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Full Length Research Paper

Correlation of lymphocyte count with serum calcium level and neutrophil-to-lymphocyte ratio in end stage renal disease patients undergoing hemodialysis in Adamawa State, Nigeria

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Low lymphocyte count in end stage renal disease (ESRD) patients receiving maintenance hemodialysis was documented. This study aims to correlate the levels of lymphocyte count with serum calcium level, and neutrophil-to-lymphocyte ratio (NLR) in ESRD patient receiving hemodialysis in Northeast Nigeria. Seventy eight (78) patients receiving hemodialysis and 75 clinically healthy subjects (control) participated in the present study. Full blood count and serum calcium level were determined by using a Sysmex XP-300 Hematology Analyzer and a Selectra Pro-S Chemistry Analyzer, respectively. The mean levels of lymphocyte count, packed cell volume, hemoglobin and serum calcium level were significantly lower ($p < 0.001$), while neutrophil, and NLR values were significantly higher ($p < 0.001$) in ESRD patients undergoing hemodialysis as compared to the healthy control subjects. Lymphocyte count was negatively correlated with NLR value ($r = -0.341$; 0.05) but positively with serum calcium ($r = 0.904$; $p < 0.001$). In end stage renal disease (ESRD) patient receiving hemodialysis, lymphocyte count was negatively correlated with NLR value and positively with serum calcium level.

Key words: Lymphocyte count, serum calcium level, neutrophil to lymphocyte ratio (NLR), hemodialysis.

INTRODUCTION

The routine medical choice for a patient with insufficient renal function (10 -15%) is maintenance hemodialysis (Block et al., 2004; Kuwae et al., 2005). Maintenance hemodialysis has been the most common method used

to treat advanced and permanent kidney failure since the 1960s (Kultigin et al., 2013). Human beings have an immune system that depends on different types of white blood cells for the protection against diseases (Johnson

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et al., 2005; Donato et al., 1992). These white blood cells include neutrophils, lymphocytes, monocytes, basophils and eosinophils. While neutrophils protect us from bacterial infections and respond to inflammation (Manal and Shaimaa, 2015; Azab et al., 2012), lymphocytes that include natural killer cells, T cells, and B cells protect us against viruses and cancer development (Templeton et al., 2014). Total peripheral blood lymphocyte count is reduced in ESRD patients undergoing hemodialysis, a finding that has been well documented since the 1980s (Manal and Shaimaa, 2015; Kuwae et al., 2005) on the other hand, neutrophile count is high in the peripheral blood of ESRD due to inflammatory processes (Turkmen et al., 2012). Cellular immunity level reflected as total lymphocyte count are reduced in ESRD patient (Johnson et al., 2005; Sakato et al., 2015), a fact that explains the frequent occurrence of infection which is the second leading cause of death in end stage renal disease patients (Egbi et al., 2014; Block et al., 2004). As it has been demonstrated, this reduction is more intense in ESRD patients undergoing maintenance hemodialysis (Block et al., 2004).

Chronic renal disease (CKD), and end-stage renal disease (ESRD) in particular, are associated with high mortality rates due to complications caused by cardiovascular diseases (CVDs) (Binnetoğlu et al., 2014), infections (Petrie et al., 2007; Reddan et al., 2003) and other inflammatory processes due to hypodynamic forces (Lavin-Gomez et al., 2011; Okyay et al., 2013).

Inflammation contributes to the development of ESRD by triggering both the release of cytokines and the increased production and activity of adhesion molecules (Memoli et al., 2000; Panichi et al., 1998), which together result in lymphocyte adhesion and movement into the interstitium (Johnson et al., 2005; Roberto et al., 2003). In ESRD, the vascular endothelium is injured by numerous potential insults, including hemodynamic forces (Hatice et al., 2012; Sakato et al., 2015). Although, circulating leukocytes do not adhere to the healthy vascular endothelium (Turkmen et al., 2012), the injured endothelium expresses different classes of adhesion molecules that selectively bind to leukocytes (Sakato et al., 2015; Templeton et al., 2014) and as a result, their presence is reduced at the site where they are needed. The changes in the hematological parameters and the correlation of lymphocyte count with serum calcium level and NLR value in ESRD patients in Northeast Nigeria have not yet been fully studied and this fact was the impetus for the present study.

MATERIALS AND METHODS

This study was carried out at the Federal Medical Center of Yola, in Adamawa State, in Northeast Nigeria. 153 subjects took part in the study, 78 of them were ESRD patients receiving hemodialysis (35 men and 43 women, with an average age of 43 ± 14 years). While the rest 75 were clinically healthy individuals used as controls (46 men and 29 women, with an average age of 35 ± 7 years). Blood

sample from the ESRD patients receiving regular maintenance hemodialysis were collected prior to their routine hemodialysis session. Blood samples of clinically healthy individuals were also collected at similar days.

Full blood count and serum calcium level were determined using a Sysmex XP-300 Hematology Analyser and a Selectra Pro-S Chemistry Analyser respectively. All analyses were performed according to the standard operational procedures.

All subjects gave their informed consent prior to their inclusion in the study. Inclusion criteria: Only ESRD patients receiving routine maintenance hemodialysis were used as test group. Exclusion criteria: ESRD patients that were not receiving routine maintenance hemodialysis and subject above 70 years old were excluded from this study.

Sample collection

Three milliliters of blood were aseptically collected through the antecubital vein of the subjects and put in both EDTA and plain vacutainer, that were further labeled with the patient number, sex and age. The full blood count for each subject was performed in the sample put in the EDTA vacutainer within one hour of collection, while the serum obtained from plain vacutainer, was centrifuged and was used for serum calcium level determination.

Sample analyses

Full blood count

Using the Sysmex XP 300 machine, the procedure for blood cell (full blood count) determination was performed as follows: EDTA samples were placed in a hematology blood mixer for five minutes and the blood cells were automatically counted through a probe fitted in the Sysmex XP 300 machine. After four minutes, the results of the blood cell count were displayed on the color LCD screen of the machine.

Calcium level measurement

The Selectra pro-S machine and direct colorimetric and complexometric methods were used. The exact procedure is described below:

- 1) Selectra pro-S machine was switch on and allow to boot and acclimatize for 30 min.
- 2) The calcium (arsenazo) reagent was automatically dispensed into the corresponding compartment of the machine.
- 3) Calibrator, control and test sample were introduced into the correspondent compartment.
- 4) The machine was allowed to calibrate automatically.
- 5) The machine was allowed to pipette, and incubate the samples automatically
- 6) The results were automatically displayed on the monitor screen.

Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) measurement

The NLR and PLR values were obtained by dividing the values of neutrophils and platelet by lymphocytes in full blood count, respectively.

Statistical analysis

Statistical analysis was performed using the Statistical Package for

Table 1. Full blood count, and serum calcium levels in ESRD patients receiving hemodialysis and clinically healthy subjects (controls).

Parameter	ESRD patients (N = 78)	Controls (N=75)	Normal values	P value
Lymphocyte count (%)	18.27±4.17	38.53±10.19	25-45	0.001
Neutrophile count (%)	70.36±7.36	52.84±16.35	40-70	0.005
Platelet count (x 10 ³ /μl)	229.4±79.54	198.6±53.47	150-300	0.005
Packed cell volume (l/l)	25.64±1.61	43.65±8.17	37-50	0.001
Hemoglobin (g/dl)	8.2±0.51	13.77±3.13	12-16	0.01
Total white cell count (x 10 ³ /μl)	9.53±2.79	6.99±1.38	4-10	0.05
Serum calcium level (mmol/l)	1.02±0.33	2.43±0.19	2.2-2.5	0.01
MCH (pg)	25.9±0.99	27.2±2.90	27-31	0.01
MCV (fl)	84.3±3.09	83.44±6.22	83-101	0.01
MCHC (g/dl)	30.99±0.89	32.55±1.23	31-34	0.01
NLR	3.7958	1.2421	1-2.1	0.05

Table 2. Correlation of lymphocyte count with other measured parameters in ESRD patients receiving hemodialysis.

