	Current standards of health care practice may be in conflict with the option	Establishing a culturally competent primary health care system by developing relevant cultural competence guide (Marshal 2005)
Motivation to change	People may not be motivated to go to health facilities regardless of the changes in health facilities	Dissemination of information that is designed to motivate the community to change their practice; financial or other incentives
Attitude of care providers	Possible resistance from care providers to allow cultural adaptations in health facilities	Establishing a culturally competent PHC system by developing relevant cultural competence guidelines (Marshal 2005). Care providers at all levels should receive ongoing education and training in culturally appropriate service delivery and/or culturally competent curriculum should be developed for health care providers. Dissemination of information regarding the size of the problem with relevant comparisons.
Lack of motivation of care providers	Health workers may not be motivated to change their practices	Dissemination of information that is designed to motivate health workers to change their practice; providing incentives, reduce the burden of changing practices

Table 4: Barriers and implementation strategies for option 3: Building Maternity waiting homes

Barriers	**Descriptions**	**Implementation strategies**
Culture	Absence of culturally appropriate practices in maternity waiting homes (WHO 1996)	Cultural adaptation of maternity waiting homes (Gabrysch *et al*. 2009)
Competency of care providers	Absence of the capacity for identification and referral of high risk women (WHO 1996)	Establishment of an effective system of community health services, staffed by providers who have been specifically trained in the identification and referral of high risk pregnancies (WHO 1996).
Blueprints for maternity waiting homes	Lack of a "blueprint" of what a maternity waiting home should constitute and provide	Developing a national guideline for maternal waiting homes
Inadequate internal communication	Lack of proper referral system might result in missing high risk women; maternity waiting homes might be occupied with women not at risk (WHO 1996)	Establishment of an effective system of community health services, staffed by providers who have been specifically trained in the identification and referral of high risk pregnancies (WHO 1996)
Absence of a guideline	Absence of standardized medical care, indications for admission, and documentation	Developing a national guideline for maternal waiting homes

Table 5: Barriers and implementation strategies for option 4: Conditional cash transfers (CCT)

Barriers	Descriptions	Implementation strategies
Fiscal sustainability	Fiscal sustainability could be a challenge (Handa & Davis 2006)	Carefully designed exit strategies consistent with CCT program objectives (Handa & Davis 2006)
Motivation to change	Participation of mothers could be low due to socio- cultural barriers	Adjusting the design of CCT programs to the heterogeneous socio-cultural factors prevailing in the country
Poor capacities of health facilities	Health facilities might find it difficult to meet additional demand likely to arise when beneficiary households try to meet the conditions.	Pilot study to assess possible rise in demand and the capacity of health facilities before full-scale implementation
Implementation capacities	Capacities for managing cash transfer schemes are weak in low-income countries. The health system may not be able to meet the additional administrative demands related to conditionality (Schubert & Slater 2006).	Preparing CCT implementation guidelines Organizational change and capacity building on CCT of the relevant bodies within the civil service (Handa & Davis 2006). Link cash transfers to existing and complementary programs
Feasibility	CCT may be difficult to implement	Pilot study to assess the feasibility of CCT
Over reporting skilled birth attendance	Abuse of money allotted for would be mothers is a possibility by over reporting skilled birth attendance	Put an appropriate auditing mechanism in place
Cumbersome bureaucracy	Burdensome paperwork to provide cash to mothers may discourage mothers not to come to a health facility again	Minimizing paper work

Discussion

This policy brief was discussed by policy makers, researchers, civil society, professional organizations, academics, and development partners at a policy dialogue meeting in Adama, Ethiopia, 5th June 2014. The following points were the major issues raised during the dialogue.

One of the major problems in the health system is there is a poor quality of health care: mothers are not satisfied with the care delivery in the country or mothers are maltreated by care providers, health facilities in the country are not mother friendly, health facilities are poorly equipped. So what is the point of increasing skilled birth attendance if mothers do not get quality care? As a result a mother might not see the difference between giving birth at home and a health facility. The use of risk approach, targeting high risk mothers to deliver in health facility, was mentioned as an option to reduce maternal mortality. However, it was also mentioned that during delivery all mothers are at risk therefore interventions should target all mothers. Besides it was also mentioned that the policy brief is not about high risk mothers but about increasing skilled birth attendance in the country.

One of the barriers for all the policy options except for community mobilization is lack of evidence. Lack of evidence therefore should be mentioned as a barrier for all options and generating local evidence on the options should also be considered as implementation strategy.

Conflict of interest

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In vitro activity of *Albizia gummifera* (J.F. Gmel.) C.A. Sm. seed extract against promastigote stages of five *Leishmania* species known to cause human leishmaniasis

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Abstract

Background: Modern drugs for treatments of leishmaniasis are expensive, bearing limited efficacy and significant toxicity as well as emergence of resistant parasite to them. Several medicinal plants are being used traditionally to treat *Leishmania* infections in Ethiopia. Therefore the discovery of safer and more efficacious drug from natural product is so essential.
Objective: To investigate the antipromastigote activity of crude extract of *Albizia gummifera* seed against five *Leishmania* species responsible to cause cutaneous and visceral leishmaniasis.
Methods: The plant materials were macerated and extracted using 70% ethanol. The extract of each plant was screened for its antipromastigote and hemolytic activities *in vitro*. Data analysis was done by using Graphpad Prism version 6 software. The criterion for activity was considered as an IC_{50}<100 µg/ml.
Results: Haemolytic test of ethanol extracts *A. gummifera* showed LC_{50} value of 453.55 ±3.9 µg/ml. The crude ethanol extract of *A. gummifera* showed the highest potency against promastigotes of *L. tropica, L. major, L. donovani, L. chagasi and L. aethiopica species* with IC_{50} values less than10 µg/ml. Among the different fractions of *A. gummifera*, the n-butanol fraction showed comparable anti-leishmanial activity (IC_{50} ranges between 0.18 and 0.28 µg/ml) with the standard drug, Amphotericin B, having IC_{50} between 0.24 and 0.29 µg/ml.
Conclusions: *A. gummifera* seed extract considered as a good candidate for further bioassay-guided fractionation and isolation of anti-leishmanial lead compound(s) that could be used for anti-leishmanial drug discovery.
Key words: *anti-leishmanial, lead compound(s), crude extract, n-butanol,* LC_{50}, *IC_{50} values,*

Introduction

Leishmaniasis is a disease caused by a protozoan parasite of the genus *Leishmania*. It is transmitted by the vector *phlebotomine* sand fly. Globally it affects more than 12 million people and 350 million are at a risk of developing the disease with 2 million new cases occurring annually (WHO 2010). Clinically the disease has got three forms: cutaneous leishmaniasis (CL), visceral leishmaniasis or Kala-azar and mucocutaneous leishmaniasis (MCL) (Desjeux 2004). In Ethiopia, CL is endemic in the highlands of the country, mainly caused by *L. aethiopica* and occasionally by *L. tropica* and *L. major*. Visceral leishmaniasis caused by *L. donovani,* is found in the arid and semi-arid area of Ethiopia (Ashford *et al*. 1973; FMoH 2013; Hailu *et al*. 2006).

Modern drugs for current therapies against leishmaniasis are pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), Amphotericin B, miltefosine, pentamidine and paromomycin *(Croft and Yardley 2002; Frezard et al*. 2009). However, all of these drugs have limited efficacy and significant toxicity as well as resistance emerges by the parasite to them (Croft *et al*. 2005). Moreover, there is no effective vaccine against human leishmaniasis (Handman 2001). Discovery for safer and more efficacious drugs is, therefore, essential. In this regard, medicinal plants claimed by traditional healers to treat leishmaniasis in Ethiopia may offer prospects for discovering new compounds with therapeutic properties. This study was, therefore, designed to scientifically validate the *in vitro* anti-leishmanial activities of five traditional medicinal plants.

Material and methods

Plant material: The medicinal plant, *Albizia gummifera* (J.F. Gmel.) C.A. Sm. seeds, was collected from its natural habitats in September and October 2010. The plant was identified and authenticated by a botanist at Traditional and Modern Medicine Research laboratory, Ethiopia Public Health institute (EPHI), the specimen was given with a voucher number of AG 2006 and was deposited in

the herbarium of Traditional and Modern Medicine Research Directorate, EPHI, Addis Ababa. The Plant materials were then air dried in the plant processing unit of Traditional and Modern Medicine Research Laboratory of EPHI.

Extraction of plant materials: The air-dried and powdered plant material (100 g) was extracted with one liter of 70% ethanol by maceration. The resulting ethanol extract was filtered and evaporated using a rotatory evaporator and then lyophilized. Ethanol extract of *A. gummifera* was dissolved in distilled water and fractionated with different solvents: petroleum ether, chloroform, and n-butanol. Fractionated extracts of *A. gummifera*, with the above solvents, and the aqueous marc were used for subsequent investigation against anti-promastigote activity. The crude and fractionated extracts were stored at 2^{o}C until used.

Promastigote cultivation: Standard strain of *Leishmania* parasites used in this study were: *L. donovani* (MHOM/50/68/15), *L. chagasi* (MCAN/84/CO9/0), *L. major* (MHOM/IR/72/NAD /MS), and *L. tropica* (IROS /NA /80 /HD3. *L. aethiopica* promastigote was isolated from patients with cutaneous leishmaniasis. The promastigotes were grown in Novy MacNeal Nicolle (NNN) medium and then cultured in RPMI-1640 supplemented with 10%FBS, (100U/100µg/ml) penicillin/streptomycin, and (2mM/ml) L-glutamine at pH 7.2, and incubated at 26^{0}C in a dark environment.

