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EDITORIAL MESSAGE

Dear Readers,

On behalf of East African Journal of Health and Biomedical Sciences *(EAJHBS)* Editorial Board, I would like to extend a warm welcome to the readers of *EAJHBS*. I take this opportunity to thank our authors, editors, anonymous reviewers, and all of whom have volunteered to contribute to the success of the journal. The *EAJHBS* is a biannual peer-reviewed journal that aims to publishing and advancing knowledge and research in the field of health and biomedical sciences.

EAJHBS publishes original research articles, case reports, review articles, and short communications. This first issue contains five peer reviewed original articles. The Editorial Board, the article contributors and the reviewers have worked for its quality.

EAJHBS is currently accepting manuscripts for upcoming issues based on original qualitative or quantitative researches, case studies, innovative conceptual frameworks, and substantial literature reviews that open new areas of inquiry and investigation. As we look at the first issue, the journal recognizes the contributions of different professionals to expanding and enriching theory, research, and practice in health and biomedical sciences. I express my gratitude to our well-wishers and contributors, and we continue to welcome, not only original and good quality manuscripts, but also constructive comments, suggestions and criticisms from our readers, which will stimulate further debates and research on health and biomedical sciences.

The East African Journal of Health and Biomedical Sciences welcomes article contributions of all interested scholars and practitioners in health and biomedical sciences to submit their manuscript to the editorial manager's office as indicated on the University's website. The editor-in-chief gratefully welcomes submissions that fit the publication guidelines, and for any other details, please contact the Editorial Manager at: *tdigaffe@yahoo.com*. Alternatively you may also access the Editor-in-chief at: *tesfayegob@gmail.com*.

Tesfaye Gobena (PhD, Assoc. Professor)

Editor-in-Chief

In vivo Evaluation of Antifertility Activity of Aqueous and Butanol Fractions of Methanolic Root Extract of *Rumex steudelii in* Female Mice and Rats

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Abstract

Background: In Ethiopia, to control fertility, traditional medicines have been used for many years. The crude methanolic root extract of *Rumex steudelii* (Tult) is believed to have anti-fertility effect.

Objectives: To identify fraction(s) of crude methanolic root extract of *Rumex steudelii* responsible for antifertility effect, to suggest the possible mechanisms, to determine the oral Lethal Dose, 50% (LD_{50}), and to screen the phytochemicals for the fractions.

Methods: Preliminary screening for the antifertility activity was conducted in female mice at 700mg/kg and 900mg/kg using aqueous and butanol fraction from January to November, 2011, Addis Ababa, Ethiopia . Besides effect of the fractions at 900mg/kg on implantation, serum estrogen, progesterone, and cholesterol, the weight of genital organ and body weight was carried out.

Results: The fractions reduced the number of litters at 700mg/kg and 900mg/kg ($p<0.01$). The aqueous fraction decreased implantation site ($p<0.05$) which was further supported by increase in serum progesterone level ($p<0.001$). The wet weight of the uterus was shown also to be reduced significantly ($p<0.05$). The LD_{50} of the aqueous and butanol fractions was found to be 10.475g/kg and 7.080g/kg, respectively. Determination of the secondary metabolites in these fractions indicated the presence of phytosterols, polyphenols and tannins, which could be responsible constituents for their antifertility effects.

Conclusion: The aqueous fraction showed more antifertility activity and higher lethal dose than butanol fraction. Further investigations on the quantity of screened phytochemicals and efficacy, safety, isolation, characterization and structural elucidation of active principles on these fractions should be conducted. Moreover the antifertility effects and phytochemical screening of other fractions need to be investigated.

Keywords: *Rumex steudelii, antifertility, aqueous fraction, butanol fraction, LD_{50}, secondary metabolite*

Introduction

The ever increasing population in the world, particularly in low income countries, has detrimental effects on life supporting system on earth. Fertility regulation comprising contraception and management of infertility forms an important component of reproductive health (Gupta and Sharma, 2006). There are different methods used for family planning methods: continence abstinence, rhythm method, barriers, hormonal contraceptives, implantable devices, and permanent birth control methods (Qureshi *et al.,* 2006). But there is no "best" method of birth control. Each method has its pros and cons making choices difficult (USAID, 2010).

The development of new fertility regulating drugs from medicinal plants is an attractive proposition for a number of reasons. Plants derived compounds or their derivatives form the basis of a large number of established drugs. Approximately 80% of the world's populations depend on health care systems that involve the use of traditional medicine including fertility regulating plants (WHO, 2001). The acceptability of new antifertility drugs may be greatly enhanced if these preparations are based on indigenous knowledge and practices (Bekele, 2007). Major economic and commercial benefits could be derived by fostering this

national self-reliance in drug development and production.

Natural plant substances that have mild inherent estrogenic and anti-estrogenic properties offer themselves as effective non-conventional sources of contraception with less deleterious side effects. For this reason, continuous efforts are being made to use natural plants to develop antifertility products (Angela and Christy, 2009).

The practice of traditional medicine for the control of fertility in rural Ethiopia is also based on folk use of numerous anti-fertility herbs. A total of 210 extracts/ fractions from 70 traditionally used Ethiopian plants were subjected to utero-tonic and anti-implantation bioassays (Desta, 1994). *Achyranthes aspera* (Shibeshi *et al.*, 2006)*, Asparagus africanus* (Tafesse *et al.*, 2006)*, Leonotis ocymifolia* (Tafesse *et al.*, 2005)*, Jatropha curcas L.* (Makonnen *et al.*, 1997) *and Rumex steudelii* (Gebrie *et al.*, 2005b) are some of those used for this purpose.

Rumex steudelii, H. whose vernacular name is "Tult" or "Yeberemelase", is found in different parts of Ethiopia, such as Tigray, Gondar, Gojam, Wollo, Shewa and Arsi highlands. It is an erect, perennial herb which grows up to one meter tall at an altitudinal range of 1200–3900m. It is traditionally used with other medicinal plants to treat various illnesses such as hemorrhoids, rectal prolapse, abdominal colic wounds, eczema, leprosy and tonsillitis. It is also used as hemostatic and oxytocic agent (Gebrie *et al.*, 2005a).

The methanolic extract of this plant showed anti-implantation effect in rats (Desta, 1994). It prolonged the estrous cycle and the diestrous phase of the estrous cycle (Gebrie *et al.*, 2005a). It also showed that the antifertility effects of the extract were transient and reversible. The extract caused significant decrease in the number of implantation sites and reduced the serum estrogen level (Gebrie et *al.*, 2005b). The phytochemical screening of the plant has showed the presence of polyphenols, phytosterols, saponins and tannins (Gebrie *et al.*, 2005a). Atrophic changes in the uterus and disruption of ovarian folliculogenesis by inhibiting further development of recruited ovarian follicles in dose dependent manner was also discovered (Tibebu *et al.*, 2010).

Despite the availability of several findings stating its antifertility effect, it was conducted using crude extracts (Gebrie *et al.*, 2005a; Gebrie et *al.*, 2005b; Tibebu *et al.*, 2010). Therefore, the objectives of the present study were to identify fraction(s) of crude methanolic root extract of *Rumex steudelii* responsible for antifertility effect, to suggest the possible mechanisms, to determine the oral LD_{50} (Lethal Dose in 50% of mice), and to screen phytochemical for the fractions in Addis Ababa, Ethiopia, from January to November, 2011.

Materials and Methods
Preparation of Animals
All anti-fertility experiments were performed on inbred adult, cyclic virgin female albino rats (4-5 months old and weighing 150-200g) except in preliminary screening where female albino mice (2-3 months old and weighing 25-30g) were used. Female albino mice weighing 25-30g were also used for the acute toxicity study. The mice used in these experiments were obtained from Ethiopian Public Health Institute. The animals were housed in polypropylene cages and maintained under environmentally controlled room provided with 12:12 hour light and dark cycle for each 24 hour period at a temperature of 25^0C. They were fed on pellets and tap water ad libitum. The animals were allowed to acclimatize to the laboratory environment for three to five days prior to the experiment (Obernier and Baldawin, 2006).

Collection and Identification of Plant Material
The root of *Rumex steudelii* was collected from a garden in Ethiopian Public Health Institute by a staff member in December, 2010. The plant was identified by a taxonomist and a voucher sample (Herbarium number AA-2135) was deposited in the herbarium of the Department of Drug Research of Ethiopian Public Health Institute, Addis Ababa.

Preparation of Plant Crude Extract
The crude extract of *Rumex steudelii* was prepared based on the manual described by Debella (2002). The root of the plant was dried under shade, ground into course powder, and percolated in 80% methanol for three days. Then it was filtered using filter paper (Whatman No. 1). The solvent was removed by using rotary evaporator. Further concentration of the extract was made by heating and the evaporation of the solvent was kept in water bath at 40°C, which finally gave a brownish dark semi-solid mass. The percent yield of 1,103g powdered *Rumex steudelii* root was 23.4%w/w (258.1g) methanolic extract.

Preparation of Solvent Fractions

The isolation of compounds from natural sources in a pure state is very important, but it can be a difficult and time-consuming process in natural product research. One of the separation techniques is the solvent partitioning method, which usually involves the use of two immiscible solvents in a separating funnel. In this method, compounds were distributed in two solvents according to their different partition coefficients. This technique is highly effective, as the first step of the fairly large-scale separation of compounds from crude natural product extracts (Otsuka, 2005).

The crude *Rumex steudelii* root extract was fractionated by dissolving 50g methanolic root extract in mild hot distilled water. After filtration, the dissolved extract was added in a separating funnel and mixed with 50ml of n-hexane three times. After shaking, it was allowed to stay for some time until a complete formation of two layers. Then the upper layer, i.e. n-hexane, was collected. After collecting the n-hexane layer, 50ml of dichloromethane was successively added three times in the left aqueous residue and then the lower layer (dichloromethane) was taken. After 50ml of n-butanol was added successively three times in the remaining aqueous residue, the upper layer, i.e. n-butanol, was collected. Finally, n-hexane, dichloromethane and n-butanol fractions were concentrated under vacuum using rotary evaporator and then dried at 40°C using water bath to give 0.029%(w/w), 0.035%(w/w), 31.7%(w/w) residue, respectively. But the aqueous fraction residue was lyophilized (freeze-dried) to give 32.5% (w/w). Since the yield for hexane and dichloromethane were too little, n-butanol and aqueous fractions were used in the experiments. The n-butanol fraction was stored in refrigerator at -8°C and aqueous fraction in desiccator until the experiment was conducted.

Test Material Administration and Dose Calculation

The administration of the extract was done with intragastric tube on the basis of the animal's body weight. The dose for each animal was calculated, considering the doses that were effective in the previous studies conducted by Gebrie *et al.* (2005a) on methanol extract of *Rumex steudelii* root. Based on the percent of yield of the crude extract for the fractions, the proportion of these doses which could give the dose for the fractions was calculated, as was done in other study (Flores *et al.,* 2008). Hence two doses were selected: 2.2g/kg and 2.8g/kg. The proportions of aqueous fraction were 0.715g and 0.910g, and butanol fractions

were also found to be 0.697g and 0.888g, respectively. Moreover, from acute toxicity study, the result of LD_{50} of crude extract and pilot study were also considered based on Organization of Economic Co-operation and Development guideline (OECD, 2008). To employ uniform doses, 0.7g/kg and 0.9g/kg were used. The aqueous and butanol fractions were reconstituted in distilled water and distilled water with 2% tween-80, respectively, to get the desired concentration for all pharmacological tests.

Pharmacological Screening for Antifertility Effect

Preliminary Screening for the Antifertility Activity

Six groups of mature female mice (6 mice per group) were selected for the experiment. Two groups were used as a negative control receiving distilled water and 2% tween-80 in distilled water vehicles for aqueous and butanol fractions, respectively. The remaining four groups received aqueous and butanol fractions at two doses levels each: 700mg/kg and 900mg/kg. All the groups received the test and control substances intragastrically daily for 7 days. All the experimental animals were then allowed to mate with mature male mice (one male for two female) and the administration of the vehicles and the fractions continued for 21 days. As described by Gebrie *et.al.* (2005a), in both the control and test groups, the number of litters was determined after the completion of one gestation period. The litters of extract treated mice were then allowed to grow in order to check for postnatal growth and congenital anomalies.

Studies on Anti-implantation Activity

Four groups of mature female rats (6 rats per group) were selected for this experiment. Two groups served as negative control and received the vehicles intragastrically for 10 days. The other two groups were used as test groups receiving the aqueous and the butanol fractions at 900mg/kg for the same number of days by the same route. Anti-implantation activity was determined based on the methods used by Ahirwar *et al.* (2010) and Gebrie *et al.* (2005b). All the groups were left over night with male (in the ratio of 2 female to 1 male) and the vaginal smear was examined for motile spermatozoa in the next morning. The day on which the spermatozoa were found in the smear was considered the first day of pregnancy (day one). The animals were separated immediately after confirming mating. On the 11th day of pregnancy, all the groups of rats were undergone laparotomy under diethyl

ether anesthesia to determine the number of implantation sites in the horns of the uterus. The presence of significant difference in the mean number of implantation sites between the fractions and the controls were taken as a positive response.

Effect on Serum Estrogen, Progesterone and Cholesterol

In this experiment the two test groups (5 female rats/group) were treated with test substances at 900mg/kg for 10 days by intragastric gavages. The negative control groups were treated with vehicle in the same way as the test groups. The level of estrogen, progesterone, and cholesterol were determined based on the methods described by Gebrie *et al.* (2005b). On the 11th day, the control and the test groups were anesthetized under diethyl ether and blood was drawn by cardiac puncture. The blood was allowed to coagulate for an hour. The separation of the serum from other cellular components of the blood was done by centrifuging the coagulated blood at 2500 revolution/minute for 15 minutes. Then sera was collected and stored in deep freezer (-20 ^{0}C). After two weeks, the sera were analyzed for estrogen and progesterone using Elecsys2010 immunoassay analyzer. All components and reagents for routine analysis are integrated in or on the analyzer. Total cholesterol in serum was analyzed using the instrument Cobas Integra 400 Plus, Roche Diagnostics.

Effect of the Fractions on the Weight of Genital Organ and Body Weight

Four groups of matured female rats (6 rats per group) were selected for this experiment. Two groups served as negative control and received the vehicles intragastrically for 10 days. The other two groups were used as test group receiving the aqueous and the butanol fractions at 900mg/kg for the same number of days by the same route. On the 11th day, all the animals in all groups were weighed and sacrificed under diethyl ether anesthesia. The ovaries and uteri were dissected out, freed from surrounding tissues, blotted on filter paper, and weighed quickly on analytical balance. The ovary and the uterine ratios were calculated by dividing the ovary and the uterine weight in milligrams by body weight in grams (Gebrie *et al.,* 2005b).