Parameters	Correlation coefficient (r)	P-value
Lymphocyte count and neutrophil count	-0.201134	0.001
Lymphocyte count and platelet count	0.090885	0.05
Lymphocyte count and packed cell volume	0.179913	0.05
Lymphocyte count and hemoglobin	0.035250	0.005
Lymphocyte count and serum calcium	0.904	0.001
Lymphocyte count and NLR	-0.341	0.005
Lymphocyte count and PLR	-0.401	0.005

Social Sciences (SPSS) 20.0 software (Chicago IL). Descriptive values were given as mean standard error of mean. Categorical variables were expressed as the number of cases and the percentage value. The Student's *t*-test was used to compare the means between ESRD patients and control subjects, while Pearson's correlation coefficient was used to calculate the relationship between lymphocyte count, serum calcium level and NLR values.

RESULTS

The mean levels of lymphocyte count, packed cell volume, hemoglobin and serum calcium levels were significantly lower ($p < 0.001$), while neutrophil and NLR values were significantly higher ($p < 0.001$) in ESRD patients undergoing hemodialysis as compared to controls as shown in Table 1. In addition, lymphocyte count was negatively correlated with neutrophil count, PLR and NLR value and positively with serum calcium level as shown in Table 2.

DISCUSSION

In this study, lymphocyte count was reduced when NLR value increased, and as it was observed, this reduction

was proportional to serum calcium levels decrease indicating that calcium may play an important role in

lymphocyte kinetics and functions in ESRD patients. In general, the healthy Kidney turns vitamin D into active hormones called calcitriols (Egbi et al., 2014) that increase calcium absorption from the intestine into the blood (Block et al., 2004). In ESRD patients receiving hemodialysis, calcitriols are deficient due to kidney malfunction (Fung et al., 2002; Lavin-Gomez et al., 2011) and so intestinal adsorption of calcium may be impaired and its concentration in the peripheral blood is reduced. In addition, packed cell volume was decreased in ESRD patients due to failure of the kidneys to release erythropoietin (Fung et al., 2002), which is the hormone that is required for adequate red cell production in humans. NLR value was increase in the 78 ESRD patients undergoing maintenance hemodialysis. It is believed that, the increase of NLR value is a result of the increased neutrophil count observed during inflammatory processes. This inflammation may be caused by hypodynamic forces on vascular epithelial cell (Lavin-Gomez et al., 2011; Roberto et al., 2003). Hypodynamic forces observed in ESRD may be due to changes in plasma protein ratio, oncotic pressure and electrolyte imbalance arising from kidney malfunction (Kato et al.,

2012; Memoli et al., 2000). Low lymphocyte count in patients is a result of Neutrophilia (that was observed among ESRD patients) and is an inflammatory response that inhibits immune system response by suppressing the activity of immune cells such as lymphocyte, activated T cells, and natural killer cells (Manal and Shaimaa, 2015; Keizman et al., 2012). In addition, lymphocyte adheres to the inflamed vascular epithelial cells (Pecoits-Filho et al., 2002) and their concentration is reduced in the peripheral blood system.

Conclusion

In a study conducted in Adamawa State in Northeastern Nigeria, the mean lymphocyte count, packed cell volume, hemoglobin and serum calcium level were significantly lower and values of neutrophil and NLR were significantly higher in ESRD patients undergoing hemodialysis as compared to clinically healthy subjects. Moreover, lymphocyte count was negatively correlated with NLR value and positively with serum calcium level indicating that, calcium may play an important role in lymphocyte kinetics in ESRD patient receiving maintenance hemodialysis in Northern Nigeria.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviations: EDTA, Ethylene diamine tetraacetic acid; NLR, neutrophil-to-lymphocyte ratio; ESRD, end stage renal disease; PLR, platelet-to-lymphocyte ratio.

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Full Length Research Paper

Categorization of relative risk of diseases with relation to stress, body mass index and anthropometric markers: A cross-sectional study in general population of Karachi, Pakistan

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This study aimed to evaluate stress among general population of Karachi, Pakistan and to categorize the relative risk of diseases through body mass index (BMI), as well as to categorize the relative risk of diabetes through questionnaire based scoring pattern. Study consisted of two phase study duration. Total 377 individuals were approached; only 269 individuals completed the study procedures. In phase-I, data was collected through standardized questionnaire of American Institute of Stress (AIS), to determine total stress scores (TSS). In phase-II, anthropometric markers (e.g., body weight, height, waist and body mass index) were measured, to evaluate the Relative Risk of Disease (RrD). With the aid of socio-demographic variables and questionnaire, the relative risk of diabetes mellitus (RrDM) was also quantified. 71% was the participation rate, in which 51.3% were male and 48.7% were female. Cronbach's alpha (α) value was 69%. The mean scores of TSS (57.31 ± 5.28) were moderate. Averages of body weight, height, waist and body mass index (BMI) were 164.6 ± 13.5 , 1.67 ± 0.06 , 35.75 ± 2.5 , 27.35 ± 1.8 , and RrDM (34.17 ± 7.8), respectively. Through categorizing the RrD, 87.7% respondents were found to have increased risk, whereas, a least proportion were with high risk. Spearman R coefficient of gender variables showed indirect significant ($p \leq 0.01$) association with RrDM score. Pearson r coefficient of age variable showed direct significant ($p \leq 0.01$) association with RrDM score. Findings concluded that moderate increase of TSS with increase RrD leads to moderate existence of RrDM.

Key words: Diabetes mellitus, Karachi, body mass index, metabolic diseases, Pakistan, stress.

INTRODUCTION

Social, physical and genetic consequences have added vast impacts on human body which have reported with high risk of different diseases (Repetti et al., 2002; Shonkoff et al., 2009). These risk of diseases generally

may relates with accumulation of chronic stress (Salleh, 2008; Azmi et al., 2010), increase body mass index (BMI) (Megis et al., 2006), with different pathological scenarios ultimately resulting to various metabolic diseases or

disorders (Mazzucato et al., 2014).

Common metabolic diseases such as hypertension and diabetes mellitus (DM) progress side by side (Konzem et al., 2002; Rydén et al., 2007), with almost similar causes and consequences (Rydén et al., 2007). DM characterized by the dysfunction in the metabolic mechanisms of carbohydrates, lipids and amino acids (Kolluru et al., 2012), whereas hypertension is characterized by persistently elevated blood pressure (Konzem et al., 2002). Worldwide, there is an increasingly high prevalence of DM from 108 million in 1980 to 422 million in 2014 (Zhou et al., 2016) and the prevalence of hypertension from the latest studies reported around 25% in Pakistan (Neupane et al., 2014).

In present era of modern global industrialization, the relationship between stress, increase weight gain, and other metabolic diseases is the subject of many diseases (Foss and Dyrstad, 2011). These studies link DM to depression, socio-economic status (Pampel et al., 2010), sedentary life style, smoking, etc. (Cameron et al., 2003; Booth et al., 2012; Tassaduqe et al., 2004). Furthermore, these studies also indicate that many of these adults are unaware (that is, undiagnosed) they have DM or associated risk factors. In adults the relation of stress with DM (type 2) is more specifically estimated in those who have previous standing with undiagnosed DM, with prominent escalation in body weights, serve as the most significant risk factors for risk of DM and metabolic diseases (Gray et al., 2015). Early detection of DM (undiagnosed) or identifying the risk of developing DM (that is, specifically type 2) is highly important to improve the quality of life, as well as reducing the burden of associated morbidities and co-morbidities, in any society (Marshall and Flyvbjerg, 2006).

In 2015, a national research survey estimating diabetic prevalence in Mongolia revealed that the majority of the population was unaware of their present health conditions, and that more than one half of the diagnosed population were not being treated (Dugee et al., 2015). In addition, categorization of stress with DM is often underestimated (Djindjic et al., 2012). In Pakistan, to date the categorization of diseases risk through anthropometric markers and questionnaire based scoring system with relation to the assessment of stress was not reported. Therefore, the present study was carried out to categorize the relative risk of disease with stress, BMI and relative risk of DM among general population of Karachi, Pakistan.

STUDY METHODS

Design of the present study was cross-sectional. The study

consisted of two (02) phase duration. In both phases, data were collected through questionnaire based interviews from the respondents, comprising the general population of Karachi, Pakistan. Enrollments of participant for this study were entirely independent with respect to their age, gender, marital status, qualification levels and race. Study duration was December 2014 to January 2016.