***Anti-promastigote assay*:** The assay was carried out in a 96-well micro-titer plate where the extracts were diluted three-fold with RPMI-1640 to obtain serial dilutions ranging from 1.2 to 900 µg/ ml in 100 µl of culture medium with each test concentration in duplicate. Then, 100 µl of suspensions containing 3.5x10^{6} promastigotes /ml at logarithmic phase were added to each well. The plates were incubated at 26^{o}C for 72 hours and growth of *Leishmania* promastigotes were determined using the Alamar blue assay (Mikus and Steverding 2000), then optical density (OD) of each plate was measured after 24 hours using ELISA reader fluorometrically at 544 nm an excitation wavelength and 590 nm as an emission wavelength. The IC_{50} value was calculated using Graphpad prism version 6 software.

Red blood cell (RBC) lysis assay: Assay for hemolytic activity of the extract was carried out as described in Tiuman *et al*. (2005). In brief, 200 µl of 4% red blood cell suspension (in sterile 5% glucose soln.), serial dilutions of the extracts (1.2 to 900 µg/ ml) and reference drugs (0.055 - 40µg/ml) were added, and the mixture was incubated at 37^{o}C for 2 hours. The suspensions were centrifuged at 1,000 rpm for 10 min, and 100 µl of the supernatants was transferred to a 96-well plate, and absorbance was measured at 540 nm using ELISA reader. Hemolytic effects were expressed as percentage of the absorbance of the positive control and the 50% lytic concentrations (LC_{50}) were determined from sigmoidal dose–response curves with Graphpad prism 6.0 software. The negative control was the red blood cell suspension with 1.0 % DMSO and the positive control was saponin. These tests were performed in duplicates on two separate occasions.

Results

Anti-promastigote activity of the crude extracts of A. gummifera: Results of the anti-promastigote activity study showed that the crude ethanol extract of *A. gummifera* possessed a promising anti-promastigote activity with an IC_{50} value less than 1.2 µg/ml against *L. tropica* (0.78 µg/ml), *L. major* (0.94 µg/ml) and *L. chagasi* (0.35 µg/ml) which are almost comparable to IC_{50} value for control drug; 0.24 µg/ml, 0.27 µg/ml and 0.28 µg/ml, respectively (Table 1). However, the IC_{50} values for *L. donovani* (8.65 µg/ml) and for *L. aethiopica* (9.21µg/ml) are much higher compared to IC_{50} value for control drug; 0.29µg/ml for each (Table 1).

In vitro activity of the fractionated extracts of A. gummifera against promastigotes: N-butanol and aqueous fractions of *A. gummifera* had significantly high anti-promastigote activity. The effects of different fractions (petroleum-ether, n-butanol, chloroform and aqueous) of *A. gummifera* extract were also evaluated against promastigotes of *L. aethiopica* clinical isolate and reference strains of *L. donovani*, *L. major*, *L. tropica* and *L. chagasi*. Results indicated that n-butanol and aqueous fractions of *A. gummifera* had significantly high anti-promastigote activity.

Table 1: IC_{50} values of medicinal plant ethanol extracts and reference drug against Leishmania species promastigotes

Plant species and Control drug	IC_{50} (µg/ml)				
	L. tropica	*L. major*	*L. donovani*	*L. chagasi*	*L. aethiopica*
***A gummifera* (seed)**	0.78	0.94	8.65	0.35	9.21
Amphotericin B	0.24	0.27	0.29	0.28	0.29

The n-butanol fractions had a respective anti-promastigote activity with IC_{50} values of 0.22, 0.18, 0.28, 0.18 and 0.27µg/ml against *L. tropica*, *L. major*, *L. donovani*, *L. chagasi* and *L. aethiopica*, respectively. Aqueous fractions with IC_{50} values of 1.11µg/ml against *L. tropica*, *L. major* and *L. donovani*; 1.41 and 2.83µg/ml against *L. chagasi* and *L. aethiopica*, respectively, were obtained. The chloroform fraction had IC_{50} values of 17.91, 31.62, 31.62, 9.38 and 52.99µg/ml against *L. tropica*, *L. major L .donovani*, *L. chagasi* and *L. aethiopica*, respectively while the petroleum-ether fraction had IC_{50} values of 80.95 µg/ml against *L. major* (Table 2).

Table 2: IC_{50} values of *A. gummifera* tested in petroleum ether, n-butanol, chloroform and aqueous fractions *against Leishmania* promastigotes

Fractions of *A. gummifera*	**IC_{50} (µg/ml)**				
	L. tropica	*L. major*	*L. donovani*	*L. chagasi*	*L. aethiopica*
Petroleum ether	> 100	80.95	>100	>100	8.56
N- butanol	0.22	0.18	0.28	0.18	0.27
Chloroform	17.91	31.62	31.62	9.38	52.99
Aqueous	1.11	1.11	1.11	1.41	2.83

Red blood cell (RBC) lysis assay

Extracts of *A. gummifera* and one reference drug used for anti-promastigote activity study were evaluated for their hemolytic effects. Haemolytic test for *A. gummifera* (seed) showed LC_{50} values to be 453.55±3.9 µg/ml, while the LC_{50} for standard anti-leishmanial drug; Amphotericin B, was 45 µg/ml. The negative control (1% DMSO) did not cause hemolysis while the positive control **(**1% saponin) showed 100% hemolysis (Table 3).

Table 3: Haemolytic property of traditional medicinal plant ethanol extracts and reference drug tested on sheep RBC

Study plants and Control drug	**Blood hemolysis LC_{50} (µg/ml) Mean ± SD**
A. gummifera	453.55 (±3.9)
Amphotericin B	45

Discussion

Leishmaniasis is a common parasitic problem in the world. The currently used treatments for leishmaniasis are pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), Amphotericin B, miltefosine, pentamidine and paromomycin. On the other hand, all of these drugs have limited efficacy and significant toxicity as well as resistance emerges by the parasite against them. The high LC_{50} values determined for the plant extract show its safety nature.

This is in agreement with previous report (Ghebreselassie *et al*. 2011) where aqueous leaf extract of *M. stenopetala* on mice treated with doses of up to 900 mg/kg did not show any morphological changes in liver cells. Moreover, acute toxicity study on the aqueous extracts of *A. gummifera* (seed) in mice showed an oral LD_{50} value of 2.50 g per kg animal body weight and an IP LD50 value of 250 mg per kg animal body weight (Debella *et al*. 2007). The very low toxicity shown in both *in vitro* and *in vivo* studies indicated that the tested medicinal plants extracts may not be toxic and do not affect red blood cells.

According to Bero *et al.* (2011), the *in vitro* anti-leishmanial activity of a given crude extract or

compound must have an IC_{50} value of ≤20 μg/ml to possess a promising activity, whereas IC_{50} values

between 21 and 50 µg/ml, 50 and 100 µg/ml are considered to have moderate and low activity, respectively and that with an IC_{50} > 100 µg/ml is considered inactive.

Accordingly, the crude ethanol extract and n-butanol and aqueous fractionation of *A. gummifera* had a promising anti-promastigote effect against *L. tropical, L .major, L. donovani* and *L. aethiopica* compared to other test plants. This is similar to the report on *in vitro* anti-leishmanial activity of the same medicinal plant species from Kenya: *A. gummifera* ethanol extract with IC_{50} value of 10 µg/ml against *L. donovani* promastigotes (Muhammad *et al*. 2011). Thus, this medicinal plant may be a promising source of lead compounds for drug development against *Leishmania* parasites.

According to report of Debella (Debella, 2002) the n-butanol and aqueous partition products of *A. gummifera* fractions contains favonoids, saponins, phenols, phenolic glycosides and carbohydrates, which are very polar compounds. These constituents had stronger activity against *Leishmania* parasites compared to chloroform fractions which contains non-polar alkaloids and diterpenes.

This indicates that the strong anti-promastigote activity of *A. gummifera* seed fractions could be due to the presence of the above mentioned compounds. The petroleum-ether fractions which contain fats and sterols, however, were found to be the least active in the present study. The anti-promastigote activities of *A. gummifera* n-butanol extracts against promastigotes of the five *Leishmania* spp. observed in this study exhibit the potential of this plant in the development of a new anti-leishmanial drug.

Conclusions

This *in vitro* study indicated that *A. gummifera* (seed) ethanol extract and n-butanol and aqueous fractions have an anti-promastigote activity with low toxicity profile. These results need, however, be confirmed using amastigotes stages of the parasite.

Conflict of interest

Authors declare that there is no conflict of interest.

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Correlation of maternal nutritional status with breast milk content of iron, zinc and vitamin A in rural southern Ethiopia

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Abstract

Background: Malnutrition in women can be categorized as the macro-and micro-nutrient malnutrition. Many women, particularly those living in developing countries like Ethiopia, are underweight and/or stunted. Nutritional status of mothers in Ethiopia is currently a major public health concern. There are three key factors of maternal nutrition that could have an impact on human milk composition: current dietary intake, nutrient store, and alterations in nutrient utilization.

Objective: The aim of this research was to study the relation between maternal nutritional status and contents iron, zinc and vitamin A in breast milk of mothers in a rural community in Southern Ethiopia.

Methods: Community-based cross sectional study was conducted from April to May 2012. Maternal anthropometric status, plasma level of ferritin, iron, zinc and retinol and breast milk composition was assessed. Energy and nutrient intakes were calculated using 24h recall method.

Results: The prevalence of iron deficiency anemia (using hemoglobin concentration), zinc deficiency (using plasma zinc) and vitamin A deficiency (using plasma retinol) among lactating mothers of 6 to 12 months of postpartum in Boricha, SNNP, district were 36%, 100% and 7.3%, respectively. The median calorie and nutrient intake of the lactating mothers were below the Estimated Average Requirement. Dietary intake of vitamin A (beta-carotene) showed a significant positive correlation ($p<0.05$, $r=0.24$) with plasma retinol concentration of lactating mothers. The plasma concentration of retinol also showed a significant correlation ($p<0.05$, $r=0.23$) with that of breast milk retinol concentration.