Determination of Oral LD$_{50}$

The LD$_{50}$ of the fractions was determined by taking probit 5 (equivalent to 50% mortality) on the Y-axis interpolated to X-axis to get log LD$_{50}$ and then antilogarithm of which was taken as the LD$_{50}$ of the fractions, as described by the Organization of Economic Co-operation and Development guideline (OECD, 2008). It was conducted in female Swiss albino mice. The fraction was administered by intragastric route starting from smaller to higher doses in 1ml of vehicle. The records of mortality and the manifestation of toxicity were made in 24 hours.

Secondary Metabolites Identification

Phytochemical tests of the aqueous and butanol fractions of methanolic root extract of *Rumex steudelii* were carried out as described by Debella (2002). Phytosterols are screened by Lieberman and Burchard's reagent whereas polyphenols with 1% Ferric Chloride and 1% Potassium Ferrocyanide and formation of honeycomb froth was used to determine saponins. Moreover, 1% Potassium ferrocyanide and concentrated Ammonia, Sodium nitrate and 0.1N Hydrochloride acid, Ferric sulphate and Dragendroffs reagent were used to screen tannin, hydrolysable tannin, phenolic glycosides and alkaloids, respectively (Debella , 2002).

Statistical Analysis

The data were expressed as mean ± Standard Error of Mean (SEM) and analyzed using GraphPad Prism Version 5.04 software. One way Analysis of Variance (ANOVA) was used to assess the variation of the means among the treatments. Student paired t-test was performed to compare body weight changes before and after treatment. P-value less than 0.05 was considered as a cutoff point as statistically significant.

Ethical Considerations

This study was approved by the Ethics Review Committee of Addis Ababa University College of Health Sciences. Animal care and handling were according to international guidelines for the use and maintenance of experimental animals (Institute for Laboratory Animal Research, 1996; National Research Council, 2011; OECD, 2008).

Results

Preliminary Screening for the Antifertility Activity

Both fractions, aqueous and butanol, decreased the number of litters significantly ($p<0.05$) at 700mg/kg.

Besides, there was no birth in those mice treated with 900mg/kg aqueous fraction and a significant ($p<0.01$) decrease in number of litters was found in the mice that received butanol fraction at this dose level (Table 1).

Table 1. Antifertility effect of aqueous and butanol fractions of methanolic root extract *Rumex steudelii* at two dose levels on mice Addis Ababa, Ethiopia, 2011.

Treatment	Dose (mg/kg)	Number of animals	Mean number of litters (*Mean ± SEM)*
Aqueous fraction	700	6	3 ± 1.897*
	900	6	0.00
Negative control of aqueous fraction	Distilled water	6	10.333 ± 1.542
Butanol fraction	700	6	4.123 ± 2.0220*
	900	6	3.625 ± 1.851**
Negative control of butanol fraction	2% tween 80 in distilled water	6	10.666 ± 0.8819

*Note: *p<0.05;   ** p<0.01;   *** p<0.001*

Anti-implantation Activity of Aqueous and Butanol Fractions of Methanolic Extract of Rumex steudelii

The aqueous fractions of methanolic root extract of *Rumex steudelii* showed a significant ($p<0.05$) decrease in implantation site in the rats but the butanol fraction did not (Table 2).

Effect of Aqueous and Butanol Fractions of Methanolic Extract of Rumex steudelii on Serum Estrogen, Progesterone and Cholesterol

There was a significant ($p<0.001$) increase in serum progesterone in the aqueous fraction treated rats but a decrease in serum cholesterol in fractions treated groups, as compared to the controls (Table 3).

Table 2. Effect of aqueous and butanol fractions of methanolic root extract of *Rumex steudelii* on implantation, Addis Ababa, Ethiopia, 2011.

Treatment	Dose (mg/kg)	Number of animals	Number of implantation site (*Mean ± SEM)*
Aqueous fraction	900	6	2.833 ± 1.797*
Negative control of aqueous fraction	Distilled water	6	8.666 ± 0.4216
Butanol fraction	900	6	4.833 ± 1.537
Negative control of butanol fraction	2% tween-80 in distilled water	6	8.333 ± 0.333

*Note: *P<0.05;   ** P<0.01;   *** P<0.001*

Table 3. Effect of aqueous and butanol fractions of methanolic root extract of *Rumex steudelii* on serum estrogen, progesterone and cholesterol, Addis Ababa, Ethiopia, 2011.

Treatment	Dose (mg/kg)	Estrogen (pg/ml) *(Mean ± SEM)*	Progesterone (ng/ml) *(Mean ± SEM)*	Cholesterol (mg/dl) *(Mean ± SEM)*
Aqueous fraction	900	15.428 ± 1.861	49.768 ± 3.739***	52.2 ± 2.973
Negative control for aqueous fraction	Distilled water	14.654±5.888	19.518 ± 5.37	60.6 ± 3.385
Butanol fraction	900	18.192 ± 3.268	15.634 ± 1.002	59 ± 8.602
Negative control for Butanol fraction	2% tween 80 in distilled water	19.742 ± 4.75	17.232 ± 7.312	72.4 ± 4.781

*Note: Number of animals=5; pg: picogram; ng: nanogram; dl: deciliter; *p<0.05; ** p<0.01;   *** p<0.001*

Effect of Aqueous and Butanol Fractions of Methanolic Extract of Rumex steudelii on the Weight of Genital Organ and Body Weight

Except in the butanol fraction group, all groups increased body weight significantly ($p<0.001$) after the study period, but a significant reduction in body weight gain was observed in aqueous fraction ($p<0.01$) and butanol fraction ($p<0.001$) treated groups as compared to the controls. Uterine ratio was also significantly ($p<0.05$) reduced in the test groups compared to the controls. Ovarian ratio was not significantly affected with any of fractions (Table 4).

Table 4. Effect of aqueous and butanol fractions of methanolic root extract of *Rumex steudelii* on weight of genital organ and body weight, Addis Ababa, Ethiopia, 2011.

Treatment	Dose (mg/kg)	Uterine ratio (mg/gm) *(Mean ± SEM)*	Ovary ratio (mg/gm) *(Mean ± SEM)*	Body weight (gm) *(Mean ± SEM)*		Change in Body weight (gm) *(Mean ± SEM)*
				Initial	Final	
Aqueous fraction	900	1.1318±0.1615*	0.5846±0.0859 NS	154±1.7703	171.9±3.002***	17.7±1.3**
Negative control of aqueous fraction	DW	1.7954±0.1022	0.7906±0.0699	153±2.864	183.98±1.195***	30.98±2.94
Butanol fraction	900	1.3678±0.0847*	0.6452±0.0162	154 ±1.461	163.233±3.092	9.23±1.804***
Negative control of Butanol fraction	2% tween 80 in DW	2.0462±0.2262	0.8566±0.083	151.4±1.077	184.08±1.951***	32.8±0.9695

*Note: Number of animals=6; *p<0.05; ** p<0.01; *** p<0.001; DW: Distilled Water*

LD$_{50}$ for Aqueous and Butanol Fractions of Methanolic Root Extract of Rumex steudelii

The LD$_{50}$ of aqueous (Figure 1) and butanol (Figure 2) fractions of methanolic root extract of *Rumex steudelii* were found to be 10.475g/kg and 7.08g/kg, respectively. During observation for toxic manifestations, most mice showed hypo-activity, piloerection, depression and breathing difficulty before death.
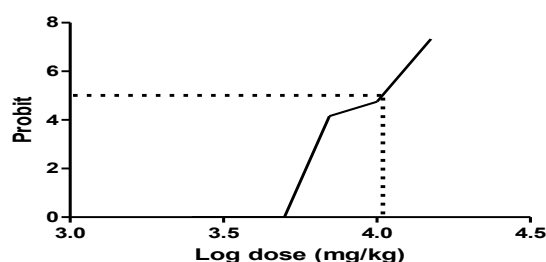

Figure 1. Lethal oral dose of aqueous fraction of methanolic root extract of *Rumex steudelii*, Addis Ababa, Ethiopia, 2011.
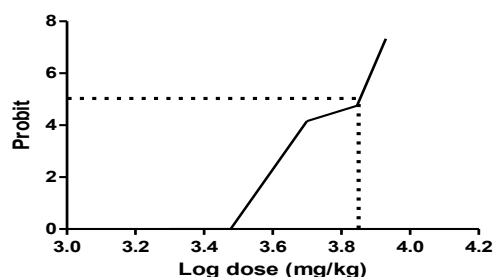
Figure 2. Lethal oral dose of butanol fraction of methanolic root extract of *Rumex steudelii*, Addis Ababa, Ethiopia, 2011.

Secondary Metabolites of the Aqueous and Butanol Fractions of Methanolic Root Extract of *Rumex steudelii* by Chemical Method

The aqueous fraction of methanolic root extract of *Rumex steudelii* was shown to have polyphenols, phytosterols, tannins and saponins (Table 5). Similar screening of butanol fraction displayed the presence of polyphenols, phytosteroides, tannins and hydrolysable tannins (Table 6).

Table 5. Secondary metabolites of aqueous fraction of methanolic root extract of *Rumex Steudelii* by chemical method, Addis Ababa, Ethiopia, 2011.

Plant material used	Secondary metabolites tested	Reagent	Result
	Polyphenols	$1\% K_3Fe(CN)_6$ & $1\% FeCl_3$	+
	Phytosterols	Liebermann and Burchard's	+
	Alkaloids	Dragedroff's	-
Aqueous fraction	Tannins	$1\% K_3Fe(CN)_6$	+
	Hydrolysable tannins	$NaNO_3$	-
	Saponins	Honeycomb froth formation	+
	Phenolic glycoside	$FeSO_4$	-

Note: (+) indicate presence and (-) indicate absence of particular metabolites

Table 6. Secondary metabolites of butanol fraction of methanolic root extract of *Rumex Steudelii* by chemical method, Addis Ababa, Ethiopia, 2011.

Plant material used	Secondary metabolites tested	Reagent	Result
	Polyphones	$1\% K_3Fe(CN)_6$ & $1\% FeCl_3$	+
	Phytosterols	Liebermann and Burchard's	+
	Alkaloids	Dragedroff's	-
Butanol fraction	Tannins	$1\% K_3Fe(CN)_6$	+
	Hydrolysable tannins	$NaNO_3$	+
	Saponins	Honeycomb froth formation	-
	Phenolic glycoside	$FeSO_4$	-

Note: (+) indicate presence and (-) indicate absence of particular metabolites

Discussion

The antifertility activity of aqueous and butanol fractions of the 80% methanolic root extract of *Rumex steudelii* (crude extract) were conducted using different parameters. In preliminary screening of these fractions at two dose levels (700mg/kg and 900 mg/kg) on female mice for the treatment period of 28 days, the mean number of litters decreased very significantly with no delivered pups in those which received the aqueous fraction at 900mg/kg, indicating that these fractions have antifertility effect. Anti-implantation activity and an increase in the level of serum progesterone were also observed with the aqueous fraction that could be possible mechanisms. The observations that both fractions had LD_{50} greater than 5gm/kg clarify the safety nature of the fractions. The phytochemical screening of the fractions indicated the presence of phytosterols, polyphenols and tannins, which could possibly reason out the antifertility effect of the plant.

Based on these promising results in preliminary screening of the fractions, further experiments were conducted to establish the possible mechanisms for antifertility activity. Fractions treated mice's litters were allowed to grow to check for postnatal growth and congenital anomalies, but there was not any physical deformity observed in any of the litters. All litters grew into adult stage which suggests that the aqueous and butanol fractions of *Rumex steudelii* methanolic root extract may not have teratogenic effect even if contraception fails. Similar results were also seen with the methanolic crude extract of the plant (Gebrie *et al.,* 2005a).

Anti-implantation activity was observed with the aqueous fraction and this can be one possible mechanism for its antifertility effect. Similar results were reported on crude extract of this plant (Desta, 1994; Gebrie *et al.,* 2005b). Anti-implantation effects were also observed with *Trigonella foenum-graecum* (Ahirwar *et al.*, 2010), *Sida acuta* (Londonkar *et al.*, 2009), *Cassia fistula* (Yadav and Jain, 2009), *Ferula jaeschkeana Vatke* (Pathak and Prakash, 1989), and *Hibiscus rosa-sinensis* Linn (Vasudeva and Sharma, 2008).

The success of implantation depends on achieving the appropriate embryo development to the blastocyst stage, which in turn depends on the development of an endometrium. Implantation is a very intricate process, regulated by a number of complex molecules like hormones (estrogen and progesterone), cytokines (Leukemia inhibitory factor, Interleukin 6, Interleukin 11), and growth factors (Transforming growth factor-b, Epidermal growth factors, Heparin binding-epidermal growth factor, Insulin-like growth factors) and their cross talk. A network of these molecules plays a crucial role in preparing receptive endometrium and blastocyst (Singh *et al.,* 2011). Any imbalances or disturbances to these compounds could result in failure of implantation.

The increase in the level of serum progesterone with the aqueous fraction could explain its anti-implantation effect. Furthermore, insignificant anti-implantation effect of the butanol extract might be associated with its insignificant effect on the hormonal level. Its antifertility activity might be due to reduced uterine ratio and repeated administration.

Progesterone containing contraceptives act by inhibiting ovulation which result in low level of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH), as well as the inadequacy of the mid cycle LH surge. Since the proper frequency of LH pulses is essential for ovulation, progesterone is likely to play a major role in contraception. Progesterone also influences the endocervical glands, and the abundant watery secretion of the estrogen-stimulated structures is changed to a scant, viscid material thereby decreasing the penetration of the cervix by sperm, delaying the tubal transport of egg or embryo, and affecting fertilization. Besides, it is involved in the alteration of endometrial receptivity for implantation (Guyton and Hall, 2006).

Endometrium is known to become receptive only for short period in both rodents and humans. Beyond this period of receptivity, the embryo is unable to establish contact with receptive endometrium successfully. Therefore, a timely arrival of embryo in a receptive endometrium is very crucial for successful implantation (Singh *et al.,* 2011). The increased progesterone level by the aqueous fraction could, therefore, be possible mechanism for its anti-implant effect. A similar finding was reported by Akpantah *et al.* (2010) on Gonadal histo-morphologies and serum hormonal milieu in female rats treated with *Azadirachta indica* leaf extract.