Ethics consideration

Prior to the start of the research, all participants were given a brief overview about the impact of this study as well as the necessary concerned information regarding the nature of this work. In addition to this, informed verbal consent was also obtained from all participants before handing over the survey instruments. Declaration of confidentiality of all their shared information as well as their personal identity was also provided.

Sample size

Sample size was calculated with the help of previously published study reported by Wahed and Hassan (2017). The minimum sample size for this study was equivalent to 377 respondents which was computed by adjusting the margin of error (d) at 5%, confidence of interval on 95%, considering the recommended population size (20000) with at least 50% response distribution, significance level of 0.05 and the power of the study was 80%.

Exclusion criteria

The following individuals or groups of people were excluded from participation in present study: individual under 18 years of age; unable to provide informed consent; pregnant females; individuals with known chronic diseases such as diabetes mellitus, coronary disease or coronary disease equivalent, etc.; any past record of psychiatric disease, intellectual deficiency, poor motivation; any sign pertinent to alcohol or cocaine use, or other conditions that would limit the validity of the informed consent, or confound the interpretation of the study; and any kind of association or affiliation with study investigators or investigational site.

Phase I

In this phase, workplace stress was calculated in term of Total Stress Score (TSS) through a pre-tested and standardized questionnaire on job stress, which was used as tool to collect data from all respondents. This research instrument was easily accessible through American Institute of Stress (AIS) and used in various researches to calculate the professional stress (Khan et al., 2013). Prior to the start of this study, special permission has been taken for the use of the aforementioned questionnaire from the AIS officials. Questionnaire (tool) for the assessment of stress consisted of total of ten (10) variables. Responses to all variables were equally scored on 10 points Likert scale (1 to 4 = strongly disagree, 5 to 7= Agree somewhat and 8 to 10 = strongly agree) with the statement. However, after the completion of phase I (with consent, JS tool was distributed to the participants and after completion of all information it was collected and recorded

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in computerized sheet), the aggregated TSS score were also classified in three distinct and even categories as, the respondents which had TSS score between 10 and 30 have handle professional stress well; the respondents which had TSS score between 40 and 60, have handle job stress moderately well and the respondents which had TSS score between 70 and 100, have encountering problems that need to be addressed and resolved.

Phase II

After the completion of phase I (approximately one week of duration), respondents were again approached for the completion of phase II of the study. In this phase, waist was measured in inches, body weight was measured in kilograms (kg) and converted to pounds (lbs) and height was measured initially in feet' inches and converted to meters (m). BMI (kg/m^2) was calculated with the help of the formula mentioned earlier by Qureshi et al. (2011). Age, gender, marital status, academic qualification, employment status with experience, economic profile and ethnic origin were investigated as socio-demographic variables from each respondent.

Thereafter, a questionnaire was designed based on the internationally pretested variables used for the categorization of the risk of diabetes (Zhang et al., 2014; Stiglic and Pajnikihar, 2015), which after modification was used as a tool for the categorization of the relative risk of diabetes mellitus (RrDM), among the general population. The questionnaire consisted of a total of 14 different variables and total aggregated score of all variables was 80. The questionnaire for the categorization of RrDM takes into account: age, BMI, waist circumference, routine of exercise habits, dietary habits (e.g., routine of vegetables intake), medication for hypertension, history of hyperglycemia, and family diabetes history (Siren et al., 2012). This tool was also administered with same manner as mentioned in phase I.

Categorization of RrD and RrDM

RrD was categorized with reference to BMI in five grades as; individual with $<24.9 \text{ kg}/\text{m}^2$ has no risk (0 point), 25 to $29.9 \text{ kg}/\text{m}^2$ has increased risk (1 point), 30 to $34.9 \text{ kg}/\text{m}^2$ has high risk (2 points), 35 to $39.9 \text{ kg}/\text{m}^2$ has very high risk (3 points) and $>40 \text{ kg}/\text{m}^2$ has extremely high risk. RrDM was categorized after the successful completion of RrDM questionnaire. The total obtained score was further grade into three even categories from low risk to high risk. The total RrDM obtained score between 01 and 27 units with 'Low Risk of Disease' subject. The total RrDM obtained score between 28 and 54 units with 'Intermediate Risk of Disease' subject, while, the total RrDM obtained score between 55 and 80 units with 'High Risk of Disease'.

Data analysis

Data were entered in Microsoft 2010 Excel Workbook and statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 16.0, statistical software for Windows version 7 professional and through online *GraphPad* Software, Quick Calcs Online Calculator for scientist. Frequencies and percent frequencies of the respondents were computed on the basis of gender for categorical variables like age group, marital status, academic qualification, affiliation, economic status and ethnic classification. Depending on the distribution of gender as well as the total obtained scores and the measurement of the anthropometric markers, viz., TSS, RrDM, body weight, height, waist measurement, and BMI, RrD for respondents were presented as mean with standard deviation (SD), median and interquartile range (IQR), respectively. In addition to this, data were also

represented in quartile series on the basis of the obtained scores and the values of the selected anthropometric markers, and to with help of independent sample t-test significance among these quartiles with aggregated scores and values was also verified. Data were correlated through Pearson's and Spearman's method (two tailed) depending on distribution of variables in order to test the correlation between selected variables with RrDM scores. Cronbach's Alpha was calculated for detecting the consistency among the responses of the both study instrument. Results were considered significant when P value < 0.05 .

RESULTS

Participants' enrollment

A total of 377 individuals were approached. After the completion of both aforementioned phases, only 269 respondents completed the study questionnaires through interviews. 28 individuals had regretted their availability in the phase I of the study while 42 individuals had regretted their availability in phase II. However, 39 did not report their response absolutely on the study instruments. Overall, 109 respondents were excluded from this study (Figure 1).

Demographic characteristics of participants

Of the total 269 study respondents, 138 (51.3%) were male and 131 (48.7%) were female. Age groups described that under 25 years of age limit, only one female was available. In age range from 26 to 35 years, 41 (29.71%) were male and 30 (22.9%) were female. Age group range from 36 to 45 years had 55 (39.86%) male and 52 (39.7%) females and age group of 46 to 55 years had 24 (17.39%) male and 23 (17.56%) female. However, in age group of 56 years and above 18 (13.04%) were male and 25 (19.08%) were female. Of 138 total male, 68 (49.3%) were single and 70 (50.7%) were married. From total 138 females, 67 (51.1%) were single and 64 (48.95) were married. 13 (9.42%) males and 9 (6.9%) females were uneducated, 20 (14.5%) males and 11 (8.4%) females were upto 8th grade. 39 (28.3%) males and 44 (33.6%) females were upto 10th grade qualified and 66 (47.82%) males and 67 (51.1%) females completed their college/university qualification. 36 (26.1%) males were unemployed and 52 (39.7%) females were housewives. 38 (27.5%) males, 25 (19.1%) were students and 64 (46.4%) males and 54 (41.2%) females were professionally employed. With reference to economic status, 18 (13.04%) males, 9 (6.9%) females belongs to lower middle class, 49 (35.5%) and 47 (35.9) females belongs to middle class. 47 (34.1%) males, 47 (35.9%) females belongs to upper middle class and 24 (17.4%) and 28 (21.4%) females belongs to upper class. Classifying the respondents with reference of ethnic origin, 72 (52.2%) males, 62 (47.3%) females were Urdu speaking, while, 17 (12.3%) males, 15 (11.5%) females