Conclusion: Maternal malnutrition is a serious problem in the study area. Maternal plasma retinol status was positively related with maternal intake of vitamin A and breast milk retinol concentration..

Key Words: Breast milk composition, dietary intake, plasma nutrient level, Boricha.

Introduction

Many women living in developing countries experience various biological and social stresses that increase the risk of malnutrition throughout life, such as food insecurity, inadequate dietary intake, recurrent infection, frequent parasites, poor healthcare, heavy work burden, and gender inequality (UNICEF, 2009). Malnutrition in women manifests itself both at the macronutrient and micronutrient levels, and is observed by inadequate weight and/or height. Deficiencies in micronutrients such as iron, zinc, and vitamin A are highly prevalent in many regions of the world (USAID and AED 2004).

The nutritional status of mothers in Ethiopia is currently a major public health concern. About 27% of Ethiopian mothers of childbearing age suffer from chronic energy malnutrition with a body mass index (BMI) less than 18.5 and 17% of women aged 15-49 years are anaemic, with 13% mildly anaemic, 3% moderately anaemic, and less than 1% severely anaemic (EDHS 2012). Malnutrition compounded by anaemia, contributes to low birth weight and high mortality, and Ethiopia is plagued by high rates of each, 11% and 88/1,000 live births, respectively (EDHS 2012).

Iron deficiency is the most important cause of nutritional anaemia and is the most common micronutrient deficiency worldwide; which leads to impairment of health, growth, and development, affecting learning ability and thus scholarly achievements. Iron supplementation is currently the most important tool for combating iron deficiency. However, intake of iron supplements in malaria endemic regions among children who are not iron deficient could lead to increased morbidity and possibly mortality (Samuel and Tom 2010).

Zinc deficiency increases incidence and severity of infection, impaired growth and development of children, and pregnancy complications of low birth weight and increased prenatal mortality. Stunting (low height-for-age) among preschool children is a common clinical manifestation of zinc deficiency (United Nation University 2004). Zinc is also thought

to play a role in vitamin A and β-carotene metabolism. Zinc supplements had shown previously to improve dark adaptation and intestinal integrity and zinc deficiency was found to aggravate the clinical effects of vitamin A deficiency (Marjoleine *et al*.2001).

A poor diet and infection frequently coexist and interact in populations where vitamin A deficiency (VAD) is widespread. In such settings, VAD can increase the severity of infection which in turn, can reduce intake and accelerate body losses of vitamin A to exacerbate deficiency. The prevalence and severity of xerophthalmia, anaemia and the (less-measurable) "vicious cycle" between VAD and infection in vulnerable groups (notably young children and pregnant or lactating mothers) represent the most compelling consequences of VAD and underlie its significance as a public health problem around the world (WHO 2009).

The prevalence of clinical vitamin A deficiency and night blindness among mothers was 1.8% nationally and 1.0% in the southern region of Ethiopia. Nationally, 37.7% of children (95% CI, 35.6% to 39.9%) had deficient serum retinol levels which may be concluded that the prevalence of vitamin A deficiency is significantly higher among children and mothers in the country (Demissie 2010).

Breast-milk vitamin A is a unique indicator for assessing the vitamin A status of lactating mothers and their breast-fed infants, and has recently been recommended by WHO for use in monitoring global elimination of vitamin A deficiency (Stolztus and Underwood 1995). Assessing breast milk vitamin A is less invasive than alternative approaches for assessing a mother's vitamin A status and her infant. Collection of milk samples in the field is generally feasible and acceptable and can serve as an indicator for measuring the impact of vitamin A interventions on women and infants.

The nutritional composition of breast milk in developing countries, especially among malnourished women, has not been fully investigated. The three key factors of maternal nutrition that could have an impact on human milk composition are current dietary intake, nutrient stores, and alterations in nutrient utilization. Alterations in maternal nutrition that change the composition of human milk may have positive, neutral, or negative consequences to the nursing infant (CNSDPL 1991). When malnutrition is sufficiently severe, lactation performance becomes compromised (UNACC 1992). Even though the prevalence of hemoglobin concentration <120 g/L (30.4%) and the prevalence of night blindness (1.8%) among lactating mothers were known (Demissie *et al*. 2010; Haider 2010), the prevalence of zinc deficiency among lactating women is unknown.

Therefore, study on nutritional status of lactating mother is an important health issue since nutritional status may have relationship with the nutrient content of her breast milk. Hence this study was designed to determine breast milk composition of iron, zinc and vitamin A from women 6 to 12 months postpartum and to correlate with the nutrient adequacy of the lactating mothers using blood biomarkers of iron, zinc and retinol and dietary assessment in a rural area of southern Ethiopia.

Materials and methods

The study area, Boricha district is found in Sidama zone, Southern Nation, Nationalities and People Regional State (SNNP) which is located at 311 km south of Addis Ababa. The district is known to have very high food insecurity level and experiences long periods of increased food deficit due to erratic rainfall pattern and lack of modern agriculture (Aster 2010**,** personal communication). The total population of the district is 282,310 with 50.1% females and 49.9% males. The estimated rural population is 266,406 among these, 3.5% are lactating mothers. Agriculture is the major source of income with the major crops being maize, haricot bean, sweet potato and *Enset* produced in the district (UNHCO 2005).

A community based cross-sectional survey was conducted from April to May 2012. Lactating mothers who were breast feeding a single child 6-12 months of age, apparently healthy, free from medication, non-pregnant, and gave their written consent were included in this study. Lactating mothers who use hormonal contraceptive were excluded from this study.

In order to get a maximum sample size; 50% population proportion was used and a total of 384 lactating mothers were randomly selected for anthropometric measurement and 24 hour dietary recall. After the first visit of the selected 384 HHs, 120 lactating mothers randomly selected and invited through health posts for plasma, breast milk and repeated 24 hour dietary recall assessment. Except one woman, the others were willing to participate in this study.

A maximum of 7ml venous blood sample was collected by a medical laboratory technologist in the morning prior to the first meal of the day.

Hemoglobin analysis was conducted using HemoCue hemoglobin analyzer. For zinc, ferrtin, iron and retinol analysis, blood samples were collected using trace element-free test tubes. Within 30 minutes of collection, blood was centrifuged (3500 rpm for 10 minutes) and plasma was transferred to trace element free Nunc tube, covered by aluminium foil to protect from light and transported using dry ice. The samples were stored at -20^{o}C until laboratory analysis. Plasma ferritin was analysed by the Elecsys 2010 immunoassay (Roche diagnostic); plasma retinol was determined using HPLC method (EHNRI 2010); and plasma iron and zinc were analyzed using atomic absorption spectroscopy (Perkin Elmer 1996).

Breast milk sample was collected during regular feeding time, two hours after the previous nursing. The breast milk samples were collected in to hard plastic containers with an airtight seal and then delivered on ice to the health centre. The breast milk samples were then stored at -20°C (WHO 2007). Breast milk iron and zinc analyses were done by atomic absorption spectroscopy; retinol by high performance liquid chromatography, protein using Kjeldahl apparatus, and fat analysis done using the *Gerber method*. Breast milk carbohydrate was found by calculating the difference after analyzing the moisture and ash concentration of breast milk.

During the 24-hour recalls, respondents were asked for detailed descriptions of all foods and beverages consumed, including cooking methods and recipes. Every household provided one recall, and from 120 sub-samples, a second recall was collected, for a weekend day. Using a software, nutrient data for each food from food composition table for Ethiopian foods (EHNRI 1997; EHNRI 1998); Tanzanian (Zohra and Ellen 2008) and American food composition tables were combined to provide dietary intakes.

Ethical issues

The study protocol was reviewed and approved by National Scientific and Ethical Review Committee of the Ministry of Science and Technology of Ethiopia. Participants were informed of all study procedures and provided informed written consent.

Results

Maternal nutritional status: Of the 384 lactating mothers, 372 completed anthropometric and dietary data collection (96.9% response rate). For the subset of 120 lactating mothers for biological samples, 109 women (90.8% response rate) had completed the data. Twenty four percent of lactating mothers had BMI below 18.5 kg/m^2 considered to be underweight, while only 1.5% had BMI above 25 kg/m^2 considered being overweight.

The prevalence of anemia among lactating mothers aged 15-49 years was 36%, with 18.4% having mild anemia, 17.5% moderate anemia, and 0.8% severe anemia. In addition, 10.6 % of lactating mothers had ferritin levels below 15µg/L. The prevalence of zinc and vitamin A deficiencies were 100 % and 7.3%, respectively (Table 1).