In the preliminary screening with the butanol fraction a significant reduction in the number of litters in the mice was shown, though the implantation sites in the rats were not affected. This could be attributed to the long term administration, which might cause fetal resorption. A similar result was seen on antifertility activity of *Derris brevipes* variety *coriacea* by Badami *et al.* (2003).

Estrogen increases body weight, uterine, and ovarian (Guyton and Hall, 2006). In this study, after treated with the aqueous and butanol fractions, there was a significant reduction in the body weight gain in both fractions as compared to the controls. Moreover, the reduced uterine and ovarian ratio which was observed with both fractions may indicate their anti-estrogenic nature which alters the biochemical milieu of the reproductive tract leading to change in the normal status of the reproduction in female reproductive tract of rat and thus may contribute for antifertility effect. Anti-estrogenic effects were also observed with *Nelumbo nucifera* (Mutreja *et al.*, 2008), *Saccharum officinarum* (Balamurugan *et al.,* 2009)*, Piper betle* (Sharma *et al.,* 2007), and *Catharanthus roseus* (Gupta, 2009).

The observation that both fractions had LD_{50} greater than 5g/kg suggests the safety nature of the fractions (OECD, 2008).

Chemical, biological, or physical assays are necessary to identify the responsible compound(s) from a complex natural product being used in any study. The target compounds may be of certain chemical classes and have

certain physical properties, or possess certain biological activities. Therefore, appropriate assays should be incorporated in the extraction and isolation protocol (Sarker, 2005). In the present study, the phytochemical screening of the aqueous and butanol fractions indicated the presence of phytosterols, polyphenols and tannins which are higher molecular weight polyphenols (Alugah and Ibraheem, 2014) and this could possibly suggest the antifertility effect of the plant. These constituents were also identified and reported in *Ficus asperifolia* (Watchoa *et al.,* 2009) and *Alangium salviifolium* (Linn. f.) Wang (Murugan *et al.,* 2000), which are traditionally employed antifertility plants.

Conclusion and Recommendations

The present study indicated that both aqueous and butanol fractions of the methanolic root extract of *Rumex steudelii* have dose dependent anti-fertility effect. The antifertility effect of the aqueous fraction may be attributed to its anti-implantation activity resulting from an increase in the progesterone hormone and reduced uterine ratio, but that of the butanol fraction might be due to reduced uterine ratio and repeated administration. The antifertility effect is more with the aqueous fraction than with the butanol fraction. Moreover, both fractions employed in the present study seem to be safe. The aqueous fraction were safer than the butanol based on LD_{50} finding. The polyphenols, phytosterols, and tannins might also be responsible for the anti-fertility activity in both fractions.

In general, comprehensive investigations should be conducted to establish additional mechanism(s) of the action of the aqueous and the butanol fractions such as effects on serum Gonadotropin Releasing Hormone (GnRH), FSH, LH and uterine contractility. Further determination on the quantity of the screened phyto chemicals, efficacy, safety, isolation, characterization, structural elucidation of active principles, and toxicity studies on chronic administration on these fractions should be conducted. Moreover the antifertility effects and phytochemical screening of other fractions need to be investigated.

Acknowledgments

Conflict of Interests

The authors declare that they have no competing interests.

Authors' Contributions

TG performed the laboratory work, acquired and analyzed the data and drafted the manuscript. EM conceived the research idea. AD identified and provided the experimental plant and facilitated for laboratory work in Ethiopian Public Health Institute. EM and AD participated in the design of the study, correction and approved the final version of the research manuscript.

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Survival Status and Predictors of Mortality among Children Aged 0-59 Months Admitted with Severe Acute Malnutrition in Dilchora Referral Hospital, Eastern Ethiopia.

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Abstract

Background: The mortality rate of children admitted to hospital with complicated severe acute malnourishment is as high as 35 %. In Ethiopia, this ranges from 6 % to 29 %, and its predictors vary contextually.

Objective: To assess the survival status and the predictors of mortality among 0-59 months children with severe and acute malnutrition and admitted to Dilchora Referral Hospital, Eastern Ethiopia.

Methods: A retrospective cohort study was conducted on children with Severe Acute Malnutrition (SAM) treated in Dilchora Referral Hospital. The data were collected from medical records from March 07 to 16, 2016. Six hundred seventeen children were randomly selected from all eligible records of 0-59 months children admitted to the center from September 2011 to August 2015. The data were collected using a pretested data extraction format and entered into EpiData software and exported to SPSS Version 20 for analysis. Survival curve was used to display the survival status among different characteristics. The Cox proportional hazard model was done to identify independent predictors of mortality. Associations were declared as statistically significant with $p < 0.05$.

Results: It was found that 47 (7.6 %) study participants had died, most of whom had died within the first week of admission to hospital, 431 (69.9 %) had been cured, and the rest were defaulted and transferred out. The mean survival of the children with HIV/AIDS, pneumonia, diarrhea, dehydration, and those who took intravenous antibiotics and fluid were significantly lower than that of their counter parts. The significant predictors of the mortality of the SAM children were loss of appetite (AHR=2.75; 95%CI: 1.08, 6.99), malaria (AHR=12.69, 95%CI: 4.57, 35.27), lower Waist for Height % (WFH %) (AHR=0.95, 95%CI: 0.9, 0.99), and HIV sero-positivity (AHR=11.57, 95%CI: 2.34, 57.2). Also, not taking Formula-100 (F-100) (AHR=3.26; 95%CI: 1.32, 8.07) and Formula-75 (F-75) (AHR=2.56, 95%CI: 1.06, 6.15) significantly increased the risk of death.

Conclusion: The level of the mortality was high. Presence of co-morbidities, intravenous fluid and antibiotics intake and not supplemented with nutritional therapy significantly increased the risk of death. Therefore, an appropriate nutritional therapy and management of co-morbidities as per the national SAM management protocol is recommended.

Key words: *Survival status, Predictors, Mortality, Severe Acute Malnutrition*

Introduction

Worldwide around 52 million under five children were acutely malnourished, of whom 13.2 million and 27.8 million are in sub-Saharan Africa and South Asia, respectively. It is also estimated that 25-35 million under five children are severely malnourished, and the severe form of the acute malnutrition is responsible for one million under five children deaths every year (Hobbs and Bush, 2014).

Children who are malnourished are at nine fold increased risk of death than well-nourished ones (Keane,

2013), and this is 5–20 fold for children with low weight-for-height (WFH), or mid-upper arm circumference (MUAC) (WHO, 2013). The mortality is high in sub-Sahara Africa due to the limited access to effective and timely treatment. In 2012, about 2.7 million children were admitted to hospitals for Severe Acute Malnutrition (SAM) treatment, but higher mortality and dropout rates were major problems (Hobbs and Bush, 2014). In Malawi, from 2006 to 2007, a mortality rate ranging from 2.2% to 59% was reported among children treated in

stabilization center (Kerac *et al.*, 2014; Maurya *et al.,* 2014). In Ethiopia, this ranges 6% to 29% (Tefera *et al.*, 2014; Kebede, 2015).

Cognizing all these, the World Health Organization (WHO) has developed a standard SAM management protocol, which is being adopted by many countries in which non-adherence results in greater mortality (WHO, 2013). Children with SAM are predisposed to serious infections like diarrhea, pneumonia, skin infection, gut bacterial overgrowth, and others. In stabilization centers, about 15% of SAM patients require enthusiastic treatment (Bhutta *et al.*, 2013; FMOH, 2013), which includes an integrated management of both nutritional deficiency and co-morbidities (Hobbs and Bush, 2014).

The implementation of the protocol reduced the mortality from 55% in 2008 to below 20 % in 2013. Formula milk or Ready to Use Therapeutic Food (RUTF) has been an important tool (WHO, 2013), as it results in rapid weight gain, recovery, and short hospital stay (Kapil, 2009). In 2013, however, the rate was unacceptably high (35%) in global situation, (Bhutta *et al.,* 2013). In Africa, the mortality was above 5%, and it also reaches 34% especially for complicated SAM based on perspective writing (Heikens, 2007).

Different co-morbidities, treatment related factors, non-adherence to the management protocol, and initial anthropometric indices are some of the factors that determine mortality from SAM (Heikens, 2007). Among infections, pneumonia, TB, HIV, diarrhea and others increase mortality among children admitted with complicated SAM (Irena *et al.*, 2011; Habtemu *et al.*, 2015).

The effectiveness of the treatment is evaluated based on recovery and death rate, average length of stay, and weight gain. Thus the acceptable level of mortality, the recovery, and default rate are below 10%, above 75% and below 15%, respectively (Thurstans, 2011). But these parameters are not achieved in many developing countries (Bhutta *et al.*, 2013).

Although some studies have assessed the mortality rate and its predictors among children with SAM admitted to hospital (Ephrem, 2010; Melaku *et al.,* 2014; Habtemu *et al.*, 2015; Kebede, 2015), most of them focused on children above six months, dealt with small sample size, and hardly showed the effects of some factors clearly (Melaku *et al.,* 2014). Moreover, as the factors vary according to context, this study assessed the mortality rate and its predictors among 0-59 months children treated for SAM in Dilchora Referral Hospital from 2011 to 2015.

Methods and Materials
Study Area, Design and Period
This study was conducted in Dilchora Referral Hospital, Dire Dawa, Eastern Ethiopia. Dire Dawa is 515 km away from Addis Ababa, capital city of Ethiopia. The city administration has population of 453, 000 (227, 000 male and 226,000 female) with 100 % health service coverage (CSA, 2013). The city has two government and four private hospitals, five higher and twelve medium private clinics, and 15 health centers and 31 health posts. In Dilchora Referral Hospital, there is a stabilization center which treats children with complicated SAM. Institution based a retrospective cohort study was conducted. The data were collected from March 07 to 16, 2016.

Study Population and Sampling Technique
The source population was all the records of 0-59 months children with SAM admitted in Dilchora Referral Hospital stabilization center. All eligible records of 0-59 months children with SAM admitted to the center from September 2011 to August 2015 were the study population. Records with incomplete data on outcome variable and with heart failure secondary to congenital heart disease were excluded from study.

The total sample size to detect the association between predictors and time to death was 631. Sample size was calculated using Stata version 13 (Stata corp., STATA 13.0 for window) for comparing survival Cox model between children not supplemented with Vitamin A in reference to Vitamin A supplemented (AHR=1.53) (Kebede, 2015). It was calculated taking two sided significant level (α) of 5%, 80% power, HR=1.53, probability of event of interest as 0.29 (Kebede, 2015). Comparing for the first and second objective, the final sample size became 631. OpenEpi software version 2.3 was used to generate random numbers. First serial number or unique SAM number was retrieved from patient registry.

Data Collection Method
The data were collected from the medical records and SAM treatment registry with a structured and cross checked data abstraction format. Diploma graduate nurses collected the data. A supervisor and the principal investigator took charge of the process.

Data Quality Control
To assure data quality, the checklist was cross-checked with the registers and cards. Recruited diploma nurses were trained for one day on how to extract the data from patient registry. The data were checked daily by the

supervisor and the principal investigator for completeness and consistency. The data were also double entered.

Data Processing and Analysis

The dependent variable was survival status/mortality. Whereas the independent variables were demographic characteristics (Age, sex of child, place of residence), appetite, hypothermia, presence of nutritional edema and clinical form of malnutrition, co-morbidity (pneumonia, HIV sero-status, diarrhea, dehydration status, hemoglobin level, malaria), routine medication and treatment (intravenous (IV) fluid intake, IV antibiotic treatment, blood transfusion, folic acid and Vitamin A supplementation, Formula-100 (F-100) and Formula-75 (F-75) intake, and anthropometric measurements at admission (WFH and MUAC).

In this study, recovered (cured) was used when the child reached ≥85% of median WFH or WFH Z- score ≥-2 or discharged cure by hospital according to protocol or child who fulfill discharge criteria (FMOH, 2013). Whereas censored observations were defined as those SAM children who were defaulted, transferred, recovered or non-responded. Anemia and severe anemia were defined with hemoglobin level below 11gm/dl (hematocrit level less than 33%) and 4gm/dl (hematocrit level less than 12%) at admission, respectively (FMOH, 2013).

The cleaned and checked data were entered into EpiData software Version 3.02. Then the data were exported to SPSS Version 20 for analysis. Survival curve was used to display the survival (time to death) among different characteristics. The Cox proportional hazard model with both binary and multivariate analysis was done. Associations were declared as statistically significant with p<0.05.

Ethical Considerations

The proposal of the study was reviewed by the Institutional Health Research Ethics Review Committee (IHRERC) of Haramaya University, College of Health and Medical Sciences. Informed written and signed consent was taken from the hospital manager and the confidentiality of the patients' records was kept. To prevent loss of patients' cards during the data collection process, the cards were immediately returned to the card room after taking the necessary data.

Results

Socio-demographic and Admission Characteristics

Out of the 631 randomly selected medical records of study participants, 617 were retrieved with card retrieval rate of 97.8%. A cohort of 617 SAM children were followed retrospectively for median time of seven days with interquartile range of nine days. Three hundred forty five (55.9%) were male, and 340 (55.2%) came from urban area. Their mean age was 23.5 months, with 14.7 standard deviation (SD), and 313 (50.7 %) of them were between 12 and 36 months. Among those who had recorded appetite test, 263 (65.1 %) had failed appetite. At admission, 325 (52.8%) were with edematous malnutrition. The rest of 281 (45.5 %), 50 (8.1%), and 286 (46.4%) were with kwashiorkor, Marasmic-Kwashiorkor, and Marasmus, respectively (Table 1). The average WFH-Z score was -4.05, with 1.9 unit of SD.

Table 1. Socio-demographic and admission of children with SAM admitted to Dilchora Referral Hospital, Dire Dawa, Ethiopia, 2016.

Characteristics		Freq.	%
Sex (n=617)	Male	345	55.9
	Female	272	44.1
Age (in months) (n=617)	0-6	29	4.7
	6-11	99	16.0
	12-23	166	26.9
	24-35	147	23.8
	36-47	90	14.6
	48-59	86	13.9
Residence (n=616)	Rural	276	44.8
	Urban	340	55.2
Appetite test (n=404)	Failed test	263	65.1
	Passed test	141	34.9
Nutritional edema (n=616)	Yes	325	52.8
	No	291	47.2
WFH % (n=579)	<70 %	161	27.8
	70-79.9 %	204	35.2
	≥80 %	214	37.0
Diagnosis (n=617)	Marasmic-kwashiorkor	50	8.1
	kwashiorkor	281	45.5
	Marasmus	286	46.4

As indicated in the table, 256 (41.5%) diarrhea and 134 (21.8%) pneumonia were the commonest co-morbidities among the children. Dehydration, Tuberculosis, HIV/AIDS, and Malaria were also observed in 110 (17.8%), 19 (3.1%), 18 (2.9%) and 16 (2.6%) of children, respectively (Table 2).