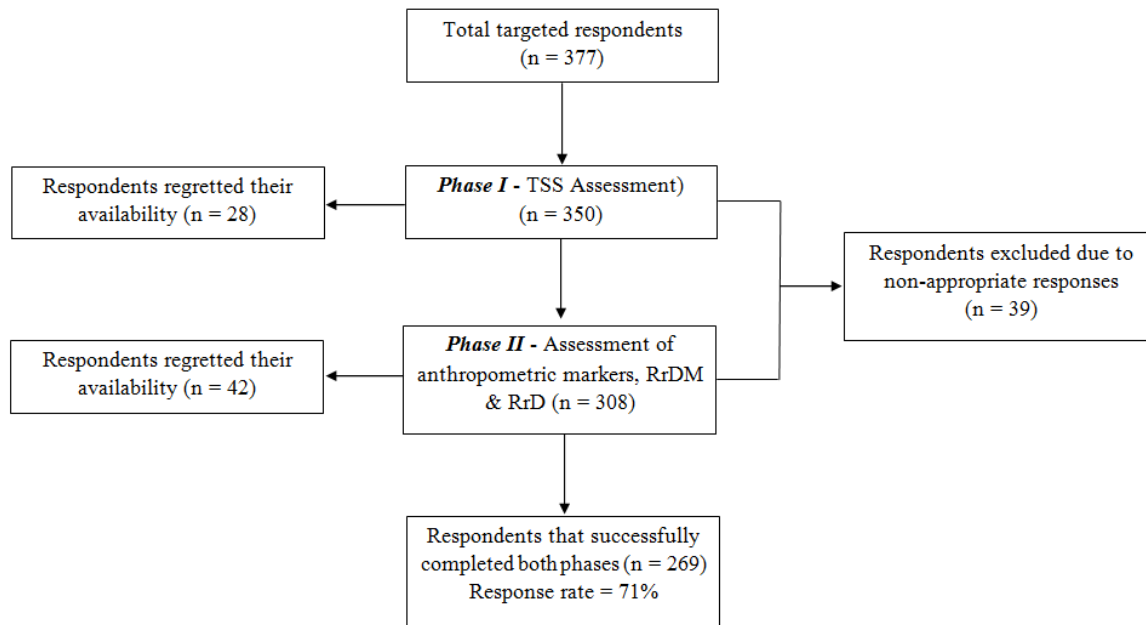


Figure 1. Participants' enrollment.

were Sindhi speaking. 11 (8%) males, 17 (13%) females were Balochi speaking, 16 (11.6%) males, 15 (11.5%) females were Pathani speaking, 22 (15.9%) males and 22 (16.8%) females were Panjabi speaking (Table 1).

Comparative analysis of TSS and RrDM

The mean total of TSS was 57.31 ± 5.28 , with median of 57 and IQR of 54-61. Comparatively male and female respondents have 56.92 ± 5.27 and 57.74 ± 5.28 , with median and IQR of 56, 53-61, 58, and 54-62, respectively. The score units of quartiles 1st and 4th showed high ($p \leq 0.0001$) significance while 2nd and 3rd quartiles showed significance ($p \leq 0.05$ and $p \leq 0.001$), respectively, when compared with the aggregated TSS (Table 2).

The mean total units of RrDM was 34.17 ± 7.8 , with median and IQR of 33 and 29-38, respectively. Comparatively, male and female have 35.84 ± 7.46 and 32.41 ± 7.79 , whereas median and IQR range were 35, 30-40.25, 31, and 27-37 units, respectively. The score units of quartiles 1st and 4th showed high ($p \leq 0.0001$) significance while 2nd and 3rd quartiles showed significance ($p \leq 0.001$ and $p \leq 0.05$), respectively, when compared with the aggregated RrDM (Table 2).

Comparative analysis of anthropometric markers

The mean total of body weight was 164.6 ± 13.5 , with median and IQR of 165 and 147.3-182, respectively. Comparatively, male and female have mean body weight

of 172.63 ± 10 and 156 ± 11.34 , whereas median and IQR range were 171.5, 167-181, 156, and 1.58-1.67, respectively. The mean weights of quartiles 1st and 4th showed high ($p \leq 0.0001$) significance while 2nd and 3rd quartiles showed significance ($p \leq 0.05$) respectively, when compared with the aggregated mean body weight (Table 2).

The mean total of body height was 1.67 ± 0.06 , with median and IQR of 1.67 and 1.64-1.73, respectively. Comparatively, male and female have mean body height of 1.72 ± 0.05 , 1.64 ± 0.05 , whereas median and IQR range were 1.71, 1.67-1.76, 1.65, and 1.58-1.67, respectively. The mean height of quartiles 1st and 4th showed high ($p \leq 0.0001$) significance while 2nd and 3rd quartiles showed significance ($p \leq 0.001$ and $p \leq 0.05$), respectively, when compared with the aggregated mean body height (Table 2).

The mean total of waist measurement was 35.75 ± 2.5 , with median and IQR of 36 and 34-38, respectively. Comparatively, male and female have mean waist measurement of 37.5 ± 1.8 and 33.9 ± 1.8 , whereas median and IQR range were 37, 36-38, 34, and 33-35, respectively. The mean waist measurement of quartiles 1st, 3rd and 4th showed highly ($p \leq 0.0001$) significance, when compared with the aggregated mean waist measurement (Table 2).

The mean total of BMI was 27.35 ± 1.8 , with median and IQR of 27.4 and 26.2-28.5, respectively. Comparatively, male and female have mean BMI of 27.5 ± 1.7 and 27.2 ± 1.8 , whereas median and IQR range were 27.6, 26.5-28.7, 27.1, and 25.9-28.1, respectively. The mean BMI of quartiles 1st and 4th showed high ($p \leq 0.0001$)

Table 1. Demographic characteristics of participants.

Variable	Male [138 (51.3)]	Female [131 (48.7)]
Age groups		
Under 25 years	0	1 (0.76)
26 - 35 years	41 (29.71)	30 (22.9)
36 - 45 years	55 (39.86)	52 (39.7)
46 - 55 years	24 (17.39)	23 (17.56)
56 years and above	18 (13.04)	25 (19.08)
Marital status		
Single	68 (49.3)	67 (51.1)
Married	70 (50.7)	64 (48.9)
Academic qualification		
Uneducated	13 (9.42)	9 (6.9)
Upto 8th grade	20 (14.5)	11 (8.4)
Upto 10th grade	39 (28.3)	44 (33.6)
College/University	66 (47.82)	67 (51.1)
Affiliation		
Unemployed/House wives	36 (26.1)	52 (39.7)
Students	38 (27.5)	25 (19.1)
Employed/Professional	64 (46.4)	54 (41.2)
Economic status		
Lower middle class	18 (13.04)	9 (6.9)
Middle class	49 (35.5)	47 (35.9)
Upper middle class	47 (34.1)	47 (35.9)
Upper class	24 (17.4)	28 (21.4)
Ethnic classification		
Urdu speaking	72 (52.2)	62 (47.3)
Sindhi speaking	17 (12.3)	15 (11.5)
Balochi speaking	11 (8)	17 (13)
Pathani speaking	16 (11.6)	15 (11.5)
Panjabi speaking	22 (15.9)	22 (16.8)

Data represent n (%) of study respondents.

significance while 2nd and 3rd quartiles showed significance ($p \leq 0.05$) respectively, when compared with the aggregated mean BMI (Table 2).

Categorization of RrD

Distribution in categorizing the RrD with reference to BMI, majority of male and female respondents, that is, 89.9 and 85.5% respectively were at increased risk. The average count of total population was 87.7%, which were at increased risk. 5.8% in male and 6.9% in female were with high risk and few, that is, 4.3 and 7.6% have no risk (Table 3).

Correlation between RrDM with demographic, TSS, RrD and other variables

Spearman correlation of RrDM with demographic factors, that is, gender showed significant ($p \leq 0.01$) negative correlation, however, a weak positive association was depicted with change in marital status. Almost economic profile of respondents showed zero correlation with RrDM, as the computed Spearman (R) coefficient was very least (0.002) in this case. RrD with relation of RrDM showed a significant ($p \leq 0.01$) positive association, as it validates the general influence of the increasing RrDM units that have increased the relative risk of disease (Table 4).

Table 2. Comparative analysis of Total Stress Score (TSS) and Relative Risk of Diabetes Mellitus (RrDM) and anthropometric markers among general population of Karachi, Pakistan.