Table 1: Anthropometry and blood analysis of the lactating mothers by months postpartum, in Boricha District, Sidama Zone, Ethiopia, 2012

Characteristics	6-8 months (n = 187)	9-12 months (n = 185)	Total (n = 372)
Anthropometry			
Height (cm)[a]	155.45 ± 6.2	156.16 ± 5.7	155.80 ± 6.0
Weight (kg)[a]	48.49 ± 6.3	48.74 ± 6.3	48.61 ± 6.3
BMI (kg/m^2)[a]	20.0± 2.1	20.0 ± 2.4	20.0 ± 2.2
% (BMI<18.5)[b]	12.04 (45)	12.04 (45)	24.07 (90)
% (BMI 18.5-24 .9)[b]	37.35 (139)	37.04 (138)	74.38 (277)
% (BMI ≥ 25)[b]	0.93 (3)	0.62 (2)	1.54 (5)
MUAC (cm) [a]	23.9± 2.2	23.8 ± 2.7	23.8 ± 2.5
% MUAC (< 21cm)[b]	3.5 (13)	1.9 (7)	5.4 (20)
% MUAC (≥ 21cm)[b]	47.2 (175)	47.5 (177)	94.6 (352)
Blood analysis			
Hb (g/L)[a]	130.5 ± 26	130.6 ± 22	13.05 ± 24
% (Hb < 120 g/L)[b]	12.5 (15)	16.7 (20)	29.2 (35)
% Mild (100 - 119g/L) [b]	6.7 (8)	11.7 (14)	18.4 (22)
%Moderate (70-99 g/L) [b]	5.8 (7)	11.7 (14)	17.5 (21)
% Severe (Hb < 70 g/L)[b]	0.8 (1)	0.0 (0)	0.8 (1)
Plasma Ferritin (µg/L)[a]	42.25 ± 32.0	45.92 ± 43.8	44.37 ± 39.1
% (PF< 15 µg/L)[b]	3.2 (4)	7.4 (9)	10.6 (13)
Plasma iron (µg/L)[c]	1.84 (1.41, 2.24)	1.77 (1.42, 2.05)	1.78 (1.42, 2.16)
Plasma Zinc (mg/L)[a]	0.11 ± 0.03	0.10 ± 0.03	0.10 ± 0.03
%(PZn < 0.7 mg/L)[b]	41.1(45)	58.9(64)	100 (109)
Plasma Retinol (µg/dL)[a]	58.6 ± 20.8	60.1 ± 33.2	59.5 ± 28.4
% (PR ≤ 30µg/dL)[b]	4.2 (5)	3.1(4)	7.3 (9)

[a]Mean + SD, bpercent (Number), cMedian (1st, 3rd quartile), BMI- Body Mass Index; Hb- Hemoglobin; PF- Plasma Ferritin; PR-Plasma Retinol; PZn- Plasma Zinc. BMI<18.5 is under weight, BMI 18.5-24 .9 is normal weight, BMI > 25 is overweight.

The median energy intake of lactating mothers was 1560 kcal/d, corresponding to 33.7 % of Estimated Average Requirement (EAR). The protein intake was 31.6 g/d. The vitamin C intake was very low at 24.8% of EAR. The median iron (assuming 5% bioavailability), zinc (assuming low bioavailability) and vitamin A intake of the lactating mothers were 1.44 mg/d, 0.95 mg/d and 113.32µg/d respectively (Table 2).

Table 2: Energy and nutrient intakes of lactating mothers in Boricha district, Sidama Zoe, Ethiopia, 2012

Indicators	Median (1st , 3rd quartiles) intake per day	EAR
Energy (kcal/d)	1560 (1150, 1950)	1757
Protein (g/d)	31.6 (18.5, 47.0)	39.2
Iron (mg/d)	1.44 (1.01, 1.94)	23.6
Zinc (mg/d)	0.95 (0.67, 1.31)	4.8
Vitamin A (RE µg/d)	113 (49.6, 472)	450
Calcium (mg/d)	880 (621, 1246)	833
Vitamin C (mg/d)	1.4 (0.1, 57.9)	58

***Breast milk composition**:* The nutrient composition of breast milk samples (n=115) is shown in Table 3. The mean energy concentration was lower (574.4± 75.1). Breast milk iron and zinc contents were very low, 0.24 ± 0.08 and 0.08 ± 0.06 mg per litter, respectively.

Table 3: Composition of Human Milk Collected from Lactating Mothers 6-12 months Postpartum in Boricha District, Sidama Zone, Ethiopia, 2012

Indicators	6-8 months (n = 47)	9- 12 months (n = 68)	Total (n = 115)	Other findings 6-12 months Postpartum
Energy (Kcal/L)	573.58 ± 77.8	575.05 ± 74.3	574.40 ± 75.1	642.90 ± 26.2[a]
Fat (g/L)	22.86 ± 8.3	24.32 ± 8.9	23.71 ± 8.6	39.60 ± 2.1[a]
Protein (g/L)	9.45 ± 1.7	9.63 ± 2.1	9.55 ± 1.9	8.20 ± 0.5[a]
Carbohydrate (g/L)	84.05 ± 6.5	84.18 ± 8.5	84.12 ± 7.7	-
Iron (mg/L)	0.24 ± 0.1	0.24 ± 0.1	0.24 ± 0.1	0.43 ± 0.15[b]
Zinc (mg/L)	0.08 ± 0.03	0.09 ± 0.07	0.08 ± 0.06	0.56(0.37,0.82)[b]
Vitamin A (µg/dL)	12.88 ± 0.02	12.85 ± 2.1	12.86 ± 2.2	-

For Fat analysis 60 samples were used for analysis because of lack of enough amount of sample for those analyses.

[a] Breast milk composition results studied by Mitoulas *et a*l.(2002).

[b] Breast milk iron level of Vietnamese women who had iron supplement reported by Nakamori *et al*. (2009).

Correlation between maternal nutritional status and breast milk content: Correlations between dietary intake of lactating mothers and their breast milk concentrations of iron, zinc and vitamin A are shown in Table 4. There were no significant correlation for iron and vitamin A. However, there was a significant inverse correlation for zinc (r= -0.184; $p<0.05$).

Table 4: Correlation (r) between breast milk trace element concentrations and dietary nutrient intake/plasma levels of lactating women in Boricha district, Sidama Zone, Ethiopia, 2012

Breast milk (N = 97)	Dietary	r	*P* value
Iron	Iron	-0.102	0.28
	Protein	0.029	0.76
	Energy	-0.020	0.84
Zinc	Zinc	-0.184	0.05
	Protein	-0.121	0.20
	Energy	-0.156	0.10
Vitamin A (retinol)	Vitamin A (β-carotene)	-0.140	0.14
	Protein	0.018	0.85
	Energy	-0.008	0.94
Breast milk (N = 96)	**Plasma**	**r**	***P* value**
Iron	Ferritin	-0.014	0.89
	Iron	0.088	0.42
	Hemoglobin	-0.31	0.75
Zinc	Zinc	-0.08	0.45
Vitamin A	Vitamin A (retinol)	0.227	0.03

There was a significant positive correlation between the concentration of retinol in breast milk and maternal plasma (r=0.227; $p<0.03$). However, there was no correlation between ferritin, iron and zinc concentrations in breast milk with the corresponding plasma level (Table 4).

Correlation between maternal plasma level of trace elements and dietary intake: There was a significant positive correlation between plasma retinol and the dietary intake of vitamin A (β-carotene) (r=0.223; $p<0.03$). However, plasma zinc concentration showed inverse correlations with dietary intake of protein and of energy (Table 5).

Table 5: Correlation (r) between plasma trace element concentrations and dietary intake of lactating women in Boricha district, Sidama zone, Ethiopia, 2012

Plasma trace elements (N = 97)	Dietary intake	r	*P* value
Iron	Iron	-0.068	0.53
	Protein	0.000	0.99
	Energy	-0.083	0.44
Zinc	Zinc	-0.166	0.11
	Protein	-0.205	0.05
	Energy	-0.205	0.05
Vitamin A (retinol)	Vitamin A (β-carotene)	0.223	0.03
	Protein	-0.053	0.60
	Energy	0.190	0.06

Discussion

This study is the first to measure the concentration of iron, zinc and vitamin A in human milk in lactating rural Ethiopian women, along with assessing dietary intake, anthropometric status and plasma nutrient levels.

The study population showed a mean height (155.8 ± 6.0 cm) similar to the national average (Abuye *et al*. 2010) and previous survey finding in the same region (Abebe and Bogale 2008). Mean weight in this study was 48.61 ± 6.0 kg showing high prevalence of chronic energy deficiency, with approximately one-quarter being classified as underweight (BMI <18.5). This value is higher than that of a survey data for the Southern Regional State (20 %) (EDHS 2012), but comparable to values reported for a study in the same region (Rosalind *et al*. 2008). Both short stature and low body mass index are significant risk factors for low birth weight deliveries (Deshmukh *et al*. 1998).

The result of this study showed that the median intake of iron (1.44 mg per day), zinc (0.95 mg per day) and vitamin A (retinol, 113. µg per day) of lactating women were very low compared to studies reported for other developing countries (Masay *et al*. 2009; Kawatra and Salil 1998). This might be due to the relatively high proportions of energy provided by *Enset* products in southern Ethiopia which contains very little zinc, iron and vitamin A, and lower consumption of animal source of foods. *Enset* products such as *kocho*, consisting of the fermented pulp of *enset* baked into bread were most frequently eaten, followed by *bulla* (white low-fiber desiccated juice from *enset* pulp) and unfermented maize bread. Analysis of samples of fermented *kocho* and *bulla* showed zinc content (per 100 g fresh weight) of 0.52 mg and 0.07 mg, respectively; of iron level of 6.2 mg and 4.8 mg, respectively with low bioavailability (Abebe *et al*. 2007).

Vitamin A-RE content of fermented *kocho* and *bulla* consumed were also very low (19.4 and 1.3 RE µg/100g fresh weight respectively). Unfermented maize bread only contains iron (2 mg,), zinc (1.24 mg) and vitamin A (β-carotene, 0 RE µg) per100g of the food, respectively (EHNRI 1998). Plasma retinol concentration of lactating mothers in Boricha district indicated that 7.3% had plasma vitamin A level below 30µg/dL (0.7µmol/L) indicating vitamin A deficiency, a level higher than a recent WHO report on Ethiopia showing a prevalence of 4.2% (WHO 2009).

Anemia was found in 36% of the lactating mothers studied, again higher than that found (25%) among women of reproductive age in southern Ethiopia (Umeta *et al*. 2008). The prevalence of zinc deficiency was 100% of lactating mothers studied, higher than the other study results (76%) conducted on pregnant women of Southern Ethiopia (Stocker *et al*.2009). The current study population lived in a food insecure district dependent on starchy foods; therefore the higher prevalence of zinc deficiency in the study area is not unexpected. These might be due to the lower value of human milk fat concentration (23.71 $\pm$ 8.6) in this study as compared to the finding of Mitoulas *et al*. (2002).