With regard to routine medication and nutritional therapy, 161 (26.2%) children had taken IV antibiotics, and 182 (29.5%) had taken IV fluid therapy. Three hundred eighty seven (62.8%) and 379 (61.5%) had received folic acid and vitamin A supplementation, respectively. Five hundred nine (83.6%) had received F-75 nutritional therapy, whereas 289 (49.7%) and 100 (17.2%) had received F-100 and Plumpy'nut, respectively (Table 3).

Table 2. Major co-morbidities among severely acute malnourished children in Dilchora Referral Hospital, Dire Dawa, Ethiopia, 2016

Characteristics		Freq.	%
HIVsero-status (n=617)	Positive	18	2.9
	Negative	416	67.4
	Unknown	183	29.7
TB (n=616)	Yes	19	3.1
	No	597	96.9
Pneumonia (n=616)	Yes	134	21.8
	No	482	78.2
Diarrhea (n=617)	Yes	256	41.5
	No	361	58.5
Dehydration (n=617)	Yes	110	17.8
	No	507	82.2
Malaria (n=617)	Yes	16	2.6
	No	306	49.6
	Unknown	295	47.8

Table 3. Patterns of routine medication and nutritional therapy intake among severely acute malnourished children admitted in Dilchora Referral Hospital, Dire Dawa, Ethiopia, 2016.

Variables		Freq.	%
IV antibiotics (n=615)	Yes	161	26.2
	No	454	73.8
Blood transfusion (n=616)	Yes	34	5.5
	No	582	94.5
IV fluid (n=616)	Yes	182	29.5
	No	434	70.5
Folic acid supplementation(n=616)	Yes	387	62.8
	No	229	37.2
Vitamin A Supplementation (n=616)	Yes	379	61.5
	No	237	38.5
F-100 intake (n=581)	Yes	289	49.7
	No	292	50.3
F-75 intake (n=609)	Yes	509	83.6
	No	100	16.4
Plumpy'nut intake (n=580)	Yes	100	17.2
	No	480	82.8

Mortality and Survival of SAM Children

With regard to the treatment outcome of the children, 47 (7.6%) died, 431 (69.9%) were cured and discharged, 47 (7.6%) were transferred out, and 88 (14.3%) were defaulters. The person time at risk for the children was 6267 days, with cumulative incidence of 0.0075 deaths per person days (7.5 deaths/1000 person days). The median of waiting time in the hospital was 7 days, with minimum and maximum hospital stay of 1 and 81 days (Inter Quartile Range (IQR) =9 days), respectively. The Survival patterns of children were dropped early in the first weeks. Most of the deaths (85%) occurred within the first ten days of the admission (Figure 1). The average length of stay in the hospital was 10 days (SD±10.4). The average rate of weight gain was 15.6g/kg/day, with lower rate of 3.4g/kg/day and higher rate of 30g/kg/day for edematous and non-edematous malnutrition, respectively. The mean survival time for the cohort was 69.28 days with median survival time of 80 days.

Those with failed appetite had lower mean survival time (57.43 days) than those with passed appetite (72.54 days) (p-value of log rank = 0.105). Additionally, the SAM children with HIV/AIDS had significantly shorter survival time than the sero-negative ones. Children with co-morbidities (HIV/AIDS, pneumonia, diarrhea and dehydration) had significantly shorter mean survival time than their counter parts.

Predictors of Mortality among SAM Children

Bivariate analysis showed that edematous malnutrition was associated with a non-significant decrease in the risk of death from SAM, one centimeter increase in WFH% index was associated with 5% significant decrease in the risk (CHR=0.95, 95%CI:0.92, 0.98). HIV sero-positivity (CHR=3.39, 95%CI: 1.22, 9.44) and pneumonia (CHR=2.43, 95%CI: 1.36, 4.35) were significantly associated with risk of death in time from SAM. Additionally, IV antibiotic medication intake also significantly increased the risk of death (CHR=2.39, 95%CI: 1.35, 4.25) (Table 4). Those who did not get F-75 had twice increased risk of death from SAM at time than those who took it (CHR=2.10, 95%CI: 1.09, 4.07).
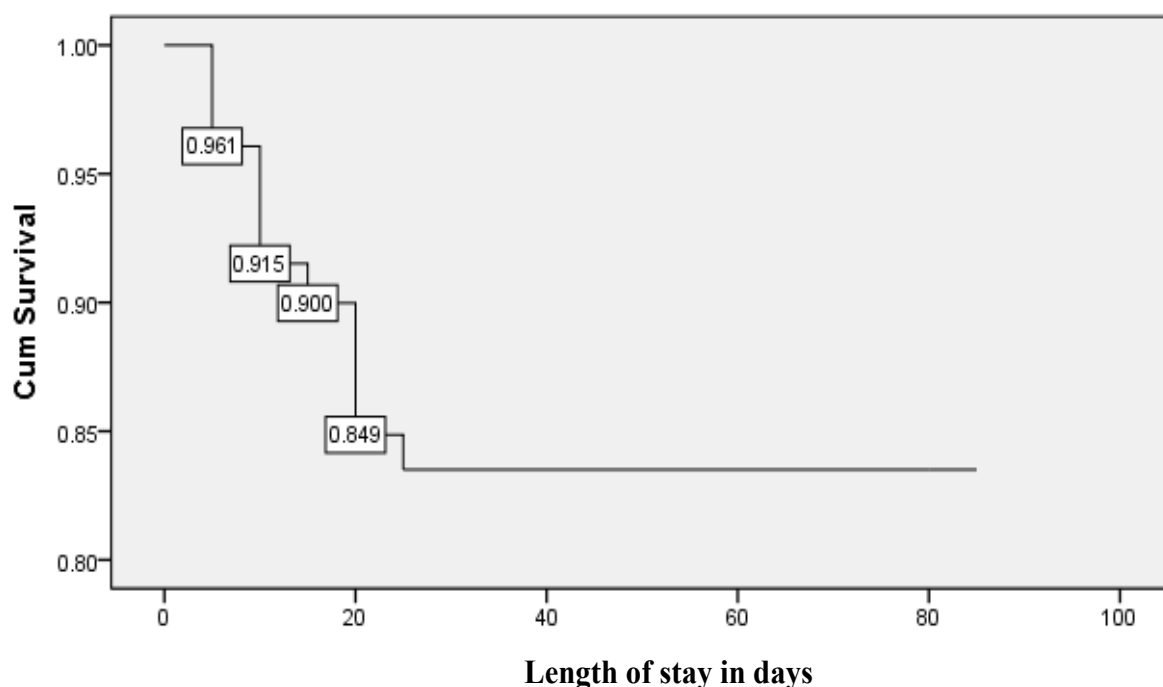

Figure 1. Survival functions of admitted SAM children from admission to death or other outcomes in Dilchora Referral Hospital, Eastern Ethiopia, 2016.

Table 4. Binary Cox proportional hazard Model for predictors of mortality from SAM among children admitted in Dilchora Referral Hospital, Dire Dawa, Ethiopia, 2016.

Variables		Outcome		CHR (95%CI)	AHR (95%CI)
		Dead Freq. (%)	Censored Freq. (%)		
Age	<12 months	9 (7.0)	119 (93.0)	1	
	≥12 months	38 (7.8)	451 (92.2)	1.04 (0.50 - 2.14)	
Sex	Male	27 (7.8)	318 (92.2)	1.12 (0.63 - 2.00)	
	Female	20 (7.4)	252 (92.6)	1	
Family residence	Rural	17 (6.2)	259 (95.8)	0.72 (0.40 - 1.30)	
	Urban	30 (8.8)	310 (91.2)	1	
Appetite test	Failed appetite	22 (8.4)	241 (91.6)	1.99 (0.85 - 4.67)*	2.75 (1.08 -6.99)*
	Passed appetite	7 (4.0)	134 (96.0)	1	1
MUAC	Per 1 cm increase			0.88 (0.72 - 1.09)	
WFH %	Per 1 % increase			0.95 (0.92 - 0.98)*	0.95 (0.90 -0.99)*
Edema	Yes	23 (7.1)	302 (92.9)	0.73 (0.41 - 1.30)	
	No	24 (8.2)	267 (91.8)	1	
Diagnosis	Marasmic-Kwashiorkor	5 (10.0)	45 (90.0)	0.93 (0.35 - 2.42)	
	Kwashiorkor	17 (6.0)	264 (94.0)	0.61 (0.33 - 1.13)	
	Marasmus	25 (8.7)	261 (91.3)	1	
HIV sero-status	Yes	4 (22.2)	14 (77.8)	3.39 (1.22 - 9.44)*	11.57 (2.34-57.22)*
	No	43 (7.2)	556 (92.8)	1	1
Tuberculosis	Yes	1 (5.3)	18 (94.7)	0.84 (0.12 - 6.07)	
	No	46 (7.7)	551 (92.3)	1	
Pneumonia	Yes	19 (14.2)	115 (85.8)	2.43 (1.36 - 4.35)*	
	No	28 (5.8)	454 (94.2)	1	
Diarrhea	Yes	29 (11.3)	227 (88.7)	2.38 (1.32 - 4.28)*	2.52 (0.93 - 6.83)
	No	18 (5.0)	343 (95.0)	1	1
Dehydration	Yes	17 (15.5)	93 (84.5)	3.07 (1.69 - 5.60)*	
	No	30 (5.9)	477 (94.1)	1	
Malaria	Yes	7 (43.8)	9 (56.3)	6.10 (2.73 - 13.66)*	12.7 (4.57 - 35.27)*
	No	40 (6.7)	561(93.3)	1	1
Anemia (Hgb <11g/dl)	Anemic	17 (10.2)	150 (89.8)	1.28 (0.38 - 4.39)	
	Non anemic	3 (8.6)	32 (91.4)	1	
IV antibiotics	Yes	22 (13.7)	139 (86.3)	2.39 (1.35 - 4.25)*	
	No	25 (5.5)	429 (94.5)	1	
Blood transfusion	Yes	5 (14.7)	29 (85.3)	2.06 (0.81 - 5.20)	
	No	42 (7.2)	540 (92.8)	1	
IV fluid intake	Yes	19 (8.1)	163 (83.6)	1.48 (0.82-2.65)	
	No	28 (6.5)	406 (93.5)	1	
Folic acid supplementation	Yes	24 (6.2)	363 (93.8)	1	
	No	22 (9.6)	207 (90.4)	1.56 (0.87 - 2.78)	
Vitamin A	Yes	27 (7.1)	352 (92.9)	1	
	No	19 (8.0)	218 (92.0)	1.10 (0.61 - 1.98)	
F-100 intake	Yes	19 (6.6)	270 (93.4)	1	1
	No	27 (9.2)	265 (90.8)	1.79 (0.99 - 3.22)*	3.26 (1.32 - 8.07)*
F-75 intake	Yes	34 (6.7)	475 (93.3)	1	1
	No	12 (12.0)	88 (88.0)	2.10 (1.09 - 4.07)*	2.56 (1.06 - 6.15)*
Plumpy'nut intake	Yes	5 (5)	95 (95.0)	1	
	No	39 (8.1)	441 (91.9)	1.92 (0.76 - 4.88)	

*Note: * p<0.05*

After adjusting for confounders through step wise backward Cox regression method, the children with failed appetite had 2.7 times increased risk of death from SAM after admission (AHR=2.75, 95%CI: 1.08, 6.99). Sero-positivity for HIV significantly increased the risk of death from SAM by 11 fold than non-reactive for HIV test (AHR=11.57, 95%CI: 2.34, 57.22). Those infected with malaria were twelve times more likely to die than those without malaria (AHR=12.70, 95%CI: 4.57, 35.27). Those who were not treated with F-100 (AHR=3.26, 95%CI: 1.32, 8.07) and F-75 (AHR=2.56, 95%CI: 1.06, 6.15) were more likely to die than their counterparts (p< 0.05) (Table 4).

Discussion

This study revealed that 7.6% had died, 69.9% had been cured, 14.3% had been defaulters and 8.2% had been transferred out and non-responders from treatment in therapeutic feeding center. The mean survival time for the cohort of SAM children was 69.28 days, with median survival time of 80 days. The level of mortality and the measure of the effectiveness of other treatments are acceptable, except the slightly lower cure rate (Thurstans, 2011). The mortality rate is a bit higher than the one reported from Jimma (6%) (Habtemu *et al.,* 2015), but lower than the ones found in Tigray (12%) (Melaku *et al.,* 2014) and in Sekota (29%) (Kebede, 2015). The humanitarian charter standards set the mortality rate below 10%, cure rate above 75%, and default rate below 15% (Thurstans, 2011). In this study, the result showed acceptably short length of hospital stay and acceptable rate of weight gain.

The mean survival time was higher than the mean reported from Sekota (10 days) (Kebede, 2015). This difference might be due to the higher number of actual death and lower censored cases which decreased the mean of survival time. Like similar findings in Uganda (Bachou *et al.,* 2006) and in Mekele (Melaku *et al.,* 2014), in this study most of the deaths occurred within the first week of admission to Therapeutic Feeding Unit (TFU).

In the current study, SAM children with pneumonia, HIV/AIDS, diarrhea, and dehydration had significantly shorter mean survival time than their counterparts. HIV/AIDS is associated with increased risk of other acute and chronic opportunistic infections which aggravate the risk of death. Not taking F-100 and F-75 was associated with significantly shorter survival time. This result is supported by the fact that the nutritional therapies are particularly suited for SAM children for reduced metabolic state (WHO, 2013).

This study also showed that failed appetite, HIV/AIDS, malaria, and not taking F-75 were significant predictors of mortality from SAM in the hospital. Loss of appetite (FMOH, 2013) is a manifestation of disease in SAM and associated with other co-morbidities which increase the severity of SAM patient's condition. But IV fluid, IV antibiotics intake, diarrhea and dehydration have not reached statistical significance level. However, other studies showed that Diarrhea, IV antibiotics and IV fluid intake were significantly associated with the risk of the death. (Munthali *et al.,* 2015; Bachou *et al.,* 2006; Melaku *et al.,* 2014; Habtemu *et al.,* 2015)

In contrast, unlike results from Kebede (2015), in the current study, routine medical therapy increased the risk of death from SAM. In our study, for each increase in anthropometric indices (WFH % and MUAC) there was a decrease in mortality from SAM which is similar with findings from Malawi, although the effect is higher in the latter (Kerac *et al.,* 2014). It is probably because as the child's nutritional status deteriorates, there is an increase in co-morbidity and other physiological stresses which increase mortality (Kapil and Sachdev, 2010).