Characteristics	Q1	Q2	Q3	Q4
TSS	51.35 (2.37)/52 (50-53)* (n= 82)	56 (0.74)/56 (55-57)*** (n=63)	59.42 (1.15)/59 (58-60)** (n=64)	64.75 (2.38)/65 (63-66)* (n= 59)
RrDM	25.53 (2.83)/27 (23-27)* (n=73)	31.38 (1.21)/31 (30-33)** (n=69)	35.92 (1.42)/36 (34-37)*** (n=61)	45.11 (4.67)/44 (41-49)* (n=66)
Body weight (lb)	147.3 (6.37)/148 (143-153)* (n=72)	160.9 (2.44)/161 (159-163)*** (n=66)	169 (2.2)/169 (167-171)*** (n=64)	182 (3.9)/182 (179-185)* (n=67)
Height (m)	1.58 (0.02)/1.58 (1.58-1.61)* (n=66)	1.65 (0)/1.64 (1.64)** (n=45)	1.69 (0.01)/1.7 (1.67-1.7)*** (n=88)	1.76 (0.02)/1.76 (1.73-1.76)* (n=70)
Waist measurement (inch)	32.8 (1.1)/33 (32-34)* (n=85)	35.6 (0.5)/36 (35-36) (n=86)	37.6 (0.5)/38 (37-38)* (n=63)	39.9 (1.1)/40 (37-38)* (n=35)
BMI (kg/m ²)	25.1 (0.9)/25.3 (24.85-25.8)* (n=68)	26.88 (0.34)/26.9 (26.6-27.1)*** (n=71)	27.88 (0.3)/27.8 (27.6-28.1)*** (n=67)	29.7 (1.1)/29.6 (28.9-30)* (n=63)
		Male (n=138)	Female (n=131)	Total Score (n=269)
TSS		56.92 (5.27)/56 (53-61)	57.74 (5.28)/58 (54-62)	57.31(5.28)/57 (54-61)
RrDM		35.84 (7.46)/35(30-40.25)	32.41 (7.79)/31 (27-37)	34.17 (7.8)/33 (29-38)
Body weight (lb)		172.63 (10)/171.5 (167-181)	156 (11.34)/156 (148-162)	164.6 (13.5)/165 (156-174)
Height (m)		1.72 (0.05)/1.71 (1.67-1.76)	1.64 (0.05)/1.65 (1.58-1.67)	1.67 (0.06)/1.67 (1.64-1.73)
Waist measurement (inch)		37.5 (1.8)/37 (36-38)	33.9 (1.8)/34 (33-35)	35.75 (2.5)/36 (34-38)
BMI (kg/m ²)		27.5 (1.7)/27.6 (26.5-28.7)	27.2 (1.8)/27.1 (25.9-28.1)	27.35 (1.8)/27.4 (26.2-28.5)

Values in each column represent means (SD)/median (IQR), respectively. Q1, Q2, Q3 and Q4 are the quartile 1st, 2nd, 3rd and 4th. ***Indicates p value ≤ 0.05 ; **Indicates p value ≤ 0.001 ; *Indicates p value ≤ 0.0001 when each quartile values (mean and SD) were compared with their respective total scores/values (mean and SD), by using independent sample t-test.

Pearson correlation of RrDM with demographic factor, that is, age showed significant ($p \leq 0.01$) positive correlation, as with increasing age, the RrDM unit score was also increased in both gender respondents. Similarly, correlation between TSS and RrDM also depicted positive association, as the person (r) coefficient indicated the positive correlation (0.122) between these two variables. However, a weak positive (0.097) association was also depicted with change in BMI status with any increase in RrDM score (Table 4).

DISCUSSION

Timely screening of individual through anthropometric markers and questionnaire based tools set the cost-effective way to manage strategically the primary care disease screening program

(Meigs et al., 2006; Qureshi et al., 2011; Stiglic and Pajnikhar, 2015). Worldwide, many questionnaire based tools exist to evaluate the risk of developing chronic metabolic disease like diabetes mellitus in their population (Stiglic and Pajnikhar, 2015). These tools can easily be available in pencil-paper based questionnaire assessment or in capacity of online risk assessment surveys to categorize RrD. The role of the authentication of biochemical screening tools like Sugar Tolerance Test (OGTT), estimation of glycated hemoglobin (HbA1c), insulin monitoring, etc., served as confirmatory analyses that rule the findings (Alqahtani et al., 2013). But as a preliminary assessment model, the importance of categorizing the relative risk of disease through pertinent scoring of available and essentially required information received from individual without any biochemical diagnostic

estimation or endocrine assays establish a predictive assessment model that aid the healthcare system with very least cost specially for under-developed countries (Sacks et al., 2011; Stiglic and Pajnikhar, 2015).

As a developing country, general population of Pakistan faced many socioeconomic burden like poverty, high cost for standardized education, health and nutrition to community, more surprisingly very least population size has access to healthcare facilities which has been reported in the past (Shaikh and Hatcher, 2005; Jalal-ud-Din, 2014). Substantial researches contributed in reporting the high prevalence of stress as well as dependently or independently linked elevated glucose level with sedentary life-style more likely establishes a link to have type2DM and other associated disease risk in future life status (Warren et al., 2010; van der Berg et al., 2016).

Table 3. Relative risk of disease with reference to BMI.

BMI categorization	Male (n = 138) (%)	Female (n = 131) (%)	Total (n = 269) (%)
<24.9 kg/m ² (No risk)	6 (4.3)	10 (7.6)	16 (5.9)
<25 - 29.9 kg/m ² (Increase risk)	124 (89.9)	112 (85.5)	236 (87.7)
<30 - 34.9 kg/m ² (High risk)	8 (5.8)	9 (6.9)	17 (6.3)

Values represent n (%) of study respondents.

Table 4. Correlation between RrDM with demographic, TSS and other variables.

Variable	Spearman R	Pearson's r	Cronbach's (α) alpha (All variables)	P-value
Gender	-0.248**	-		0.0001
Marital status	0.034	-		0.576
Economic profile	0.002	-		0.979
Relative risk of disease (RrD)	0.179**	-	0.69 or 69%	0.003
Age	-	0.576**		0.0001
TSS	-	0.112		0.066
BMI	-	0.097		0.111

**Correlation is significant at the 0.01 level (2-tailed) when compared with RrDM.

Therefore, in countries like Pakistan public perspective regarding the adaptation of preventive strategies needs to be strengthened to become a part of national strategic processes with prime focus for cure purposes. The purpose of this study was to investigate or to categorize the risk of diabetes and other diseases through simple questionnaire based approach with relevance to stress among the individuals of Metropolitan Karachi, with substantial participation of individuals from most communities (ethnicity) were involved. The socio-economic profile of respondents stratified the maximum participation of respondents from middle class economic group and studies had documented the relation of poor socioeconomic status and the associated complications (which might contribute the cause of undiagnosed diabetes), eventually result in all of society being affected (Rabi et al., 2006). Society benefits when the basis of socioeconomic inequity become a greater focus of attention and increased efforts are made to minimize the vast breaches in socioeconomic status as well as to manage the health related hazards worldwide (Baum et al., 1999; Azmi et al., 2010).

Inequities in wealth distribution, resource distribution, and quality of life are increasing and these imbalances are steadily becoming a global concern. A comparatively new theory has surfaced that these effects are due to chronic stress that is associated with socioeconomic status; lower socioeconomic status has been consistently affiliated with various significant social and environmental conditions that add to the burden of chronic stress which if undiagnosed or non-treated is linked with diabetes and other diseases (Steyn and Damasceno, 2006). The first

phase of this study estimated the TSS in all respondents which showed the presence of moderately elevated level of stress, which in general reflects the advancement in psychosocial and professional aspects of life, with more demanding daily routine. Researches interrelated the impact of socioeconomic stress with commonly standing health problems (hypertension diabetes and other dyslipidemic complications), and with health-impairing behaviors and in future the probability of the increment of this TSS may also encounter health related problems in individuals (Tamashiro et al., 2011). Another aspect to contemplate is the stress on females in society who are expected to raise their children and manage their work life simultaneously, which could be a further source of stress (Poduval and Poduval, 2009).

Probing the premise mechanism for studying the impact of stress with relevance to undiagnosed diabetes; this relates stress to triggers the hypothalamic pituitary-adrenal axis and the sympatho-adrenal-medullary axis (Smith and Vale, 2006). Sequentially, this biochemical connection results in an increased blood pressure and elevated level of blood glucose, the purpose of which is to prompt a fight-or-flight response situation (Gerra et al., 2001; Foss and Dyrstad, 2011). Moreover, prolong accumulation of stress also causes cortisol levels to remain consistently high (Kirschbaum et al., 1995); subsequently, it promotes the hypertension, accumulation of body fat, and persistent standing leads to insulin resistance and dyslipidemia (Azmi et al., 2010).