Breast milk concentrations are affected by the time of day and the time of sampling during feeding (Masay *et al*. 2009). Breast milk was collected in the morning; between 8 and 9 am more than an hour after the previous breastfeeding. Variation of breast milk zinc concentration in the study participant showed relatively lower standard deviation (0.06 mg/L) and CV (75%) compared to other studies regulated by the timing of collecting breast milk (Shahnaz 2010; Heloisa *et al*. 2002). The concentration of zinc, iron, fat and energy were relatively lower than other comparable studies conducted in developing countries (Masay *et al*. 2009; Mitoulas *et al*., 2002).

The concentration of iron, zinc and retinol in breast milk is considered low in relation to plasma iron, zinc and retinol concentration. Breast milk iron concentration was 14% of plasma iron, breast milk zinc was 80% of plasma zinc and breast milk retinol was 22 % of plasma retinol concentration. In this study, breast milk concentration of zinc is not correlated with either dietary intake or plasma zinc concentration. This result is similar to the study conducted on Vietnamese lactating mothers where their zinc store and intake was higher (Masay *et al*. 2009).

Although anemia was highly prevalent among lactating mothers, it was not correlated with breast milk iron concentration. This result was comparable with the study results of Shashirja *et al*. (2006). Breast milk iron concentration appears to be constant whether women have low blood levels, as found in this study which is in agreement with values from a study done on Nigerian lactating women supplemented with iron (Arnaud *et al*. 1993).

In this study, there was no correlation of maternal dietary intake of vitamin A (primarily as β-carotene) and breast milk concentration of retinol. In contrast, a study reported by Grazyna *et al*. (2009) stated that dietary vitamin A (β-carotene) intake was correlated with the concentration of retinol in breast milk although vitamin A status and their intake had differences with this study population. The present study results showed that breast milk vitamin A concentration was positively correlated with plasma retinol in lactating mothers in agreement with study results of Grazyna *et al*. (2009).

Conclusion

In this study, the prevalence of iron deficiency anemia, zinc and vitamin A deficiency among lactating mothers, 6 to 12 months postpartum were high: 36%, 100% and 7.3%, respectively. The median calorie and intakes of iron, zinc and vitamin A were inadequate. The average energy, fat, iron and zinc content of breast milk were lower than other comparable studies done in other developing countries. The breast milk content of iron and retinol is not correlated with dietary intakes; however, breast milk zinc showed a negative correlation with dietary intake of zinc. Plasma concentration of retinol showed a significant correlation with that of breast milk retinol concentration, but plasma concentrations of iron and zinc showed no correlation with breast milk levels.

Competing interests

The authors declare that they have no competing interests.

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Effect of 1.5% sodium hydroxide final concentration on recovery rate of Mycobacterial Species and decontamination of other Bacterial and Fungal contaminants on sputum

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Abstract
Background: Digestion and decontamination of non-sterile clinical specimens such as sputum are an essential step in the isolation of mycobacteria. Masking of mycobacteria in Mycobacterial growth indicator tube (MGIT) 960 liquid culture system by fungi and bacteria other than mycobacteria is a major problem.
Objective: To assess the effect of 1.5% sodium hydroxide final concentration on recovery rate of mycobacterial species and decontamination of other bacterial and fungal contaminants from sputum sample.
Methodology: Laboratory based cross sectional study with convenient sampling technique was carried out on subjects referred to the National Tuberculosis Reference Laboratory of Ethiopian Public Health Institute from November 2015 to February 2016. Single morning sputum was collected from each patient and analyzed.
Results: A total of 264 subjects were enrolled in the study. The mean age of participant was 31 (SD 20.14 - 41.42) years old. The majority (61%) were male. Increasing the final concentration of NaOH from 1% to 1.5% reduced the contamination rate from 22.4% to 6.8% (P<0.001) without affecting mycobacterial recovery (P=1.00). A total of 26 different species of microbial contaminants were identified as being associated with BACTEC MGIT 960 culture system.
Conclusion: Results presented in this study demonstrated that the use of a final concentration of 1.5% NaOH with NALC method aids in reducing culture contamination rate for decontaminating sputum samples referred for tuberculosis culture diagnosis. Among the identified microbial contaminants, the most predominant was coagulase negative *Staphylococcus* species.
Key words: Decontamination methods; Mycobacteria; NALC- sodium hydroxide

Introduction
Tuberculosis (TB) is a disease of major public health concern globally with an estimated 9.6 million new cases of TB and about 1.5 million deaths in 2014(Global Tuberculosis Report, WHO 2015). It is caused mainly by a single infectious agent, namely, *Mycobacterium tuberculosis*, which is transmitted easily in overcrowded settings and in conditions of malnutrition and poverty (Pereira *et al.*,2005). There has been an increase of cases in multidrug resistant (MDR) tuberculosis as well as extensively drug resistant tuberculosis at global and national level.
In 2014, 3.3% of new TB cases and 20% of previously treated cases are estimated to have MDR-TB; among this 9.7% of people with MDR-TB are expected to have extensively drug resistant TB (XDR). Globally, out of 9.6 million estimated TB cases only 6 million TB cases were reported. This indicates that above 3 million estimated TB cases were undiagnosed and not reported. Of the 480,000 estimated multidrug-resistant TB cases, only 120,000 cases were detected. So far, more TB patients were tested for drug resistance in 2014 than ever before. Rapid case detection is critical, and attempts to shorten the time for detection deserve attention (Global Tuberculosis Report, WHO 2015; TB DOTS Strategy Coordination 2014, Republic of South Africa).According to 2015 WHO TB report, Ethiopia is one of the high TB burden countries. The estimated country TB prevalence and incidence is 200 and 207 per 100 000 population in 2014, respectively(Global Tuberculosis Report, WHO 2015).

Demonstrating acid fast bacilli by Ziehl Neelsen (ZN) smear microscopy procedure from various clinical samples has been the mainstay for the diagnosis of mycobacterial infections(Chakravorty *et al.* 2005).Although many new molecular diagnostic methods have been developed, AFB smear microscopy is widely applied and TB culture remains the "gold" standards for the diagnosis of active TB. These old methods have been routinely used for the confirmation of TB in patients particularly in Africa(Della Latta P 2004).Definitive diagnosis of mycobacterial disease demands that the causative agent be recovered and identified in culture. Specimens collected from sterile body sites of presumptive TB patients can be inoculated directly onto primary isolation mycobacterial growth supporting media without prior decontamination as long as they are properly handled and homogenized

before inoculation. However, most of clinical specimens submitted to the mycobacteriology laboratory for cultural confirmation of the TB are non-sterile; contain microbes other than mycobacterial (contaminants). *Mycobacterium tuberculosis* culture usually requires sputum decontamination and centrifugation to prevent cultures from being overgrown by contaminating bacteria and fungi(Grandjean *et al*. 2008).

The use of sodium hydroxide (NaOH) -sodium citrate along with the mucolytic agent N-acetyl L-cystein (NALC) (NALC-NaOH method) is one of the most widely used methods, as it is rapid and relatively effective in reducing the number of contaminating organisms(Yajko *et al*. 1993). Decontamination of clinical samples submitted for the diagnosis of mycobacteria has two objectives: destruction of bacteria other than mycobacteria and homogenization of the specimen to allow for adequate exposure of the bacilli. Decontaminating clinical samples enables killing much of the contaminants while harming as few mycobacteria as possible(Global Tuberculosis Report, WHO 2009).

The increase in the concentration of NaOH has effect on the mycobacterial recovery rate due to its killing effect. In addition, high final NaOH concentration leads to false positive results in MGIT liquid culture system. Since, detection of growth in MGIT is based on an oxygen sensor system, as a result high concentration of NALC or NaOH might result in false fluorescence (Peres *et al*. 2009; Becton Dickinson 2006).

In fact, the high contamination rate affects the diagnosis of TB; and has a direct impact on the sensitivity and specificity, time for the delivery of laboratory results, and the capacity to trace people with positive culture results. Therefore, an economical, straightforward decontamination and concentration method would improve case detection rate in a setting such as Ethiopia. Hence, in this study 1.5% final concentration of NaOH decontamination solution were evaluated in comparing with 1% standard decontamination method, for the successful cultivation of *Mycobacterium species*. Then contaminants prominent in TB culture laboratories were identified. Therefore, the main aim of this study was to compare 1.5% final concentration of NaOH with the standard 1% NaOH decontamination method for the successful cultivation of *Mycobacterium* species and to identify the most common contaminants in TB culture laboratories.

Methods and Materials

Study design and period: A cross sectional study design with convenient sampling technique was used to assess the effect of 1.5% NaOH final concentration on detection and recovery rate of mycobacterial species and decontamination of bacterial and fungal contaminants from sputum collected in patients referred to the Ethiopian Public Health Institute from November 2015 to February 2016 Addis Ababa, Ethiopia.

Study area and population: The study was conducted at the National TB Reference Laboratory (NTRL) of Ethiopian Public Health Institute (EPHI). All patients referred from 12 health facilities (Addis Hiwot Health Center, Armed Force General Teaching Hospital, Alem Tena Higher Clinic, ALERT, Ambo Hospital, Kara Health Center, Fiche Hospital, Police Hospital, St Paul Hospital, St Peter Hospital, Tullu Bolo Health Center, and Universal Higher Clinic) for routine MDR-TB diagnosis and MDR-TB treatment follow-up culture diagnosis were enrolled in the study. Specimens were collected at the referring health facilities.

Specimen Collection, Storage and Transportation: Morning sputum was collected from 264 patients. Each sputum was spilt into two. Half were processed for culture using 1% final concentration of sodium hydroxide-sodium citrate with NALC, and the remaining half were processed by 1.5% final concentration of sodium hydroxide-sodium citrate with NALC. Clinical specimens were collected in wide-mouth containers at the referring health facility. Specimens were either processed in the same day of collection or refrigerated at -4°C for an average of 3 to 5 days until transported to the EPHI. Specimens were transferred using cold chain through the network of national sample transportation system.