Additionally this study showed that those who did not take F-75 intake are at higher risks of death than who took F-75. It is associated with the fact that F-75, which decreases the risks of fluid overload and other related abnormalities, is best suited for the reduced physiological capacity of SAM children and reduces the mortality rate (Bhutta *et al.,* 2013; FMOH, 2013). Appropriate nutritional therapy in conjunction with the national SAM management protocol promotes early recovery and decreases the risk of death.

Missed data, like bottle feeding and breast feeding histories and inconsistency of patient medical records, together with the absence of the actual outcome of defaulters, were some of the limitations of the present study.

Conclusion and Recommendations

In this study there was substantial level of mortality among the children admitted with SAM. Dehydration and malaria were the common medical complications affecting SAM children. Malaria, HIV status, failed appetite, not taking F-100 and F-75 were identified as the independent predictors of the death due to SAM.

This study showed substantial level of mortality, and also identified some treatment and morbidity related factors for increased risk of deaths among SAM children. Thus Dilchora Referral Hospital, in collaboration with

other NGOs and concerned bodies, should put an effort to improve the outcome in terms of mortality, recovery rate and default rate. There should be strengthened efforts to decrease mortality as a result of SAM from current level to below 5%.

Further researches should be conducted on the level of the implementation of the national protocol with especial focus on using the new WHO growth standard, as it will identify SAM children early in order to enhance child survival below the current level. In addition, the adherence level of patients to the application of the treatment protocol should be studied.

Acknowledgment

Conflict of Interests

The authors declare that there is no conflict of interest.

Authors' Contributions

AO participated from inception of idea, proposal development, data collection, analysis and final write up. FM and MD has participated in the amendment of proposal and the write up of final result. All authors approved the final manuscript.

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Level of Adherence and Factors Associated with Antiretroviral Therapy among HIV Infected Children in Selected Public Hospitals, Addis Ababa, Ethiopia

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Abstract

Background: Non-adherence to Antiretroviral Therapy (ART) has serious consequences both on the individual and the society. It diminishes the effectiveness of viral suppression, promotes viral resistance, and places the individual at risk of hospitalization, opportunistic infection, and HIV transmission.

Objective: To assess the level of adherence and factors associated with Antiretroviral Therapy among HIV infected children in selected public hospitals, Addis Ababa, Ethiopia.

Methods: A hospital based cross-sectional study was conducted on 380 under-fifteen years old children who had been taking ART for at least 6 months, and on their respective caregivers. It was conducted at pediatrics ART units of the selected hospitals from January to March, 2014. From five public hospitals that were giving the service, two were selected through simple random sampling technique. The data were entered into Epi Info 3.5.3 and analyzed using SPSS Version 20. The statistical association between the outcome and predictor variables were assessed by logistic regression model, using adjusted Odds Ratio with its 95% CI. P-value < 0.05 was considered statistically significant

Results: As 380 of the caregivers of the children were interviewed, a response rate of 95% was obtained. The level of adherence to ART was 355 (93.4%). The main reason for 7(28%) of the non-adherent participants skipping their treatment was forgetting. The factors that had statistical association with the adherence were the caregivers' with secondary and above educational status (AOR=4.17, 95%CI: 1.30, 13.32); the Child's age <5years (AOR=11.24, 95%CI: 1.32, 95.45); lack of nutritional support (AOR=0.40, 95%CI: 0.005, 0.308); and the children who were aware of their HIV sero-status (AOR=4.26, 95%CI: 1.48, 12.18).

Conclusion: The level of adherence to ART in the study area was suboptimal. The caregivers' educational status, the age of the children, lack of nutritional support, and non-disclosure sero-status were associated with non-adherence to ART. Since forgetting was the main reason reported for missing ART doses, use of reminders should be encouraged. The caregivers should inform the children about their sero-status. Additionally, the caregivers and the clinicians should look for various means to meet the nutritional requirements of the children. In general, a collaborative effort of all stakeholders is recommended to enhance adherence to ART among HIV infected children.

Key Words: *Adherence, ART, Care giver, HIV/AIDS*

Introduction

HIV has always been a major health problem globally. In 2014 in sub-Saharan Africa, about 25.8 million people lived with the virus. In 2015, UNAIDS reported that among the children who were less than 15 years, 190,000 were newly infected ones (UNAIDS, 2015; WHO, UNICEF, UNAIDS, 2011). In the same year, there were 123,550 HIV infected children in Ethiopia, of whom 58,806 needed ART (AIDS Resource Center, 2015).

Adherence to ART is as important as access to it (CSA, 2011, 2012). To fully realize the therapy's life-extending benefits (Starace *et al.*, 2006), more than 95%, which is consistent and nearly perfect, adherence is recommended (Heyer *et al.*, 2006). Poor adherence to ART diminishes the effectiveness of viral suppression, promotes viral resistance, and places the People Living with HIV (PLHIV) at risks of hospitalization,

opportunistic infection, and HIV transmission (Mohammadpour *et al.*, 2010).

However, a child's adherence can be influenced by many factors which can be categorized as characteristics of the child, the caregiver, the family, and the regimen. Many of these complicate the measurement of pediatric adherence, as well (Haberer *et al.*, 2009; Ugwu *et al.*, 2013). Unlike adults, children rely upon their caregivers for their medicine. The caregivers may lack commitment or be busy with other activities (WHO, 2006). Poor palatability, lack of fixed dose, and side effects of the drugs hinder the children to take optimal doses (Federal Ministry of Health, 2008). Furthermore, parents' death and their unwillingness to disclose the status also limit the involvement of others into the care (Osterberg *et al.*, 2005).

Several studies have been conducted to determine the level of adherence and its associated factors. Studies conducted in South Africa and Nigeria reported that 94% and 86% of the children were adherent to ART, respectively (Davies *et al.*, 2015; Iroha *et al.*, 2010). A study in Addis Ababa also revealed that 93.3% of the children had 95% adherence level (Biressaw *et al.*, 2013). In Ethiopia, little has been known about the pediatric adherence to ART and its associated factors. There are also inconsistencies among the findings.

Therefore, this study tried to assess the level of adherence and factors associated with ART among HIV infected children in selected public hospitals, Addis Ababa, Ethiopia. Its findings would contribute to the body of knowledge in the problem area.

Methods and Materials

Study area, Design, and Period
A hospital based cross-sectional study was carried out in Addis Ababa, the capital city of Ethiopia. About 3 million population live in the city (CSA, 2015). In Addis Ababa City Administration, 9 public and 18 private hospitals; 24 public health centers and 3 private clinics deliver HIV care. However, only 5 of the public hospitals provide pediatric Antiretroviral Treatment (ART) services (AIDS Resource Center, 2015). For this study, two public hospitals, Saint Peter TB Specialized Hospital and Yekatit 12 Teaching Hospital, were randomly selected. Saint Peter TB Specialized Hospital provides care and treatment for patients with TB, Multi Drug Resistant Tuberculosis (MDR-TB), and HIV. This study was conducted from January to March, 2014, among under 15 years of age who were enrolled in ART program in both hospitals.

Study Participants
The study participants were under-fifteen years of age children living with HIV and taking ART for at least 6 months in pediatrics ART units of the selected hospitals during the study period. Caregivers of the children who were below 6 months of age and who were seriously ill were excluded. The sample size was determined using single population proportion formula. The final calculated sample size was 401.

Data Collection Procedure
Data were collected with an interviewer guided structured questionnaire adopted from different literatures and pre-tested on 10% of the children taking ART in Alert Hospital, Addis Ababa. Initially, it was prepared in English and then translated into Amharic by language experts. The data collectors were trained for two days about data quality and interviewing techniques. Two BSc. nurses conducted the interview in private rooms nearby the ART units. The principal investigator checked the data for completeness on daily basis.

Data Processing and Analysis
The outcome variable was adherence to ART, and adherence was defined as taking more than 95% of the prescribed doses; no dose of pill or suspension missed or delayed for more than or equal to 90 minutes in the past seven days (Markos *et al.*, 2016). The independent variables includes socio-demographic characteristics (Age, sex, ethnicity, educational status, marital status, and relationship of care giver with child, parents' vital status) of the caregivers, who were persons in charge of routinely administering antiretroviral drugs to the enrolled children. Moreover, the independent variable included the children's age and sex, the duration on ART, the recommended regimen, the CD4 count, the WHO clinical staging, the opportunistic infections' (OI's) prophylaxis, the nutritional support, and the disclosure status of the children.

The data were coded and entered into Epi Info 3.5.3 and analyzed using SPSS Version 20. Descriptive statistics were computed and presented in tables and graphs. Logistic regression model was used to determine the statistical association between the outcome and the predictor variables using adjusted Odds Ratio (OR) with 95%C.I. P-value<0.05 was considered as a statistically significant association.

Ethical Considerations

The study was reviewed and approved by Institutional Health Research Ethics Review Committee (IHRERC) of Haramaya University, College of Health and Medical Sciences. Data collectors were ensured assent and consent from children and caregivers, respectively.

Results

Socio-demographic Characteristics of Caregivers and Children

Three hundred eighty caregivers of children on ART were interviewed, which was a 95% response rate, and their mean age was 39 (SD±12). One hundred thirty two (24.7%) of the caregivers belonged to Amhara ethnic group, followed by Oromo 76(20%). Out of all caregivers, 109 (28.7%) were unable to read and write. Two third of the children, 254 (66.8%), were male. More than half of them (52.2%) were between 5 and 9 years of age, and the mean age was 8 years (SD±3) (Table 1).

Clinical Markers and Treatment Related Characteristics

During the clinical record review, the CD4 count of 168 (44.2%) children ranged from 200 to 499 cells/mm^3, 221 (58.2%) were in stage I of WHO clinical classification at base line, 113 (29.7%) were receiving fixed dose regimen of AZT-3TC-EFV (Zidovudine-Lamivudine-Efavirenz) and more than three fourth, 289(76.1%), were taking prophylactic drugs for opportunistic infections (OIs). Nearly half of the children, 180 (47.4%), received nutritional support from the clinics, and only 60 (15.8%) of the children were aware of their HIV sero-status (Table 2).

Level of Adherence

The study found out that 355 (93.4%) of the children were adherent to ART: they missed no dose or not delayed it for more than or equal to 90 minutes. Only 25 (6.6%) of the children missed doses of their drugs in the last 7 days of the study period. The main reason for 7 (28%) of the non-adherent participants skipping their treatment was forgetting.

Table 1. Socio-demographic characteristics of caregivers and HIV infected children on ART in Saint Peter TB Specialized and Yekatit 12 Teaching Hospitals, Addis Ababa, Ethiopia, 2014.

Characteristics		Frequency	%
Caregivers age (in years)	18-40	192	50.5
	41-59	155	40.8
	>60	33	8.7
Sex	Male	180	47.4
	Female	200	52.6
Educational status	Unable to read and write	109	28.7
	Primary	98	25.8
	Secondary & above	173	45.5
Marital status	Single	51	13.4
	Married	254	66.8
	Divorced/Separated/Widowed	75	19.8
Ethnicity	Amhara	132	24.7
	Oromo	76	20.0
	Gurage	55	14.5
	Tigre	33	8.70
	Others	84	22.1
Monthly income (in USD)	<24.51	81	21.3
	24.56-73.54	159	41.8
	73.59-122.57	98	25.8
	>122.62	42	11.1
Kinship to child	Mother	123	32.4
	Father	96	25.3
	Grandmother/father	61	16.1
	Others	100	26.2
Child age (in years)	< 5	97	25.5
	5-9	198	52.2
	10-14	85	22.4
Child sex	Male	254	66.8
	Female	126	33.2

Table 2. Clinical markers and treatment related characteristics of HIV infected children on ART in Saint Peter TB Specialized and Yekatit 12 Teaching Hospitals, Addis Ababa, Ethiopia, 2014.

Variable		Frequency	%
CD4 count/ mm³ (Recent)	< 200	80	21.1
	200-499	132	34.7
	>=500	168	44.2
WHO Clinical stage(Base line)	Stage I	221	58.2
	Stage II	132	34.7
	Stage III	27	7.1
ART used (Fixed dose)	ABC-3TC-EFV	93	24.5
	AZT-3TC-EFV	113	29.7
	AZT-3TC-NVP	22	5.8
	TDF-3TC-NVP	95	25.0
	Others	57	15.0
OIs prophylaxis (during interview)	Yes	289	76.1
	No	91	23.9
Duration on ART (month)	6-12	189	49.7
	13-24	108	28.4
	>25	83	21.8
Nutritional support	Received	180	47.4
	Not received	200	52.6
Aware of their HIV sero-status	Yes	60	15.8
	No	320	84.2

Note: OI: Opportunistic infection; ART: Antiretroviral Therapy; ABC: abacavir, 3TC: lamivudine, AZT: Zidovudine, EFV: efavirenz, NVP: nevirapine, TDF: tenofovir

Predictors of Pediatrics Adherence to ART

In this study, the children below 5 years were more likely adherent (AOR=11.24, 95%CI: 1.32, 95.45) than those above 10 years. The children with caregivers who attended secondary education and above were also more likely adherent (AOR= 4.17, 95% CI: 1.31, 13.32) than those who were illiterate. Lack of nutritional support decreased adherence to ART by 60% (AOR=0.40, 95%CI: 0.01, 0.31) than those children received nutritional support. Additionally, children who were aware of their sero-status were more likely to be adhered (AOR=4.26, 95%CI: 1.49, 12.18) to ART compared with their counter parts (Table 3).

Besides, further investigations will be required to straighten out these contradictory results.

The current study revealed that lack of nutritional support decreased adherence to ART by 60%. The World Bank reported that nutritional interventions can optimize the benefits of ART and may increase compliance with treatment regimens (World Bank, 2008). A study in Uganda also reported that lack of nutrition was a significant barrier of adherence (Kitaka *et al.*, 2007). Provision of food and micronutrients improves outcomes (Fawzi *et al.*, 2004; Ndekha *et al.*, 2005; Villamor *et al.*, 2005). Additionally, Biadgilign *et al.* (2008) reported that those children received nutritional support from the clinic (66.3%) showed poor adherence. Therefore, the issue of nutritional support needs further exploration.