The linkage between work stress and diabetes could partly be facilitated by gain in body weight and it was previously well correlated that sedentary and unhealthy

life style directly associated with increased consequences in obesity and development of secondary risk factors in individual (Steyn and Damasceno, 2006; Foss and Dyrstad, 2011). Similarly, the present effort also identified the presence of increase in body weights in male as compared to female respondents. This may be linked as nowadays, everyone has faced more demanding professional environment, that consistently generate stress by disturbing the circadian rhythms, having intake of energy (in term of high fat and carbohydrate) with least physical activeness serve as a crucial risk factor in this regards (Johnston, 2014). Beside this, an international research in 2012 claims that assessment of waist circumference is also characterized as an identifying tool for assessing the increased risk for type 2 diabetes (Siren et al., 2012). In the present work, the increase waist measurement (both in male and female) establishes the interfering impact of stress in respondents that not only relate with gain in body weights but also in waist circumference, as abdominal obesity is more specifically connected as a physical investigative anthropometric tool to characterize metabolic risk (Chan and Woo, 2010; Meigs et al., 2006).

In the present findings, the BMI results suggested that on average, the respondents (both genders) were overweight. Term 'overweight' in categorization of BMI was classified as the increased risk status that substantially increases lifetime risk of diagnosed diabetes (Meigs et al., 2006). Moreover, Ganz et al. (2014), strongly correlated the levels of BMI with risk of being diagnosed with T2DM, as individual with higher BMI has higher risk of T2DM as compared with a lower BMI (Ganz et al., 2014).

The existing American Diabetic Association (ADA) guidelines for type 2 diabetes screening among the asymptomatic population are based on laboratory testing (American Diabetes Association, 2013). However, there is always a trade-off between simplicity and accuracy for each screening method. Therefore, a much more cost-effective and practical process would be to use a valid, basic questionnaire as a preliminary screening method, followed by more invasive and accurate diagnostic tests in primary care and/or community settings. Globally, various diabetic risk assessment tool is a means to predict the future risk of diabetes based on certain known or self-assessed risk (physical) factors that mainly includes age, sex, ethnicity, parental history of diabetes, history of elevated blood glucose level, use of antihypertensive medications, smoking, physical inactivity, waist circumference, body weight and some others (Christian et al., 2009; Siren et al., 2012; Stiglic and Pajnikihar, 2015). Similar tool from Australia, predicts five-year risk of diabetes, and the risk of developing diabetes over a longer period would be greater (Chen et al., 2010; Adegbija et al., 2015). In a published study in 2015 with reference to Asian population, it was reported that these global tools were not directly adopted without preliminary validation and after the modification its

effectiveness in term of reliable screening in undiagnosed diabetes were also reported as Mongolian population (Dugee et al., 2015). In 2012 and 2015, a diabetes risk score based study was reported that established the mechanism for identification of high risk individuals for early intervention to delay or prevent type 2 diabetes in Pakistani population (Riaz et al., 2012; Basit et al., 2015). In the present research, with the aid of modified tool and methodologies, the respondents were identified at intermediate risk, with increased risk for relative risk of disease which may also increase with certain passage of time in life.

Conclusion

The present findings conclude that increased RrD was linked with intermediate stress and RrDM obtained scores, among general population of Karachi. In future, this type of assessment should be more probed and extended with reference to biochemical diagnostic markers. Proper planning for health counseling and life-style based interventions needs to be establish.

Study limitation

The following were the limitations of study: questionnaires were used to collect data; assurance for the accuracy of response provided by respondents was also considered as a limitation; the size of sample taken from study subjects; response accuracy and data validation; cultural and other type of differences; language differences while dealing with the respondents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Full Length Research Paper

Knowledge, attitudes and practices towards rabies in Dedo district of Jimma zone, southwestern Ethiopia: A community based cross-sectional study

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Rabies is a fatal neglected viral zoonosis which causes encephalitis in many warm-blooded animals and humans. It is an incurable disease once the clinical signs appear. However, it can be prevented via vaccination and community awareness. This study was therefore designed to determine the level of community knowledge, attitude and practices (KAP) regarding rabies. Cross sectional study was conducted from December 2016 to January 2017 in Dedo district, Jimma zone, southwestern Ethiopia. Multistage sampling technique was employed for selection of sample unit and data were collected from 135 household by face-to-face interview using a semi structured and validated questionnaire. Logistic regression was used for assessing the association of independent variable with KAP score. Out of 135 participants interviewed, 71.1% were urban and 57% were male respondents. All (100%) participants had heard about rabies and 88.9% were aware that rabies is zoonotic. However, there were misperceptions about the causes, transmission and incubation period of rabies. Overall KAP score revealed that 51.9% of the respondents had a good KAP, whereas 48.1% was found to have poor KAP score. Multivariable analysis showed that a good KAP score was significantly higher in male than female (OR= 37.16, CI=4.7- 92.1, $p= 0.001$), in age group >46 years than 15 to 30 years (OR=8.9, CI=1.6-47.2) in urban than rural residents (OR=4.35, CI=1.3-14.14, $p= 0.014$), education ($P= 0.040$), in respondent who were at secondary school education level than illiterate (OR=7.7, CI=1.05 - 56.64), in dog owners than those who had no dog (OR=2.9, CI=1.12-7.93, $P=0.029$), and in trained respondents than non-trained respondents (OR=3.37 CI=1.17-9.69, $p=0.024$). Generally, the overall, KAP score recorded in this study is an indication of low overall awareness level in the study area. This reflects the urgent need for rabies awareness programmes within the community. Thus, close collaboration of public health, veterinary sector and local authorities is a key element for preventing this fatal incurable disease.

Key words: Attitudes, knowledge, rabies, practices, Dedo.

INTRODUCTION

Rabies is an acute viral infection of the central nervous system, caused by a lyssavirus in the family Rhabdoviridae (Nilsson, 2014). It is a fatal neglected viral zoonosis which causes encephalitis in many warm-

blooded animals and humans. This virus is a highly neurotropic pathogen that typically leads to mortality of infected animals and humans. Globally, it is estimated that at least 55,000 people die of rabies each year

(Andrea and Jesse, 2012). Wild animals serve as a large and mainly uncontrollable reservoir of sylvatic rabies, which is an increasing threat to the human population and domestic animals in many countries (Rupprecht et al., 2002). Worldwide, the dog is the principal vector in transmitting rabies to human, irrespective of whether the reservoir hosts are wild animals or dogs themselves (Williams and Barker, 2001; Abbas et al., 2011).

The virus is present in the saliva of affected animals, and the most frequent method of transmission to humans is by bites, scratches or licks from infected animals in open wounds or on mucosal membranes (Jackson, 2010; Depani et al., 2012; WHO, 2013; Nilsson, 2014). The clinical features of rabies are similar in most species, but there is great variation between individuals. Following the bite of rabid animal, the incubation period varies from 5 days to several years (usually 2 to 3 months; rarely more than 1 year) depending on the amount of virus in the inoculum, the density of motor endplates at the wound site and the proximity of virus entry to the central nervous system (WHO, 2013).

Rabies is one of the most neglected infectious diseases affecting mainly the low and middle income countries (Abbas et al., 2011). It constitutes a serious public and animal health problem in Africa including Ethiopia. In Ethiopia, rabies has been known for centuries in society as "Mad Dog Disease (Fekadu, 1997) and has been recorded scientifically since 1903 (Pankhrust, 1990). To date, rabies is an important disease in Ethiopia both in human and animals (Tschopp et al., 2002; Deressa et al., 2010; Yimer et al., 2016; Teklu et al., 2017). In four-year retrospective study by Teklu et al. (2017) in Northwestern Tigray, the incidence of human rabies exposure cases calculated per 100,000 populations was 35.8, 63.0, 89.8 and 73.1 in 2012, 2013, 2014 and 2015, respectively.

Rabies is incurable once the clinical signs of the disease appear (Deressa et al., 2010). However, it is possible to prevent a person exposed to the virus from getting ill to rabies by neutralizing virus with antibodies before the virus invades the nervous tissue. This is done through vaccination and/or use of immunoglobulins, so called post-exposure prophylaxis (PEP) (Permpalung et al., 2013; Nilsson, 2014). Controlling rabies in dogs, and especially free-roaming (stray) dogs, is also the first priority for prevention of human rabies (Nilsson, 2014). Moreover, successes of rabies control through mass vaccination of dogs have been reported in South America and Asia (Abbas et al., 2011). Vaccinating 70% of the dog population will eliminate dog rabies and hence human rabies (Cleaveland et al., 2014).