Specimen Digestion and Decontamination: Each specimen was mixed and equally divided into two parts. One part of the specimen was decontaminated with NaOH-NALC and sodium citrate solution to make a final concentration of 1.5% NaOH while the other half of the specimen was processed with same solutions to have a final concentration of 1%. The standard procedure is recommended by the Centers for Disease Control and Prevention (Patricia *et al*.,1985), while the comparative procedure with 1.5% NaOH final concentration is recommended by WHO(GLI 2014). All specimens were processed and inoculated into the MGIT tube for cultivation using the MGIT-960 system according to the manufacturer's recommendations, including the use

of PANTA (Polymixin B, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin) antibiotic supplement(Becton Dickinson 2013).

Equal amounts of solution containing 0.5% (w/v) NALC, 3% (w/v) NaOH and 1.47% (w/v) sodium citrate (final concentrations (w/v): 0.25% NALC, 1.5% or 1% NaOH, 0.735% sodium citrate) were added to a sputum in a 50 ml BD Falcon centrifuge tube. All tubes were incubated at room temperature for 15 min. After incubation, the mixture was neutralized with PBS (Phosphate Buffer Saline), bringing the total volume to 50 ml. The homogenate was centrifuged with a relative centrifugal force of 3,000xg for 15 minutes. The supernatant was decanted/discarded into a splash-proof container containing a tuberculocidal disinfectant. The sediment was re-suspended using 1ml phosphate buffer (pH 6.8) and was used for smear preparation and inoculation of MGIT tube containing modified Middlebrook 7H9 medium. All inoculated MGIT tubes were incubated in BACTEC MGIT 960 system at 37°C for 42 days. All tubes that flag positive after incubation in BACTEC MGIT 960 system were considered positive for microbial growth. All steps and procedures used for processing samples with the standard 1% NaOH final concentration were the same in both methods except for higher NaOH concentration added for improved decontamination purposes.

Materials required for collection and culture processing were taken from the National TB Reference Laboratory, and an experienced laboratory technologist have performed the work at the National TB Reference laboratory in a Class IIB biosafety cabinet. All possible efforts were made to prevent laboratory cross-contamination; including the collection of clinical specimens with a leak proof sterile container. The stock of decontamination fluid was sterilized before use. The specimens in the laboratory were processed by batch and two negative (starting and ending) controls were included in each batch. All reagents used for sample processing were checked for sterility.

Identification of Mycobacteria: The BACTEC MGIT 960 system is designed for the rapid detection of mycobacteria in all types of clinical specimens except blood. The system consists of a culture tube containing modified Middle brook 7H9 medium with a fluorescent growth indicator embedded in silicone on the bottom of each tube. This compound is sensitive to the presence of dissolved oxygen in the broth medium. As microorganisms grow, the oxygen in the medium is depleted with a subsequent increase in the fluorescence of the indicator. The enhanced fluorescence in tubes can be monitored automatically over time using the BACTEC 960 detection module(TB DOTS Strategy Coordination 2014, Republic of South Africa; Lu *et al.* 2002).

Smears were prepared from both the sediment and culture positive MGIT tubes. Mycobacteria in all tubes that flag positive with MGIT-960 system were identified by performing acid-fast staining and SD Bioline MPT64 TB antigen tests(SD Bioline Kit, Standard Diagnostics, Inc., South Korea) to rapidly confirm the presence of MTB complex(TB DOTS Strategy Coordination 2014, Republic of South Africa).

One drop from suspended pellet and an aliquot of broth from culture positive MGIT tube about 100 µl (2 drops), were stained with Ziehl-Neelsen as WHO recommendations (Mycobacteriology laboratory manual, GLI 2014). Each slide was coded and read blindly by a qualified technologist. When comparing the results of AFB smears, only their negativity or positivity for AFB was used because of the expected difference in quantitative results between the concentrated and un-concentrated portions of the same sample.

TB Ag MPT64 Rapid test can detect MPT64antigen (Immunogenic Protein (alternate gene name: mpb64) specifically secreted from tuberculosis bacteria. The MPT64 antigen is the secretory protein (24 kDa) that is one of the secreted major antigens from *Mycobacterium tuberculosis* complex (MTBC). Then, 100µl of liquid cultures were added to the sample pore and incubated for 15 minutes. *Identification of bacteria other than mycobacteria:* A portion of positive culture (10µl) was inoculated on Blood Agar (Titan Biotech, Ltd, of India), Chocolate Agar using soluble hemoglobin powder (Oxoid, Ltd, United Kingdom) and MacConkey Agar (Oxoid, Ltd, United Kingdom). The inoculated media were incubated at 37°C for at least 48 hours. Colonies typically of bacteria were identified by Vitek 2 compact system (BioMérieux Diagnostics 2007, of France).

Fungal Identification: All positive tubes were also sub-cultured onto Sabouraud Dextrose Agar (Central Drug House (CHD), Ltd, of India) and Brain Heart Infusion Agar (Carl Roth, Ltd, of Germany) for fungal growth. Culture plates were examined twice a week for any fungal growth for about four weeks. Cultures of mycelia fungi (molds) were identified by examining macroscopic and microscopic characteristics of their colony. Texture, rate of

growth, topography and pigmentation of the front and the reverse side of the culture were employed. Microscopic identification of mold isolates was performed by placing pieces of a colony from Sabouraud Dextrose Agar to clean microscopic slide and staining with lacto phenol cotton blue.
After placing a cover slip, each preparation was observed microscopically.

Quality control: The sterility of the sample processing reagents was tested by inoculating the portion of the reagent onto blood agar media and incubated at 37ºC for 72 hours (Mycobacteriology laboratory manual, GLI 2014). Similarly, the sterility of culture media was also checked by incubating 5% of the batch media. The performance of culture media was tested by using control strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228) and H37Rv (ATCC 27294). For instance, 2% of a batch of 100 tubes of MGIT Culture media was tested with 10^{-2}, 10^{-3} and 10^{-4} dilutions of *M. tuberculosis* control strain (H37Rv) for monitoring the performance of the new lots media. A positive and negative panel slides were used for assessing the quality of newly prepared staining reagents (ZN stains). Data were captured at each and every activity of quality control process.

Data collection and analysis: The values for Positive percent agreement, Negative percent agreement and overall accuracy were calculated using a 2x2 table.
The degree of association was calculated using the kappa statistic and difference of proportions and their significance were calculated using X^2 and the Z test using SPSS version 20 and Stata/SE Ver. 13. The time to positive culture were compared between the two concentrations of sodium hydroxide methods using the Wilcoxon signed-ranks test. The following variables were collected using a structured questionnaire from registers and patient request form such as categorical data: sex, medical history, referring health facility, previous treatment, co-infection, reasons for test requested and microbial profile (bacterial and fungal contaminants), and as continuous variables: age, time for positive test results. Categorical data were presented using percentage, and continuous variables were presented using mean and standard deviation. *P value*<0.05 was considered to be statistically significant.

Results

***Demographic and clinical characteristics*:** A total of 264 study participants were enrolled in the study. Majority (61.0%) were male. The mean age was 30.78±10.64 years old. The co-infection (TB/HIV) rate in both sexes was 5.3%. Among the participants, 241 (91.3%) of them were referred for follow-up culture examination of patients on MDR-TB treatment (Table 1).

Effect of 1% and 1.5% Sodium Hydroxide final concentration in processed samples: AFB smear positivity rate was 13.6%. There is no difference between the two concentration (with 1.5% and 1% NaOH) in the isolation of mycobacterial species from both smear positive and negative samples, include MTBC (n=51), and NTM (n=3).

Table 2: Sex and age category of study participants referred for tuberculosis culture diagnostic tests at EPHI NTRL in the period from November 2015 to February 2016

Variables		Co-infections		
		No N (%)	**Yes** N (%)	**Total** N (%)
Age category	18 to 24	82(31.1)	3(1.1)	85(32.2)
	25 to 34	94(35.6)	5(1.9)	99(37.5)
	35 to 44	42(15.9)	5(1.9)	47(17.8)
	45 to 54	22(8.3)	1(0.4)	23(8.7)
	> 55	10(3.8)	0(0.0)	10(3.8)
Gender	F	99(37.5)	4(1.5)	103(39.0)
	M	151(57.2)	10(3.8)	161(61.0)
Previous TB history	New Case	6(2.3)	1(0.39)	7(2.7)
	Retreatment	14(5.03)	2(0.98)	16(6.1)
	MDR-TB	230(87.1)	11(4.2)	241(91.3)

Total no. of participants	264

Majority of the study participants (51%) were from ALERT, followed by St Peter (36.4%) (Figure1).