In this study, adherence to ART decreased with an increase in age of the children. A study done in Gonder also reported a similar finding (Dachew *et al.*, 2014). This might be due to fear of stigma. Therefore, encouraging disclosure with specific support might be central to increase children's adherence as they get older.

This study had some limitations. The use of caregivers' report of adherence to ART might lead to over estimation. Using a single method to assess adherence could not yield more reliable results. The social desirability and recall biases might not be completely eliminated. Besides, small sample size had shown some limited effect on the model we used.

Conclusion and Recommendations

The level of adherence to ART in the study area was suboptimal. It is comparable to other studies conducted in Ethiopia and other developing countries. Forgetting was the main reason reported for missing ART doses. The caregivers' educational status, the age of the children, lack of nutritional support, and non-disclosure sero-status were the statistically significant factors that showed association with non-adherence to ART.

Therefore, disclosure of HIV status to children is so controversial that further study are required. However, encouraging caregivers might be essential to disclose the HIV status to the children and use of reminder to minimize forgetting of caregivers to give ART. Case managers should link the caregivers with organizations that provide nutritional support. It is also worth mentioning that the caregivers should search for other means to meet the nutritional requirements of the children. Furthermore, a collaborative effort is recommended to enhance adherence to Antiretroviral Therapy among HIV infected children.

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Conflict of Interests
The authors declare that they have no competing interests.

Authors' Contributions
BF carried out the study starting from conception to analysis and interpretation of data. SL participated in topic selection, data analysis, and interpretation and manuscript writing. AK participated in topic selection, reviewing the proposal, data analysis and commented on drafted manuscript. All authors read and approved the final draft of the manuscript.

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Knowledge of Women about Tuberculosis and the Role of Health Extension Workers among Households in East Hararghe Zone, Ethiopia

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Abstract

Background: Tuberculosis (TB) is still one of the major public health problems in Ethiopia. This is mainly due to lack of knowledge about its cause, mode of transmission, symptom, and appropriate treatment, which in turn affect the health seeking behavior. Little is known about the women's knowledge of TB and Health Extension Workers (HEWs) contribution on information dissemination about TB.

Objective: To assess knowledge of women about tuberculosis and the role of Health Extension Workers among households in East Hararghe Zone, Eastern Ethiopia.

Methods: A community based cross-sectional quantitative study was conducted from February to March, 2014 on 2,319 households selected by multistage stratified sampling technique from six districts in East Hararghe Zone. Twelve trained data collectors collected the data from household women using a structured questionnaire. Data were analyzed using descriptive statistics and predictors were identified with logistic regression.

Results: One thousand six hundred eighty seven (86%) study participants were interviewed to assess the comprehensive knowledge of TB. Frequent visits to disseminate health information about TB made by Health Extension Workers were significantly higher among rural study participants (94.4%) than urban study participants (76.6%). Being age > 45 years (AOR=2.08; 95%CI: 1.37, 3.16), divorced marital status (AOR= 3.49; 95%CI: 1.37, 8.91), 5-8th grade student (AOR=2.72; 95%CI: 1.25, 5.91), 9-10th grade student (AOR= 4.12; 95%CI: 1.19, 14.19) and unemployed (AOR=0.74; 95%CI: 4.64, 16.47) were predictors of comprehensive knowledge of household women. Meanwhile, out of 2319 study participants, 91.8% household women reported that the HEW had visited their house regularly.

Conclusion: Study participants' comprehensive knowledge about TB was satisfactory. However, significant proportion of the respondents had less knowledge about major symptoms and main routes of transmission. Thus, Health Extension Workers should strengthen targeted health information on TB focusing on the route of transmission and prevention.

Key words: *Community, Health Extension Workers, Knowledge, TB*

Introduction

Tuberculosis (TB) is caused by bacteria, *Mycobacterium tuberculosis*, that most often affect the lungs. It spreads from person to person through the air when people with pulmonary TB cough, sneeze or spit, they propel the TB germs into the air. A person needs to inhale only a few of these germs to become infected. People infected with TB bacteria have a 10% lifetime risk of falling ill with TB. However, people with compromised immune status, such as living with HIV, malnutrition or diabetes, or people who use tobacco, have a much higher risk of falling ill. In 2014, 9.6 million people fell ill with TB and 1.5 million died from the disease and 480 000 people developed Multidrug Resistant TB (MDR-TB). Over 95% of TB deaths occur in low- and middle-income countries. The disease is curable and preventable (WHO, 2006; WHO 2016). Ethiopia is one of the 22 High

Burden Countries (HBCs). According to the 2014 WHO report, the prevalence and incidence of all forms of TB are 211 and 224 per 100,000 of the population, respectively (WHO, 2014).

It has been said that HIV/AIDS, smoking and malnutrition are substantial contributors to the epidemiological burden of active TB (Murray *et al.* 1990; Cegielski and McMurray 2004; WHO 2009). Meanwhile, poverty and lack of awareness are the most important factors that increase the risk of exposure to TB (Lienhardt, 2001; Lienhardt *et al.*, 2005), and that lack of knowledge about the cause, mode of transmission, symptoms, and appropriate treatment of TB affect not only the health-seeking behavior of patients, but also the controlling strategy, thereby sustaining the transmission of the disease within the community (Auer *et al.*, 2000; Yimer *et al.*, 2005; Mesfin *et al.*, 2009). For these reasons, creating general awareness about tuberculosis among communities and initiating community participation in the control of the disease make up one component of the six basic components of the "Stop TB Strategy" of the World Health Organization (WHO) (WHO, 2006).

In 2003, in response to the country's health problem, the Ethiopian Federal Ministry of Health (FMoH) launched a new health care plan: the "Accelerated Expansion of Primary Health Coverage" through a comprehensive Health Extension Program (HEP). The program has introduced 16 packages in four areas of care, one of which is Disease Prevention and Control (HIV/AIDS, TB and malaria) (Banteyerga, 2011). The major task of HEWs who are young women who took a one-year course of skill oriented basic training on HEP packages, is increasing the knowledge and skills of communities and households to deal with preventable diseases and be able to access services available at the nearby health institutions (Sebhatu, 2008; Banteyerga, 2011). However, little has been achieved (Yimer *et al.*, 2009). The limited studies that have tried to discover the underlying reasons why information is not widely and effectively disseminated by HEWs are the workers' limited access for updated information, low level of comprehensive knowledge, and community's misconceptions about HEWs (Mesfin *et al.*, 2005; Yimer *et al.*, 2009; Abebe *et al.*, 2010).

Though much emphasis is given on prevention and control of TB and the role of HEWs in averting the burden of the disease, little is known about community perception and awareness in management of tuberculosis. Therefore, this study was intended to assess knowledge of women about tuberculosis and the

role of Health Extension Workers (HEWs) among households in East Hararghe Zone, Eastern Ethiopia.

Material and Methods
Study Area and Period
The study was conducted from February to March, 2014 in East Haraghe Zone, which is one of the 20 Zones in Oromia Regional State and which is divided into 19 districts and many kebeles (CSA, 2011). Kebele is the smallest administrative unit which consist at least five hundred families, or 3,500 to 4,000 persons. According to Zonal Health Bureau, in each rural kebele, two HEWs have been assigned since 2003.

Study Population and Sample Size Determination
To calculate the required sample, a single population proportion formula was used. As a result the calculated sample size was 2,343 households. A household woman who was above 18 years old, able to speak and hear at the time of the interview, not critically ill, and volunteer to participate on the study was legible for the study. The research focused on household women heads because the health extension program itself focused on female and all the HEWs are female.

Sampling Procedure and Data Collection Technique
To select the required households, multi-stage stratified simple random sampling technique was used. Among 19 districts of East Hararghe Zone, six districts were selected using simple random sampling technique. Then, kebeles of the districts were stratified into urban and rural kebeles; and two kebeles were randomly selected from each randomly selected district. Finally. the households were proportionally allocated to each kebele based on the total number of households in each kebele. The study participants were household women and the data were collected through a face to face interview using structured questionnaire by 12 high school graduate women who were given training for the purpose. Three experienced public health officers supervised the data collection process.

Data Processing and Analysis
In this study, the outcome variable was knowledge about TB, composed comprehensive TB knowledge questions. The independent variables were residence, age, marital status, religion, ethnicity, educational status, occupation, availability of health facility, distance of health facility, and frequency of HEWs visit.

The questionnaire, which was adopted from numerous literatures, consisted 24 items: 12 items are about socio-demographic, 6 items are related to TB knowledge, and 6 items are related to HEWs and health facility. Each item had alternative responses. A score of one was given for each correct answer and a zero for an incorrect and 'I don't know' answers. The knowledge levels of the participants were dichotomized based on their overall knowledge level about TB. Accordingly, those who answer all questions asked to assess the overall knowledge about TB were considered as knowledgeable, whereas those who did not answer all questions asked to assess the overall knowledge about TB were considered as less knowledgeable. The data were cleaned and double entered into EpiData Version 3.1 and analyzed using STATA version 11.0. Adjusted Odds Ratio (OR) with 95% CI was used to determine the association between the outcome variable (knowledge about TB) and the predictor variables.

Ethical Considerations

The study was reviewed and approved by Institutional Health Research Ethics Review Committee (IHRERC) of Haramaya University, College of Health and Medical Sciences. Written consent was obtained from each study participant prior to the commencement of data collection. Study participants were represented by coded identifiers to maintain confidentiality,

Results

Socio-demographic Characteristics of the Study Participants

Of the 2,343 legible household women assumed, 2,319 participated in the study, which was with a response rate of 99%. Their mean age was 36.7 years (SD± 14.02). One hundred eighty two (85.5%), 2036 (87.8%), 2,024 (87.3%), and 2,070 (89.3%) were rural dwellers, married, Muslim, and Oromo ethnic group, respectively (Table1).

Knowledge of Study Participants about Tuberculosis

Most of the respondents, 1,972 (85%), reported that they had heard about TB before, of whom 1,687 (86%) correctly answered to all questions asked about TB. Additionally, 1,338 (67.8%) mentioned HEWs as a source of information about TB (Figure 1), 1,885 (95.6%) identified cough greater than two weeks as the major symptom of TB, and 99.4% described TB as a transmittable disease. Moreover, among the respondents

who had heard about TB, 1,861 (95%) reported that TB can be transmitted through droplets during sneezing and coughing, 1,843 (93.5%) mentioned that TB as a preventable disease, and 1,909 (96.8%) as a curable disease.

Table 1. Socio-demographic characteristic of study participants in East Hararghe, Ethiopia, 2014.

Variable	Category	Freq.	%
Residence	Urban	337	14.5
	Rural	1,982	85.5
Age in years	18-30	1,044	45.0
	31-45	804	34.7
	>45	471	20.3
Marital status	Married	2,036	87.8
	Single	115	5.0
	Divorced	106	4.6
	Separated	61	2.6
Religion	Christian	291	12.5
	Muslim	2,024	87.3
	Others	4	0.2
Ethnicity	Oromo	2,070	89.3
	Amhara	242	10.4
	Other	7	0.3
Educational status	Unable to Read/Write	1,657	71.4
	Read/Write	152	6.6
	1-4th Grade	167	7.2
	5-8th Grade	196	8.5
	9-10th Grade	110	4.7
	11-12 Grade	4	0.2

The proportion of urban study participants (97.9%) who had heard about TB before (p<0.005) and who replied TB as a curable disease (99.4%) was significantly higher than that of the rural study participants (p= 0.003). The number of rural study participants who mentioned HEWs as a source of information about TB 1,196 (72.8%) were higher than that of the urban study participants (P< 0.005) (Table 2).

Based on multivariate results, being age > 45 years (AOR=2.08; 95%CI: 1.37, 3.16), divorced marital status (AOR=3.49; 95%CI: 1.37, 8.91), 5-8th grade student (AOR=2.72; 95%CI: 1.25, 5.91), 9-10th grade student (AOR=4.12; 95%CI: 1.19, 14.19), and being unemployed (AOR=0.74; 95%CI: 4.64, 16.47) were identified as predictors of comprehensive knowledge level of household women (Table 3).

Role of Health Extension Workers to Disseminate Information about TB

Out of 2,319 study participants, 2,128 (91.8%) of the study participants reported that the Health Extension Workers had visited their house regularly to disseminate health information about TB and many of whom were rural study participants. Thus, frequent visits to disseminate health information about TB made by Health Extension Workers were significantly higher among rural study participants (94.4%), than urban study participants (76.6%) (p<0.005) (Table 4).

Table 2. Study participants' knowledge about TB in East Hararghe, Ethiopia, 2014.

Variables	Urban n (%)	Rural n (%)	Total n (%)
Ever heard about TB before			
Yes	330 (97.9)*	1,642 (82.8)*	1,972 (85)
No	7 (2.1)	340(17.2)	347 (15)
Cough greater than two weeks as a major symptom of TB			
Yes	318 (96.4)	1,567 (95.4)	1,885 (95.6)
No	12 (3.6)	75 (4.6)	87 (4.4)
TB is transmittable disease			
Yes	329 (99.7)	1,631 (99.3)	1,960 (99.4)
No	1 (0.3)	11 (0.7)	12 (0.6)
Air droplets during sneezing & coughing as main route of transmission			
Yes	319 (97)	1,542 (94.5)	1,861 (95)
No	10 (3)	89 (5.5)	99 (5)
TB is preventable			
Yes	305 (92.4)	1,538 (93.7)	1,843 (93.5)
No	25 (7.6)	104 (6.3)	129 (6.5)
TB is curable			
Yes	328 (99.4)*	1,581 (96.3)*	1,909 (96.8)
No	2 (0.6)	61 (3.7)	63 (3.2)

*Note: *p<0.05*

Table 3. Factors associated with TB knowledge among households in East Hararghe, Ethiopia, 2014.