Community awareness about rabies has significant role in rabies prevention and control (Chernet and Nejash,

2016; Abdela and Teshome, 2017). Knowledge, attitudes and practices (KAP) studies have been used widely to enhance community knowledge and thus change attitude and improve practices that may aid in disease prevention and control (Sambo et al., 2014; Tiembré et al., 2014; Digafe et al., 2015; Abdela and Teshome, 2017). Despite the endemic nature of rabies in Ethiopia, little is known about level of community awareness in Ethiopia in general and in Dedo in particular. However, for efficiently increasing awareness, the knowledge gap among the community should be identified and targeted. Thus, this KAP study aimed at generating information that will help to identify knowledge gaps and act as baseline data for evaluation of community awareness; thereby helps in planning and implementation of rabies control programs.

MATERIALS AND METHODS

Study area

The study was conducted from December 2016 to January 2017 in Dedo district. Dedo is one of the district in Jimma zone of Oromia regional state, southwestern Ethiopia. It has an area of about 1459.1 km². Dedo shares common boundaries with Omo Nada, Kersa and Seka Chekorsa district, and regional state of Nations, Nationalities and Peoples of South Ethiopia (Figure 1). Dedo is located at distances of 12 km from Jimma town. Jimma town is the capital of jimma zone and found at distance 352 km from Addis Ababa, the capital of Ethiopia. Topographically, mountains, plateaus, hills, plains and valleys characterize the district. About 63.1, 13.6 and 9.3% of the district's total area were arable, grazing and forest lands, respectively. The altitude of this district ranges from 880 to 2400 m above sea level. Furthermore, this district is known by mixed crop – livestock agricultural production system. The 2007 national census reported a total population for Dedo district to be of 288,457, of whom 143,935 were men and 144,522 were women; 5,755 or 2% of its population were urban residents and majority of the inhabitants were Muslim, with 92.98% of the population.

Study methodology

Community based cross-sectional study was conducted from December 2016 to January 2017 to assess the knowledge, attitude and practice towards rabies in Dedo district. The community of Dedo district who are above 15 years and lived in the district for at least six months were included in this study. The required number of population to be sampled was calculated using the formula given by Arsham (2005). $N = 0.25 / SE^2$, Where N = sample size, S = standard error, 5%. Accordingly, the required sample size was 100. However, to increase the precision and representativeness, the sample size was increased, 135. The calculated sample was proportionally distributed to the selected kebele based on their number of households.

A multi-stage sampling technique was employed for the selection of the sampling units. From the entire study, population in the

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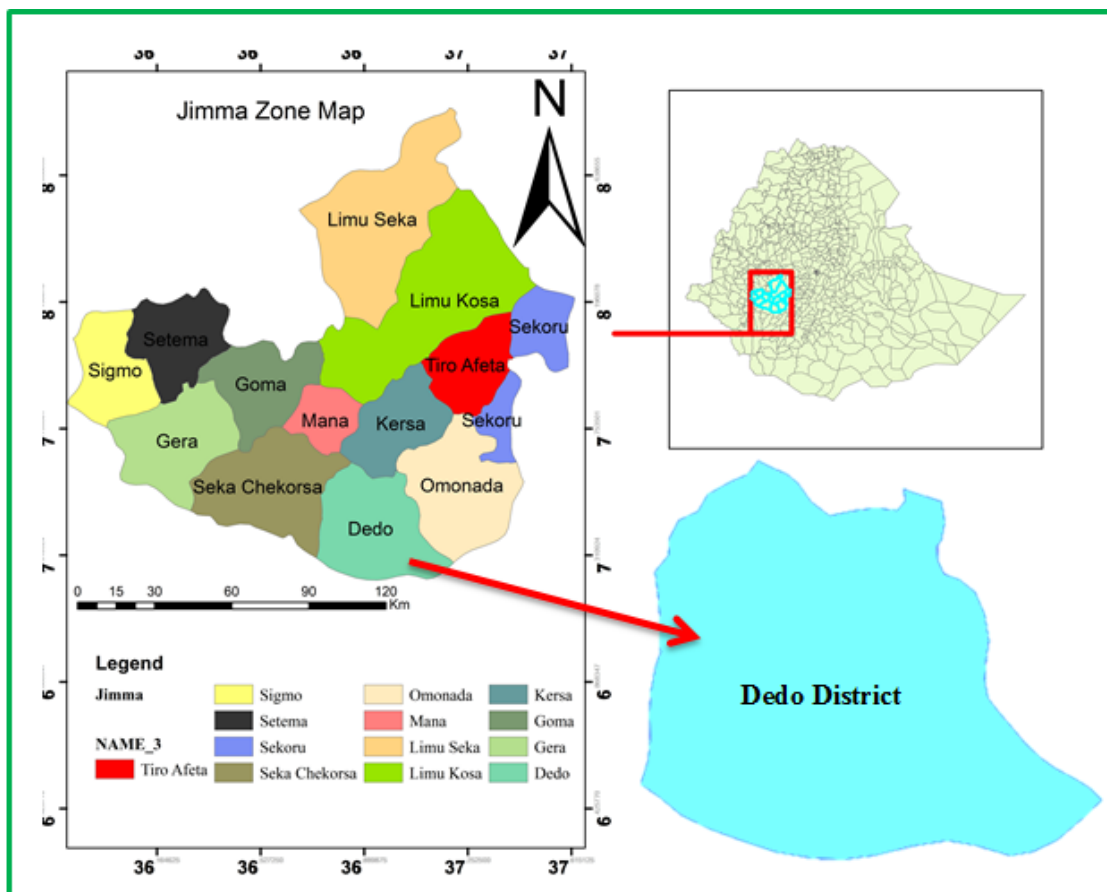


Figure 1. Map showing the study area.

district five kebeles (the smallest administrative unit) were selected by simple random sampling methods. Then, the household in the selected kebeles was further selected using a systematic random sampling technique. Finally, from all the eligible respondents in a household, only one was selected randomly for the interview. However, in the absence of eligible respondent in a given household, a replacement was immediately made by an individual in the next household until required sample size was obtained.

Data were collected by face-to-face interview by using a semi structured and pre-tested questionnaire. The questionnaire was first prepared in English and translated to Afaan Oromo for appropriateness and easiness in approaching the study participants. Before the interview begun, they were briefed about the purpose of the study and asked for their consent. Only voluntary participants were involved in the study. All the information obtained from the study participants was kept confidential.

For validation of questionnaire, a sample of 15 randomly selected individuals in the study area who are not included in the main study was used. The questionnaire was assessed for its understandability, clarity, completeness, reliability, and sociocultural acceptability and the edited as required. The questionnaire had different sections including socio-demographic factors like age, sex, marital status, religion, monthly income, educational status and occupation. Moreover, the questionnaire also incorporated questions concerning the knowledge about rabies aetiology, transmission, clinical manifestations, prevention and control measure.

For assessing the community knowledge, attitude and practices (KAP) about rabies each respondent were asked for twenty questions regarding cause, sources, mode of transmissions, clinical

signs, prevention practices and treatment measures. The questions were multiple choices question with both open and close ended. Respondents who answered the questions correctly had got one mark and zero for incorrect or do not know responses. Then, the responses for which respondents give correct answer was counted and scored.

This score was then pooled together and the mean score was computed to determine the overall KAP of respondents. Respondents who score greater than or equal to the mean value (Mean= 11.33, SD=2.185) were grouped to good KAP and coded as 1 whereas, the respondent who scored less than the mean value were grouped to poor KAP level and coded as 0.

Data management and analysis

After collecting, the data were cleaned and checked for its completeness and entered into Microsoft Excel 2010 spread sheet. The data generated were analyzed using the Statistical Package for Social Science (SPSS) Version 20.

The descriptive statistics was used for calculating frequency and percentage of both dependent and independent variable. Logistic regression was used for calculating the association between independent variables and dependent variable (KAP scores) of community regarding rabies. A 95% confidence interval of the OR and p-values were used to describe statistical significance associations. The association is judged as significant when p-value is less than 0.05.