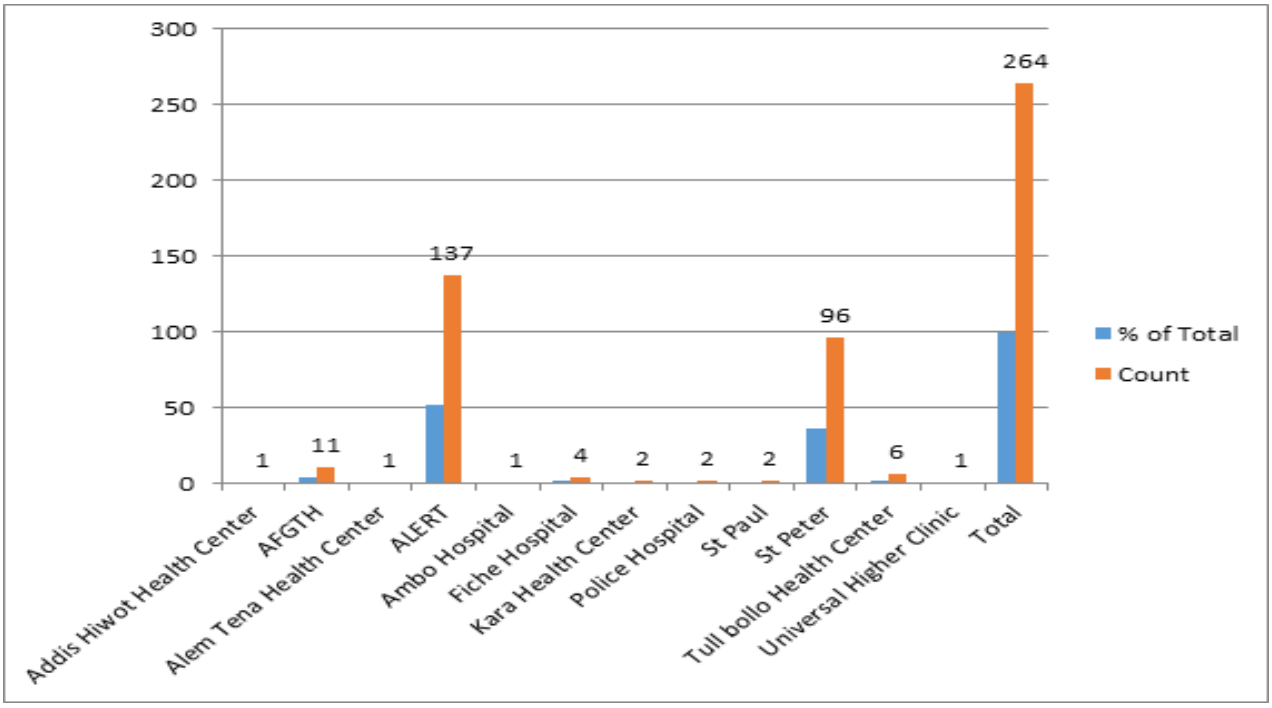

Figure 1: Distribution of study participants with referring health facility for mycobacterial culture at EPHI NTRL in the period from November 2015 to February 2016

Table 3: Classification of culture results and AFB results among sputum's collected from patients referred to EPHI NTRL in the period from November 2015 to February 2016.

AFB from Sputum	1.5%NaOH				1%NaOH mmm			
	Contaminated N (%)	Negative N (%)	Positive		Contaminated N (%)	Negative N (%)	Positive	
			NTM N (%)	MTBC N (%)			NTM N (%)	MTBC N (%)
Negative	15(5.7)	187(70.8)	3(1.1)	23(8.7)	58(22.0)	146(55.3)	3(1.1)	21(8.0)
Positive	3(1.1)	5(1.9)	0(0.0)	36(13.1)	1(0.4)	5(1.9)	0(0.0)	30(11.3)
Total	18(6.8)	192(72.7)	3(1.1)	51(19.3)	59(22.4)	151(57.2)	3(1.1)	51(19.3)

The overall accuracy of MGIT culture was 92.68% and 77.24%, when specimens decontaminated with 1.5% and 1% NaOH with NALC, respectively (Table 3).

Table 4: Positive percent agreement, Negative percent agreement and overall accuracy of the two decontamination methods (1% and 1.5% NaOH) at EPHI NTRL in the period from November 2015 to February 2016.

Decontamination methods (Concentration of NaOH)		Positive Percent Agreement	Negative Percent Agreement	Over all Accuracy
1.5% NaOH		85.71	98.01	92.68
1% NaOH		93.33	95.48	77.24
Differences	Z value	7.62	1.24	4.48
	P value	0.225	0.214	<0.001

The difference between the two methods in total was statistically not significant (P<0.001) and the two decontamination methods were moderately associated (Kappa=0.562) (Table 4).

Table 5: A cross tabulation for the 1% and 1.5% NaOH decontamination methods evaluated at EPHI NTRL in the period from November 2015 to February 2016.

			1%NaOH Positive	Negative	Contaminated	Total
1.5% NaOH	Positive	N (%)	42(15.9%)	3(1.1%)	9(3.4%)	54(20.5%)
	Negative	N (%)	7(2.7%)	148(56.10%)	37(14.0%)	192(72.70%)
	Contaminated	N (%)	5(1.90%)	0(0.0%)	13(4.90%)	18(6.80%)
Total		N (%)	54(20.40%)	151(57.20%)	59(22.40%)	264(100%)
Statistics		χ^2	184.12			
		Kappa	0.562			

The percentage of culture correlation (smear positivity versus culture positivity rate) when using either the final concentration of 1% sodium hydroxide or 1.5% sodium hydroxide were 83.3% and 77.8%, respectively, as shown in table 5.

Table 6: Comparison of decontamination methods on contamination rate, recovery rate, culture correlation and culture contribution (smear negativity versus culture positivity rate).

Indicators	Decontamination methods			Statistics	
	1% NaOH (%)	1.5%NaOH (%)	Difference	Z value	P value
Contamination	22.4	6.8	15.6	5.08	<0.001
Recovery	20.45	20.45	0	0	1.000
Correlation	83.33	77.78	5.56	0.59	0.552
Contribution	10.53	11.40	0.877	0.31	0.785

The mean incubation period to positive culture determined with the 1% and 1.5%NaOH as a component of decontamination method were (10.24±6.59 and 12.87±9.65 days), respectively, however, this difference was not significant (P=0.123) (Figure 2).

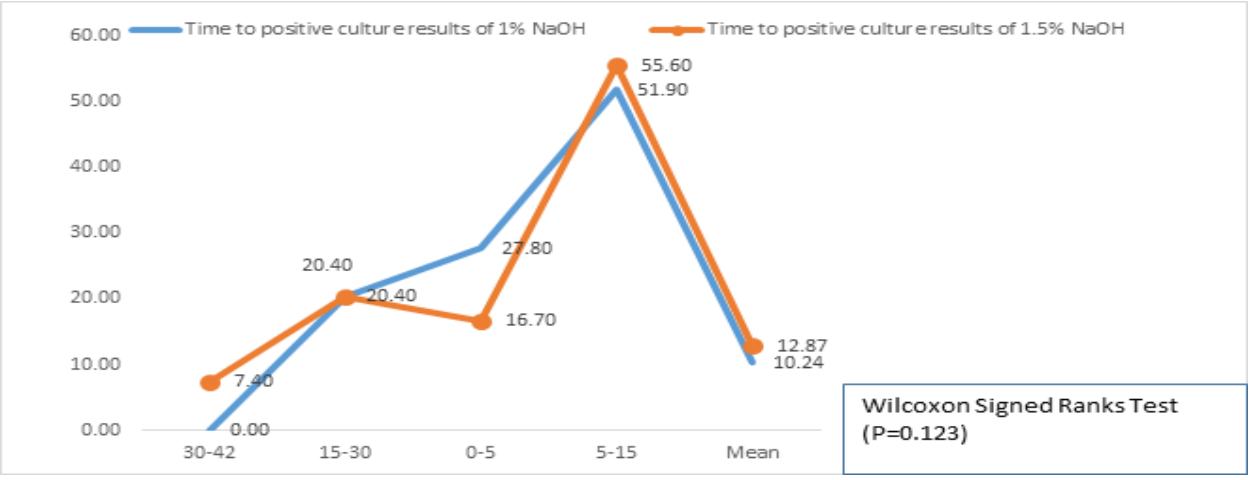

Figure 2: The mean incubation period of culture positive isolated from smear positive and negative cases at EPHI NTRL in the period from November 2015 to February 2016

The AFB negative versus culture negative, and AFB negative versus culture contaminated results with the 1% and 1.5%NaOH concentration method were (55.3% and 22.0%;70.83% and 5.68%), respectively, as shown on table 6.

Table 7: Smear examination (AFB) and culture correlation for sputum specimens processed by the NALC-NaOH method using standard (1%) and (1.5%) final concentrations of NaOH from patients referred to EPHI NTRL in the period from November 2015 to February 2016.

AFB and MGIT Culture results	Decontamination methods (Concentration of NaOH)				Statistics	
	1.5% NaOH		1% NaOH		Z	P value
	N	%	N	%		
AFB positive Vs Culture positive	28	10.61	30	11.3	-0.2538	0.7996
AFB positive Vs Culture negative	5	1.93	5	1.9	0.0251	0.9799
AFB positive Vs Culture contaminated	3	1.1	1	0.4	0.9322	0.3513
AFB negative Vs Culture positive	26	9.85	24	9.1	0.2942	0.7686
AFB negative Vs Culture negative	187	70.83	146	55.3	3.697	0.002
AFB negative Vs Culture contaminated	15	5.68	58	22.0	8.1824	<0.001
Total	264	100	264	100		

Common bacterial and fungal contaminants:

The most prevalent bacterial and fungal contaminants in the MGIT were identified. A total of 73 microbial contaminants were isolated from 59 (22.4%) contaminated MGIT tubes of 264 sputum's processed using the standard 1% NaOH final concentration. A total of 26 different microbial species were identified from contaminated MGIT tubes. Of the 26 identified microbial contaminant 24(92.3%) were bacterial and 2(7.7%) were fungal contaminants. From 59(22.4%) contaminated MGIT tubes, 10(16.9%) were isolated with more than two microbial contaminants (Figure 3).