Variable		Overall knowledge about TB		COR(95% CI)	AOR (95%)
		High, n (%)	Low, n (%)		
Residence	Urban	287 (17)	42 (15.4)	1	
	Rural	1,400 (83)	231 (84.6)	0.89 (0.62-1.26)	1.54 (0.97-2.44)
Age	18-30	735 (43.6)	128 (46.9)	1	
	31-45	587 (34.8)	107 (39.2)	0.95 (0.72-1.26)	1.27 (0.94-1.71)
	>45	365 (21.6)	38 (13.9)	1.67 (1.14-2.45)*	2.08 (1.37-3.16)*
Marital status	Married	1,474 (87.4)	241 (88.3)	1	
	Single	78 (4.6)	18 (6.6)	0.71 (0.42-1.20)	0.64 (0.35-1.17)
	Divorced	92 (5.5)	5 (1.8)	3.01 (1.21-7.48)*	3.49 (1.37-8.91)*
	Separated	42 (2.5)	9 (3.3)	0.76 (0.37-1.59)	0.85 (0.39-1.84)
Religion	Christian	255 (15.1)	21 (7.7)	1	
	Muslim	1,429 (84.7)	251 (91.9)	0.47 (0.29-0.75)*	0.84 (0.38-1.82)
	Others	3 (0.2)	1 (0.4)	0.25 (0.02-2.48)	0.14 (0.01-1.77)
Ethnicity	Oromo	1,462 (86.7)	258 (94.5)	1	
	Amhara	218 (12.9)	15 (5.5)	2.56 (1.49-4.34)	1.93 (0.79-4.69)
	Others	7 (0.4)	0 (0)		
Educational status	Illiterate	1,124 (66.6)	219 (80.2)	1	
	Read /write	111 (6.6)	25 (9.2)	0.86 (0.55-1.37)	0.77 (0.47-1.28)
	1-4 Grade	134 (7.9)	14 (5.1)	1.86 (1.06-3.29)*	1.18 (0.65-2.17)
	5-8 Grade	179 (10.6)	8 (2.9)	4.36 (2.11-9.00)*	2.72 (1.25-5.91)*
	9-10 Grade	106 (6.3)	3 (1.1)	6.88 (2.16-21.88)*	4.12 (1.19-14.19)*
	11-12 Grade	3 (1.8)	1 (0.4)	0.58 (0.60-5.64)	0.39 (0.03-4.77)
	>12 Grade	30 (1.8)	3 (1.1)	1.95 (0.59-6.44)	1.38 (0.31-6.08)
Occupation	Farmer	990 (58.7)	237 (86.8)	1	
	Daily laborer	182 (10.8)	21 (7.7)	2.07 (1.29-3.33)*	1.62 (0.91-2.87)
	Gov't employee	56 (3.3)	4 (1.5)	3.35 (1.20- 9.33)*	2.13 (0.57-7.96)
	Unemployed	459 (27.2)	11 4)	9.99 (5.40-18.47)*	8.74 (4.64-16.47)*
Availability of health facility	Yes	1,665 (98.7)	268 (98.2)	1	
	No	22 (1.3)	4 (1.5)	0.88 (0.30-2.59)	1.28 (0.42-3.89)
Distance from health facility	≤1 hour	1,665 (98.7)	267 (97.8)	1	
	> 1 hour	22 (1.3)	6 (2.2)	0.59 (0.24-1.46)	0.82 (0.29-2.29)
†Frequent visit done by HEWs	Yes	1,567 (92.9)	249 (91.2)	1	
	No	120 (7.1)	24 (8.8)	0.79 (0.50-1.26)	0.64 (0.38-1.07)

Note: †*More than one visit within a month; *p<0.05; COR= crude odds ratio; AOR= adjusted odd ratio; CI: Confidence interval*

*Note: *p<0.005*

Table 4. Health related characteristics among households in East Hararghe, Ethiopia, 2014.

Variables	Respondent's residence		
	Urban, n (%)	Rural, n (%)	Total, n (%)
Presence of health institution (any type)			
Yes	334 (99.1)	1,932 (97.5)	2,266 (97.7)
No	3 (0.9)	50 (2.5)	53 (2.3)
Type of health institution			
Health post	138 (40.9)*	1,486 (75)*	1624 (70)
Clinic	142 (42.1)*	62 (3.1)*	204 (8.8)
Pharmacy/ rural drug vender	112 (33.2)*	45 (2.3)*	157 (6.8)
Health center	90 (26.7)	552 (27.8)	642 (27.7)
Hospital	4 (1.2)	13 (0.7)	17 (0.7)
Walking distance for health institution			
≤1 hour	312 (92.6)	1,976 (99.7)	2,288 (98.7)
>1 hour	25 (7.4)	6 (0.3)	31 (1.3)
HEWs frequent visits			
Yes	258 (76.6)*	1,870 (94.4)*	2,128 (91.8)
No	79 (23.4)	112 (5.6)	191 (8.2)
Frequency of HEWs visit			
≥ once in a month	246 (95.3)	1,829 (97.8)	2,075 (97.5)
< one in a month	12 (4.6)*	41 (2.3)*	53 (2.5)

Discussion

The study demonstrated that there is a knowledge gap between urban and rural respondents regarding previous experience on TB. In this study, 85% of the respondents had information about TB, and this is compatible to similar study findings reported from South West Ethiopia (83.0%) (Abebe *et al.*, 2010), Malaysia (96%) (Koay, 2004), and rural south east part of China (99.2%) (Wang *et al.*, 2008), where the respondents had heard about TB. It is also similar with findings in South-west Ethiopia (Abebe *et al.*, 2010), North Ethiopia (86.8%) (Mesfin *et al.*, 2005; Yimer *et al.*, 2009), and in Afar Regional State (95.6%) (Legesse *et al.*, 2010), where the study participants had heard about pulmonary tuberculosis.

Unlike the finding in Lahore, where for 41% of the respondents' mass medias were their main source of information about TB (Bacay-Domingo and Ong-Lim, 2009), and in Iraq, where physicians (27.5%) and television (23.2%) were the most important sources of the information (Hashim *et al.*, 2003), in the current study HEWs were source of the information for 1,338 (67.8%) of the 1972 respondents who had had

information about the disease. This might be due to community's improved access to primary health care units and success of health extension program on disseminating health information for the community member about diseases prevention. However, our finding is in agreement with the one in Mwanza region, Tanzaina, where the primary Health workers are the main source of information about TB (Wandwalo and Mørkve, 2000).

Despite a higher proportion of the study participants mentioned that cough greater than two weeks is the major symptom of the disease and inhaling respiratory droplets are the main route of disease transmission, the comprehensive knowledge of study participants regarding TB is low. The study result regarding major symptoms of PTB is in agreement with the findings of other studies conducted in pastoral communities in the middle and Lower Awash Valley of Afar region, Ethiopia which revealed that a larger proportion of participants from the Dubti area mentioned persistent cough as a major symptom of Pulmonary TB (PTB) than participants from the Amibara. However, unprotected

coughing/breathing (80.8%) and sharing cups with a patient (77.6%) were the most frequently mentioned routes of transmission by participants from both areas (Legesse *et al.,* 2010). This might be due to good information dissemination strategy applied by HEWs.

In the present study, the study participants reported that PTB is treatable and curable with modern drug, and this is compatible with the findings of previous studies in other parts of Ethiopia (Mesfin *et al.*, 2005, Legesse *et al.,* 2010), and in Iraq (Hashim *et al.,* 2003). This might be due to improved access to primary health care units, and the frequent visits to the households by HEWs, and the success of Health Extension Program in disseminating health information for the community.

This cross sectional survey may be subjected to recall bias. However, its effect was minimized by reducing the recall period during interview. It would be also more appropriate to use analytical approaches to assess the roles of HEWs on the level the community's knowledge; and to complement the study with qualitative data.

Conclusion and Recommendations

Based on the findings of the study, study participants' overall knowledge about TB was satisfactory and frequent visit of households by HEWs has been observed. However, significant proportion of the respondents had less knowledge about symptoms and routes of transmission. Thus, HEWs should strengthen targeted health information on TB focusing on the route of transmission and prevention.

Acknowledgments

Conflict of Interests

The authors declare that they have no competing interests.

Authors' contributions

The study was conceived and designed by AS, who also retrieved the data sets for analysis and drafted the manuscript. KU and TG participated in study design, analysis, write-up, and manuscript revision. All authors approved the final manuscript.

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Under Nutrition and its Associated Factors among Lactating Mothers in Rural Ambo District, West Shewa Zone, Oromia Region, Ethiopia

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Abstract

Background: Lactation has different effects on maternal nutritional status. Even though, growing number of studies reported the existence of maternal under nutrition, very few studies in Ethiopia highlighted under nutrition among lactating mothers.

Objective: To assess the prevalence of under nutrition and associated factors among lactating mothers in rural areas of Ambo district, West Shewa zone, Oromia region, Ethiopia, 2016.

Methods: A community based cross-sectional study was conducted among randomly selected 619 lactating mothers. A pretested and structured questionnaire was used to collect socio-demographic data. Weight, height and Mid Upper Arm Circumference were measured. Body Mass Index was measured to assess the nutritional status. Descriptive statistics was used to describe the data. Binary logistic regression was performed to assess the association between each independent and dependent variable. Adjusted Odds Ratios with its 95% confidence interval were estimated to identify factors associated with the outcome variable in multivariable logistic regression.

Results: The overall prevalence of under nutrition (BMI < 18.5 kg/m2) was 21.5%. Lactating mothers within the age group of 17-25 were more likely to be undernourished than those mothers in the age group of 36-49 (AOR=6.82, 95%CI: 1.84, 25.27). Mothers who were unable to read and write (AOR=2.45, 95%CI: 1.22, 4.94) and from poor wealth index (AOR=1.76, 95% CI: 1.05, 2.95) were also more likely to be undernourished than those who had formal education and rich mothers, respectively.

Conclusion: One fifth of lactating mothers were undernourished. Thus, strengthening health education on proper maternal and child dietary practices and the need of adequate dietary intake during lactation should be emphasized. Additionally, strong multi-sectorial collaboration targeted at improving women's status in the community is very essential in order to improve health and nutritional outcomes of lactating women.

Keywords: *under nutrition, lactating mother, Ambo, Ethiopia*

Introduction

In 2015, about 795 million people, of whom 780 million were from the developing regions, were undernourished globally. This means that nearly one in nine individuals did not have enough to eat. In sub-Saharan Africa, the projected rate of undernourishment for the 2014–2016 period was almost 23 percent. Though the hunger rate has fallen, the number of undernourished people has increased by 44 million since 1990 (UN, 2015).

Lactation has different effects on maternal nutritional status depending on its duration, intensity, as well as cultural diversity. Lactating mothers from low-income settings are considered as nutritionally vulnerable group (Kiday *et al.*, 2013). Lactation puts high demands on maternal stores of energy and protein than in any other stage of a woman's reproductive life. To support lactation and maintain maternal reserves, most mothers in developing countries need to eat about 500 additional

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kilocalories every day. Extra food, therefore, must be made available to the mother to meet the additional demands of pregnancy and lactation (LINKAGES, 2004).

Maternal under nutrition, including chronic energy and micronutrient deficiencies, accounts for 11% of the global burden of disease (Cuziat and Mattinen, 2011). It is highly prevalent in low-income and middle-income countries, resulting in substantial increases in mortality and overall disease burden. The problem ranges from 10% to 19% in most countries. Its seriousness is evident in most countries in sub-Saharan Africa, south-central and southeastern Asia, and in Yemen, where more than 20% of women have a body-mass index of less than 18·5 kg/m² (Black *et al.*, 2008). In Ethiopia 27% of women aged 15-49 years were undernourished (BMI of less than 18.5 kg/m²) which remained relatively constant since the year of 2005 (CSA, 2012). Improvements in nutrition still represent a massive unfinished global agenda (Cuziat and Mattinen, 2011).

Even though a growing number of studies reported the existence of maternal under nutrition, very few studies in Ethiopia highlighted the issue of under nutrition among lactating mothers. The objective of this study was to assess the prevalence of under nutrition and its associated factors among the lactating mothers in rural areas of Ambo district, West Shewa zone, Oromia Region, Ethiopia.

Materials and Methods

Study Area and Period

The study was conducted in rural areas of Ambo district, West Shewa Zone, Ethiopia, from February 15-30, 2016. The population size was 138,612, of whom 94% were rural residents who were living in 29,102 households. During the study, there were 4,223 lactating mothers in the rural area.

Study Design and Study Participants

A community-based cross-sectional study was conducted on 638 lactating mothers, who were between 15 and 49 years of age, from five randomly selected kebeles (clusters). The lactating mothers who were seriously ill during the data collection, pregnant, and with physical deformities during anthropometric measurement were excluded from the study to allow consistent record of the measurement.

Data Collection Instruments and Procedure

The data were collected using a structured and pretested questionnaire. It was initially developed in English, and then translated into the local language, Afan Oromo; then back translated into English.The households' food insecurity level was measured using Household Food Insecurity Access Scale (HFIAS), which is a structured, standardized, and validated tool developed by FANTA (Coates *et al.*, 2007; Deitchle *et al.*, 2010). The scale is a valid tool to measure a household's food insecurity in rural and urban areas of Ethiopia (Seifu *et al.*, 2015). The mothers' dietary intake pattern was measured by a qualitative recall of all the foods they consumed in the 24 hours before the interview. The foods were grouped to calculate the Women's Dietary Diversity Score (WDDS) of the respondents, and the mean score was used to classify their food intake as adequate or not (Kennedy *et al.*, 2013). To measure the outcome variable, the weight, height, and Mid-Upper Arm Circumference (MUAC) of the lactating mothers were measured. Ten students who completed high school and who were living in the study area collected the data via home to home visits and all the anthropometric measurements were taken based on the standard procedure (Rockville, 1988; NHANES, 2007).

Data Quality Control

To assure the quality of the data, a structured and pretested questionnaire was used. The data collectors and the supervisors were given a two-day intensive training. Relative Technical Error of Measurement (%TEM) was calculated to minimize a random anthropometric measurement error. The data collectors' accuracy of the measurements was standardized with their trainer during training and pretesting. A respondent's weight and height were measured at least twice and more times when the difference between the two weight measures was greater than 0.1kg and when the difference between the two height measures was greater than 0.1 cm. The functionality of the digital weight scale was checked before use. It was checked using a known weight and assuring it read exactly at zero (NHANES, 2007). Intensive supervision was done and the data were double entered.

Data Processing and Analysis

After the data had been checked for completeness and internal consistency, the data were coded and double entered into EpiData 3.1 computer software package and cleaned for inconsistency. For further analysis, data

were exported to Statistical Package for Social Science (SPSS) Version 22 software. The descriptive statistics was used to describe the data. The Women's Dietary Diversity Score (WDDS) was calculated and the mean score was used to describe their nutrient intake status (Kennedy *et al.*, 2013; FAO, 2008). Based on the data obtained from HFIAS, the households were categorized as food secured or not secured (Coates *et al.*, 2007). By considering the locally available household assets and using Principal Component Analysis (PCA) method, the families' wealth index was constructed. The outcome variable was re-coded to dichotomous outcomes: undernourished or not undernourished. The co-linearity effect was checked using Variance Inflation Factor (VIF) and non-collinear covariates were included in the independent binary logistic regression model. All the covariates that were significant at p<0.2 in the bivariate analysis were considered for further multivariate analysis. Using a multivariable logistic regression, Odd Ratios, along with 95% Confidence interval, were estimated to identify the factors associated with the outcome variable. The level of statistical significance was declared at p<0.05. The model was tested by Hosmer- Lemeshow goodness of fit test.

Ethical Consideration

Ethical clearance was obtained from Institutional Health Research Ethical Review Committee (IHRERC) of Haramaya University College of Health and Medical Sciences. Iindividual Informed written consent was obtained from each study participant. The respondents were assured of the confidentiality by excluding their names during the data collection. They were informed well that they had full right to totally refuse to participate

and/or with draw from the study at any time without any precondition.

Results

Socio-demographic Characteristics of the Study Participants

Among the assumed 638 lactating mothers aged 15-49 years, 619 (97%) participated in the study. More than half of the respondents, 344 (55.4%), were in the age group of 26-35 years with a mean age value of 28.1years (SD±5.3 years). Of the total, 395 (63.8 %) of the women were unable to read and write, and only 178 (31.3%) of their husbands were able to read and write. Regarding the family wealth index, 247 (39.9%), 124 (20%) and 248 (40.1%) of the households were in the poor, medium and rich wealth terciles, respectively. Among the households, 238(38.4%) were food insecured whereas the remaining 381(61.6%) were food secured (Table 1)

Maternal Health Care and Feeding Practices

Among the respondents, 287 (46.4%) had been pregnant once or twice, 311 (50.2%) visited ANC 4 or more times during their last pregnancy, 483 (78%)) were using family planning methods, and only 90 (14.5%) had experienced diarrhea in the two weeks before the study, which was probably because 428 (69.1%) of the households had their own latrine, and pipe water was the major source of drinking water for 488 (78.8%) study participants.

The mean Dietary Diversity Score (DDS) of the lactating mothers was 4 ±1.5 and many of them, 446 (72.1%), had DDS of ≥4. Of the babies whom the mothers breast feeding, 238 (38.4%) were in the age group of 12-24 months and nearly one fourth of them, 147 (23.7%), started additional food at 6 month (Table 2).

Table 1. Socio-demographic characteristics of lactating mothers in Ambo district, West Shewa zone, Oromia region, Ethiopia, 2016

Variables	Category	Frequency	%
Age of mother (in years)	17-25	216	34.9
	26-35	344	55.6
	36-49	59	9.5
Religion of mother	Orthodox	404	65.3
	Protestant	184	29.7
	Muslim	4	0.6
	Others	27	4.4
Ethnicity of mother	Oromo	581	93.9
	Amhara	34	5.5
	Others	4	0.6
Current marital status of mother	Married	561	90.6
	Divorced	24	3.9
	Widowed	12	1.9
	Single	22	3.6
Educational status of mother	Unable to read and write	224	36.2
	Able to read and write	146	23.6
	Primary level	144	23.3
	Secondary level	73	11.8
	College diploma and above	32	5.2
Educational status of husband	Unable to read and write	120	21.1
	Able to read and write	178	31.3
	Primary level	126	22.1
	Secondary level	101	17.8
	College diploma and above	44	7.7
Occupation of mother	Farmer	343	55.4
	Housewife	122	19.7
	Daily laborer	74	12.0
	Private employee	14	2.3
	Merchant	30	4.8
	Government employee	24	3.9
	Not working	11	1.8
	Others	1	0.2
Family size	1-3	148	23.9
	4-6	339	54.8
	>6	132	21.3
Number of children < 2 years	1	605	97.7
	≥2	14	2.3
Household head	Male	575	92.9
	Female	44	7.1
Family wealth index	Poor	247	39.9
	Medium	124	20.0
	Rich	248	40.1
Household food security	Food secure	381	61.6
	Mildly insecure	65	10.5
	Moderately insecure	64	10.3
	Severely insecure	109	17.6

Nutritional Status of Lactating Mothers

The overall prevalence of under nutrition (BMI<18.5 kg/m²) among the lactating mothers was 21.5% (95%CI: 18.3, 24.9), whereas 78.5% (95%CI: 75.1, 81.7) of the mothers were not undernourished. Among the total lactating mothers included in the study, 68.5%; (95%CI: 64.6, 72.2) and 10% (95%CI: 7.6, 12.3) of them were

normal (BMI=18.5-24.99kg/m²) and overweight (BMI 25.5-29.99kg/m²), respectively, and there was no case of obesity. Using MUAC, 79(12.8%) (95%CI: 10.0, 15.5) of lactating mothers were undernourished (MUAC<21cm) while the remaining 540(87.2%) (95%CI: 84.5, 90.0) had MUAC ≥21cm.

Table 2. Maternal health care and feeding practice of the study participants in Ambo district, West Shewa zone, Oromiya region, Ethiopia, 2016

Variables	Category	Frequency	%
Age of breast feeding child (in months)	<6	189	30.5
	6-11	145	23.4
	12-24	238	38.4
	>24	47	7.6
Number of pregnancy	≤2	287	46.4
	3-4	216	34.9
	5-6	93	15
	>6	23	3.7
Birth interval for the index child	First birth	148	23.9
	1-2 years	151	24.4
	>2 years	320	51.7
Number of ANC visit	No ANC visit	68	11
	< 4 times	240	38.8
	≥ 4 times	311	50.2
Family planning utilization	Do not use FP method	136	22
	Pills	32	5.2
	Injectable	264	42.6
	Norplant	145	23.4
	IUCD	25	4
	Condom	2	0.3
	Breast feeding	15	2.4
Last menstruation seen	Within the last 30 days	234	37.8
	More than 30 days ago	229	37
	Did not remember	156	25.2
Duration of breast feeding	< 6 months	185	29.9
	6-11 months	168	27.1
	12-24 months	216	34.9
	>24 months	46	7.4
	Did not remember	4	0.6
Introduction of complementary feeding	Yes	457	73.8
	No	162	26.2
Age of introduction of complementary feeding	< 6 months	50	8.1
	At 6 month	147	23.7
	>6 month	246	39.7
	Do not remember	14	2.3
Diarrhea in the past two weeks	Yes	90	14.5
	No	529	85.5
Nutrition education	Yes	415	67
	No	204	33
Latrine availability	Yes	428	69.1
	No	191	30.9
Women dietary diversity score	<4	173	27.9
	≥4	446	72.1

Factors Associated with Under Nutrition among Lactating Mothers

In the multivariable logistic regression analysis, the mother's' age, education, and family wealth index were statistically significant at 5% level and were found to be the predictors of under nutrition among the lactating. The mothers within the age group of 17-25 years were 6.8 times more likely to be undernourished compared with those mothers in the age group of 36-49 years (AOR=6.82, 95% CI: 1.84, 25.27). Mothers who were unable to read and write were 2.45 times more likely to be undernourished than those mothers who had formal education (AOR=2.45, 95%CI: 1.22, 4.94). Mothers from poor households were 1.76 times more likely to be undernourished than their counterparts (AOR=1.76, 95%CI: 1.05, 2.95) (Table 3).

Table 3. Factors associated with under nutrition among lactating mothers mothers in Ambo district, West Shewa zone, Oromia region, Ethiopia, 2016

Covariates	Category	Under nutrition (BMI<18.5)			
		Yes	No	COR (95% CI)	AOR (95%CI)*
Age of mother (in years)	17-25	56	160	2.60(1.12, 6.05)	6.82(1.84, 25.27)*
	26-35	70	274	1.89(0.83, 4.36)	3.49(1.00, 12.14)
	36-49	7	52	1	1
Educational status of mother	Unable to read and write	60	164	2.10(1.33, 3.31)	2.45(1.22, 4.94)*
	Able to read and write	36	110	1.88(1.12, 3.13)	1.45(0.70, 3.00)
	Have formal education	37	212	1	1
Educational status of husband	Unable to read and write	25	95	1.43(0.83, 2.49)	0.85(0.40, 1.80)
	Able to read and write	54	124	2.38(1.50, 3.76)	1.50(0.78, 2.89)
	Have formal education	42	229	1	1
	Poor	66	181	1.90(1.22, 2.95)	1.76(1.05, 2.95)*
Family wealth index	Medium	27	97	1.46(0.84, 2.50)	1.49(0.79, 2.80)
	Rich	40	208	1	1
	Food secure	88	293		
Household food security	Mildly food insecure	9	56	0.54(0.26, 1.13)	0.44(0.17, 1.13)
	Moderately food insecure	16	48	1.11(0.60, 2.05)	1.37(0.68, 2.78)
	Severely food insecure	20	89	0.75(0.44, 1.28)	0.66(0.33, 1.31)
Age of breast feeding child (in months)	≤12	87	270	1.51(1.02, 2.26)	1.21(0.72, 2.03)
	>12	46	216	1	1
	First birth	30	118	1.13(0.69, 1.84)	0.75(0.38, 1.47)
Birth interval for the index child	1-2 years	44	107	1.82(1.16, 2.86)	1.44(0.82, 2.52)
	>2 years	59	261	1	1
Frequency of ANC visit	< 4 times	65	175	1.73(1.15, 2.60)	1.51(0.91,2.50)
	≥ 4 times	55	256	1	1
Introduction of CF	Yes	92	365	1	1
	No	41	121	1.34(0.88,2.05)	1.26(0.72, 2.20)

Note: AOR, Adjusted Odd Ratio; CF, Complementary Food; CI, Confidence Interval, COR, Crude Odd Ratio; *, p-value <0.05

Discussion

This study revealed that the prevalence of under nutrition (BMI<18.5kg/m²) among the lactating mothers was 21.5%. Mothers who were between 17 and 25 years of age, not able to read and write, and whose family wealth index was poor were found more likely to be undernourished. This finding is closer to the one reported from Nekemte (20.5%) (Temesgen *et al.*, 2015), but a bit less than the finding in Tigray (25%) (Kiday *et* *al.*, 2013). When compared with the result from Babile (41.7% during post-harvest and 54.7% during pre-harvest season) (Kedir *et al.*, 2015), the prevalence in this research is much lower. The difference could be attributed to the interventions on maternal health, nutrition and other women empowering programs by the government and other non-governmental

organizations in the study area and the time of survey as well.

However, the prevalence we found is much higher than the ones reported from India (16%) (Ghosh-Jerath *et al.*, 2015), Guatemala (5%) (Oyesiku *et al.*, 2013), Nigeria (<5%) (Sanusi and Falana, 2009), and Kenya (10%) (Gewa *et al.*, 2012). The difference might be related to a seasonal variation of the study period, the difference in socio-economic and demographic factors like educational status of mothers and family income. As per MUAC measurement, the proportion of the undernourished lactating mothers (MUAC< 21cm) was 12.8%, which is comparable with the finding from Tigray (13%) (Kiday *et al.*, 2013).

The present study has showed that the age of the lactating mothers was significantly associated with their nutritional status. The mothers within the age group of 17-25 were 6.8 times more likely to be undernourished compared with those mothers in the age group of 36-49. This is in line with a result from Bangladesh, in which, younger women in the age group 15–24 were more likely to be malnourished (Rahman *et al.*, 2015). This might be related with an increased nutritional requirement during adolescent age for normal growth and development of the mother and lack of sufficient food intake to meet this requirement.

Educational status of the mothers was also another factor that predicted their nutritional status in this study. The respondents who can't read and write were 2.45 times more likely to be undernourished than those mothers who had formal education. Similar studies from Sri Lanka (Jayawardena, 2014) and Congo Democratic Republic (Adebowale *et al.*, 2015) also reported that undernourished women were more common among those with no formal education. This could be related with an increased awareness of self care, health service utilization, and acknowledging the importance of increasing meal frequency during lactation among educated mothers.

In this study, statistically significant association was found between the covariate family wealth index (poverty level) and the nutritional status of the lactating mothers. The mothers from poor terciles family wealth were 1.76 times more likely to be undernourished than from the rich tercile. This is also supported by the study from Bangladesh (Rahman *et al.*, 2015) and Congo Democratic Republic (Adebowale *et al.*, 2015). This association could be due to the fact that the women from lower tercile family wealth were in difficulties of meeting their dietary requirement, engaged in strenuous works like daily laborer and have minimal health care practice.

Food insecurity affects the intake of adequate quantity and quality of diet that in-turn contributes to maternal under nutrition. In the present study, the lactating mothers from moderately food insecure households were 1.37 times more likely to be undernourished compared with those mothers from food secure households. The analysis did not identify statistically significant association between the households' food insecurity level and the nutritional status of the lactating mothers. However, other studies from Kenya (Gewa *et al.,* 2012) and Bangladesh (Rahman *et al.*, 2015) reported that food insecurity was significantly associated with maternal BMI. The absence of association in this study might be due to the differences in the measurement tools, the season of the study and the relatively low prevalence of food insecurity in the study area.

In this study, even if the association is not statistically significant, the odd of being undernourished were 1.26 times more likely among the mothers who did not introduce complementary feeding for their child than their counterparts. But, when we see the age of the introduction of complementary feeding, only 23.7% started additional food at the recommended age (at 6 months) whereas the 39.9% started at >6 months of age. This increases the burden of the lactating mothers to meet the nutritional demand of their child in the cost of their own nutritional status (nutrition depletion).

Recall bias is one of the limitations of this study since some of the questions were asked the event that occurred 4 weeks back. This was minimized by probing the respondents about the event. Anthropometric measurement error is also another limitation. To minimize this; data collectors were well trained, standardization of anthropometric measurers were done and the instrument was calibrated.

Conclusion and Recommendations

The result of this study revealed substantial proportion of under nutrition among the lactating mothers. The predictors of the under nutrition were the mothers' age (17-25 years of age), education (unable to read and write), and poor family wealth. Thus, strengthening health education programs on proper maternal and child dietary practices and the need of adequate dietary intake during lactation should be necessary. Additionally, strong multi-sectorial collaboration targeted at improving women's educational status in the community is very essential in order to improve health and nutritional outcomes of lactating women.

Conflict of Interests
The authors declare that there is no any conflict of interest.

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attending Jimma Hospital Diabetic Clinic. *Ethiop J Health Sci,* 15(2): 187-196.

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