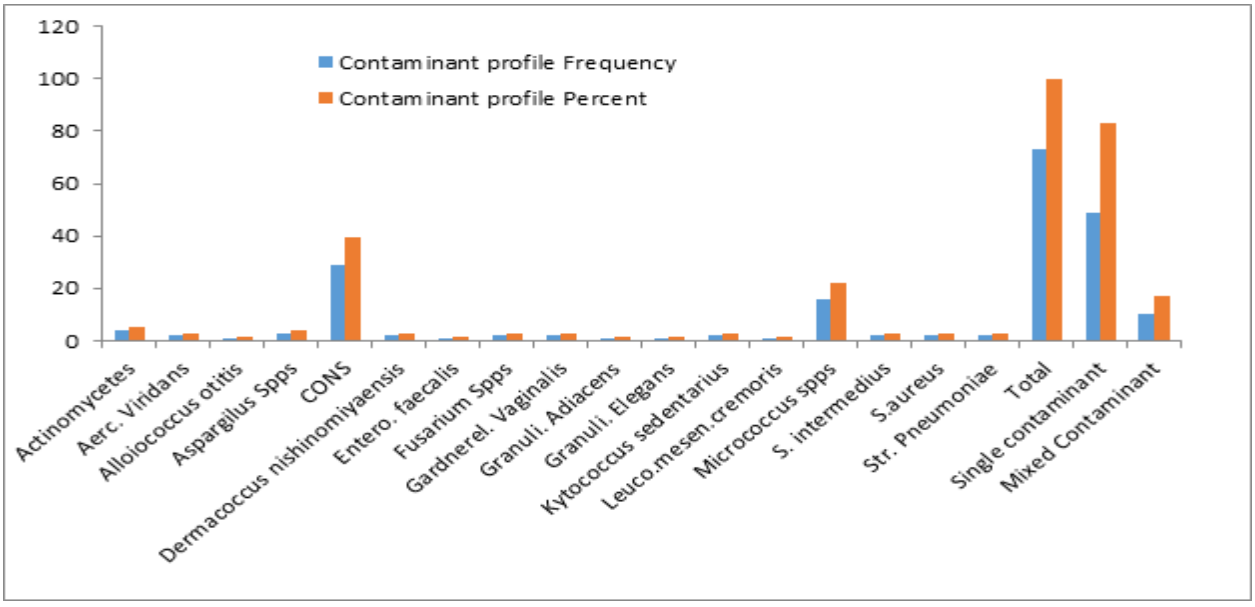

Figure 3: Distribution of bacterial and fungal contaminants associated with MGIT culture at EPHI NTRL in the period from November 2015 to February 2016

Discussion

The BACTEC MGIT 960 culture system improves the duration of time for detection and identification of mycobacterial species. Despite the fact that, the MGIT liquid culture system is associated with a high contamination rate compared to the conventional solid culture. Currently, an increased contamination rate with MGIT 960 culture system is a major challenge in tuberculosis culture laboratories. However, in this study and other similar studies(Bradner & Stabel 2012) the 1.5% sodium hydroxide final concentration with NALC were found to reduce the culture contamination rate when compared to the commonly used standard decontamination method 1% sodium hydroxide with NALC.

Treating clinical specimens by 1.5% sodium hydroxide with NALC have reduced the contamination rate from 22.4% to 6.8% compared to 1% sodium hydroxide with NALC. A statistically significant (P<0.001) reduction in the contamination rate was observed after increasing the final concentration of sodium hydroxide to 1.5% for samples incubated on MGIT 960 culture system. The contamination rate recorded in this study using the 1.5% NaOH-NALC was also reasonably lower compared to those reported by Peres *et al. (*2009) with a final concentration of 1.25% NaOH-NALC.

The contamination rate recorded in a study done at St John's Laboratory in Canada, and a multi-center study in Italy by the modified NALC-NaOH method with 1.5% NaOH were relatively lower, i.e., in the range 4-5% (Ratnam *et al.* 1987; Piersimoni *et al*. 2001), and decreased to non-detectable levels (Chien *et al.* 2000; Bradner *et al*. 2013). The varied performance of decontamination methods might be due to the slight selectivity of culture medium used. This selectivity is as a result of different; modifying ingredients, antibiotics, and the state of the medium either solid or liquid as described before (Chien *et al*. 2000; Somoskövi *et al.* 2000; Lu *et al.* 2002; Hines *et al.* 2006).

Findings in this study showed neither harmful nor beneficial effect on the mycobacterial recovery (P=1.000). Likewise, other studies on comparisons of decontamination methods reported the same rate of mycobacterial viability (Bradner *et al.* 2013; Ratnam *et al.* 1987; Piersimoni *et al.* 2001). In contrary, evaluation of culture results for specimens decontaminated with either 1.25% or higher concentration of NaOH-NALC showed a significant difference in the viability of mycobacterial species (Peres *et al*. 2009; Corner *et al.* 1995). A reduction in mycobacterial recovery might be due to the culture method used or the specimen type. Supporting our findings, above twenty (20.2%) differences in recovery of mycobacterial isolate was also observed between the MGIT 960 versus solid media in a study done by Hines *et al*. (2006).

No statistical significant differences was observed (P=1.000) when mycobacterial isolate detection on MGIT cultures from both smear positive and smear negative clinical specimens when decontaminated with 1% final concentration of NaOH-NALC compared to 1.5% NaOH-NALC. Similarly, in a study by Bradner and Stabel (2012), no significant difference was seen on the cultures contributions and recovery rate for the clinical specimens in a study conducted to optimize methods for the detection of *Mycobacterium avium*s ubs p.In contrast, better performance in isolate detection was observed by comparing four decontamination methods for recovery of *Mycobacterium avium* complex from stools (Yajko *et al.* 1993). This study was done to evaluate the four decontamination methods that the entire nine smears positive were culture positive for *Mycobacterium avium* complex with the standard concentration of NaOH with NALC. But, in a study conducted in Brazil, specimens with paucibaciliary and negative smear results were found to reduce mycobacterial isolation (Peres *et al*. 2009).Increasing the concentration of NaOH to the level evaluated in this study might not significantly affect the culture contribution rate.

The mean incubation period to positive culture determined with the 1% NaOH as a component of decontamination method (10.24±6.59 in days) was shorter than 1.5% NaOH (12.87±9.65 in days), however, the mean time for detection observed in this study between the two decontamination methods has no significant difference (P=0.123). as indicated elsewhere, specimens subjected to sodium hydroxide concentration ranging from 0.5% to 2% had no effect on the time to positive culture result (Bradner *et al*. 2013), nevertheless, a relative increment of mean time with 1.5% was seen compared to studies done abroad (Piersimoni *et al*. 2001).

The two decontamination methods, concentrated 1.5 % and 1% NaOH, have showed moderate association (Kappa=0.562). Increasing concentration of sodium hydroxide from 1% to 1.5% had improved the performance characteristics of BACTEC MGIT 960 culture system for successful yield of valid results on

the cultivation of mycobacterial species. Overall accuracy of the two methods was 77.24 and 92.68, respectively.

In this study, the false positive (instrument positive and smear/sub-culture negative) and false negative (instrument negative and smear /sub-culture positive) results were not identified. This might be due to the total specimens included in this study could not be sufficient to detect the instrument false positive or false negative culture results.

The most frequently isolated contaminants observed were coagulase negative staphylococcus (CONS) followed by *micrococcus species*. The most prominent bacterial contaminants associated with MGIT system, Coagulase-negative staphylococci, isolated in this study were similar with a rate of 34.8% recorded in a study done in Philadelphia in 1997(Cornfield *et al.* 1997) and by McClean (2011). In the same way, gram-positive organisms were highly favored in the MGIT system(Piersimoni *et al.* 2001).

Many of the isolates were grown on 5% Sheep Blood Agar, Brain Heart Infusion Agar and Sabouraud Dextrose Agar but none of the isolates were culture positive on MacConkey agar. This result showed that the standard decontamination method used and the concentration of antibiotics consisting; Polymixin B, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin (PANTA), added to the MGIT tube were inefficient in the standard decontamination method (1%NaOH) to kill or suppress the growth of identified bacterial and fungal contaminants.

The concentration of PANTA supposed to be added as recommended by the manufacturer per ml of MGIT tube were (Polymixin B, 46unit/ml; Amphotericin B, 5ug/ml; Nalidixic acid, 18ug/ml; Trimethoprim, 5ug/ml; and Azlocillin, 5ug/ml) (Becton Dickinson 2013). The concentration used in this study has a relatively slight difference (Polymixin B, 50unit/ml; Amphotericin B, 5ug/ml; Nalidixic acid, 20ug/ml; Trimethoprim, 5ug/ml; Azlocillin, 10ug/ml) than with a study conducted in Switzerland by Realini *et al. (*2000).

Most of the contaminants isolated in this study were gram positive and fungi. This implies the concentration of antibiotics assumed to be effective against these microorganisms (Trimethoprim and Nalidixic acid, respectively), might not be effective. In the other hand, antibiotics added for gram negative bacteria (Polymixin B) could effectively suppress their growth. Differences on the three drugs could be a reason for high contamination recorded by CONS. Consequently, other study showed that increasing the PANTA concentration might significantly reduce the contamination rate (Peres *et al.* 2011).

In general, the final concentration of 1.5% NaOH with NALC as a component of decontamination method was found to be equivalent to the standard method in culture recovery rate with the standard. Whereas reduction of the contamination rate is the most important advantage of this decontamination method for the reason that deprived of affecting the smear positive culture positivity and smear negative culture negativity outcomes. Additional benefits of increasing the concentration of sodium hydroxide to the level of evaluation in this study were found to increase the overall performance of MGIT liquid culture system.

Conclusion and Recommendations

Results presented in this study demonstrated that a final concentration of 1.5% sodium hydroxide with NALC decontamination method have acceptably improved the culture contamination rate without affecting the recovery of mycobacterial species, smear positivity versus culture positivity rate and smear negativity versus culture positivity rates. In addition, the increase of the final concentration of NaOH by 0.5% has no effect on the recovery of *Mycobacterial species*. Consequently, the use of a final concentration of 1.5% NaOH with NALC method are suitable and aids in reducing culture contamination rate for decontaminating clinical specimens referred for tuberculosis culture diagnosis particularly in settings where culture contamination is a high quality problem. It also might be very important for clinical specimens usually collected from follow up patients referred for culture diagnosis in Ethiopia. With the intension of supplementing the current study, further studies are recommended to assess the factors associated with increased culture contamination rate, and to examine antimicrobial susceptibility of those more prominent contaminants associated with MGIT liquid culture system are recommended.

Acknowledgement

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