RESULTS

Socio demographic character of the participants

A total of 135 community members were interviewed during the study period and all respondents were responded to the questioner. The majority of the respondents in this study were males 77 (57.0%), while the number of females was 58 (43.0%). From all participants of the study, about 83.0% (112) were married and 17.0% (23) were unmarried. The ages of respondents were classified as 15-30, 31-45 and above 46 years old, which comprises about 35 (25.9%), 62 (45.9%) and 38 (28.1%), respectively. The majority of the respondents were rural, 96 (71.1%) while that of urban was 39 (28.9%). Concerning the religion, the majority of respondents were Muslim 128 (94.8%), followed by Orthodox 7 (5.2%). Regarding the educational status of respondents, 51.1% were illiterate, 33.3% had elementary education, 11.9% had secondary education, and the rest 3.7% had tertiary education. With regards to the family size, about 53 (39.3%) participants were from family size of one to three persons and similarly most of the respondents, 57 (42.2%) were farmers and 72 (53.3%) had no defined income. From the whole respondents/participants (135) in this study, about sixty five (48.1%) had dogs while seventy (51.9%) respondents had no dogs. Socio demographic characters of the participants are summarized in Table 1.

Knowledge of respondents in relation to cause, host range, clinical signs and transmission of rabies

The knowledge on cause, host range, clinical signs and transmission of rabies were summarized in Table 2. All respondents (100%) had heard about rabies. However, only small proportion (13.3%) of respondents knew the causative agent of rabies. 39.3% of the participants responded that they do not know the causative agent and 40% were found to have misperception about causative agent which was starvation and thirst. The majority of study participants (57.0%) knew that rabies can affect both human and other domestic animals and mentioned dogs (61.5%) as a major source of rabies. Moreover, 88.9% of respondents knew that rabies can be transmitted from animal to human. However, most of respondents (51.9%) replied dog bite as the only transmission method. Bite and saliva contact with open wound were replied by 8.9% of respondents of which 7.41% were rural residents and 1.48% were urban residents (Figure 2). Regarding the clinical signs of rabies in animals, biting and change in behaviour were reported by 50.4% of the participants.

Community attitudes and practices regarding rabies

The result of the study revealed that majority of the

respondents (80.0%) considered rabies as a fatal disease and almost half (46.7%) reported that they fear rabies. Regarding observation of rabid animal(s) and human(s), 73.3 and 48.9% of the participants observed rabid animal(s) and human(s), respectively. Furthermore, out of total interviewed participants 71.1% respondents replied killing as the preferred method for rabid animal(s) and 33 (24.4%) of respondents had experienced a dog bite at least once in their life. Regarding incubation period of rabies, the most frequently mentioned incubation period was less than 40 days (46.7%). Furthermore, children were mentioned by 100 (74.1%) of the respondent to be at higher risk than old people. Regarding the preferred action taken for bitten human, post exposure vaccination was responded by 89 (65.9%) of the participants whereas, traditional treatment was responded to by 41 (30.4%) of the participants. Moreover, 23.7% of the respondents were rural residents and 6.67% were urban residents (Figure 3). Moreover, 49.6% of the respondents reported that they would wash the wound using soap and water as a first aid for bitten human whereas, tying with cloth and application of herbal extract were reported by 18.5% of the respondents. 66.6% of the participant considered rabies not to be prevented by vaccination and 68.8% do not believe that eliminating stray dog or confining dog helps to prevent rabies. The respondent's attitudes and practices towards rabies are summarized in Table 3.

Factors associated with knowledge, attitudes and practices (KAP)

The overall knowledge, attitudes and practices (KAP) revealed that 51.9% of the respondents had a good KAP, whereas 48.1% was found to have poor KAP score. Table 4 shows the multivariable logistic regression analysis output of community knowledge attitude and practice (KAP) in relation to socio-demographic characteristics. Multivariable analysis revealed that good KAP score was significantly associated with gender being higher in males than females (OR= 37.16, CI=4.7- 92.1, $p= 0.001$), in age group >46 years than 15- 30 years (OR=8.9, CI=1.6-47.2) in urban than rural residents (OR=4.35, CI=1.3-14.14 $p= 0.014$). Furthermore, the association of education level with KAP score revealed statically significant difference ($P= 0.040$). The respondent who were at secondary school education level were found to have 7.7 times more good KAP score than illiterate (OR=7.7, CI=1.05 - 56.64). Significantly higher overall knowledge on rabies was also found in dog owners than those who had no dog. Dog owners were found to have 2.9 times more good KAP score than those who had no dog (OR=2.9, CI=1.12-7.93, $P=0.029$). The participants who got training on rabies were found to have significantly higher good KAP score than non-trained participants. The odd of higher good KAP score in trained respondents was 3.37 times more likely than non-trained respondents (OR=3.37 CI=1.17-9.69, $p=0.024$).

Table 1. Socio-demographic information of the study participants in Dedo district.

Variable	Category	Frequency	Percentage
Gender	Male	77	57.0
	Female	58	43.0
Age	15-30	35	25.9
	31-45	62	45.9
	>46	38	28.1
Residence respondents	Urban	39	28.9
	Rural	96	71.1
Religion	Muslim	128	94.8
	Orthodox	7	5.2
Marital status	Married	112	83.0
	Unmarried	23	17.0
Educational status	Illiterate	69	51.1
	Elementary	45	33.3
	Secondary	16	11.9
	Tertially level	5	3.7
Household Size	1-3	53	39.3
	4-6	43	31.9
	> 6	39	28.9
Current occupation	Employed	9	6.7
	Unemployed	6	4.4
	Housewife	42	31.1
	Farmer	57	42.2
	Unskilled worker	4	3.0
	Private business	8	5.9
	Student	9	6.7
Household income per month	no defined	72	53.3
	irregular	53	39.3
	Regular	10	7.4
Dog ownership	Yes	65	48.1
	No	70	51.9

Insignificantly higher good KAP score was observed in Muslim respondent than orthodox (OR=2.9, CI=0.17-49.19, $P=0.458$), in unmarried than married (OR=1.3, CI=0.203- 8.58, $P=0.772$) and in respondent with household size of 4-6 than above 6 (OR=1.8, CI = 0.487- 6.9). Moreover, the occupation of the respondents were also found to be not significant ($P= 0.355$). The highest good KAP score was observed in students than those that were found to have 28.8 times more overall knowledge than employed (OR=28.8, CI=0.282- 2959.8). Regarding the the association of average household income per month and KAP score of respondents, no statically significant difference was observed ($p= 0.540$) (Table 4).

DISCUSSION

This study revealed that community in Dedo district are familiar with general information on rabies as 100% of the respondent had heard about rabies. This is in agreement with study by Singh and Choudhary (2005) in rural community of Gujarat, India, Digafe et al. (2015) in Gondar Zuria District and Yalamebrat et al. (2016) in Debark district, North Gondar, Ethiopia who reported 98.6, 99.3 and 100% awareness about rabies, respectively. Besides, Serebe et al., (2016), Serebe et al. (2014) and Jemberu et al. (2013) also reported a high level of awareness on rabies from different areas of

Table 2. Knowledge of respondents in relation to cause, host range, clinical sign and transmission of rabies in Dedo district, Southwest, Ethiopia.

Variable	Category	Frequency	Percentage
Heard about rabies	Yes	135	100
	No	0	0
Ever get training	Yes	70	51.9
	No	65	48.1
Cause of rabies	Virus	18	13.3
	Starvation and thirst	54	40
	Spirit	10	7.4
	Do not know	53	39.3
Species affected by rabies	dog only	22	16.3
	dog and human	26	19.3
	human and other domestic animals	77	57.0
	don't know	10	7.4
Source of Rabies	Dog	83	61.5
	Dog and cat	3	2.2
	Dog and wild canine	27	20.0
	Do not know	22	16.3
Transmit from animal to human	Yes	120	88.9
	No	15	11.1
Means of transmission	Bite only	70	51.9
	Contact with saliva only	33	24.4
	Bite and saliva contact with open wound	12	8.9
	Raw meat and milk	4	3.0
	Do not know	16	11.9
	stop eating and drinking	30	22.2
Sign and symptom in animals	Biting and change in behaviour	68	50.4
	Salivation	18	13.3
	Paralysis	11	8.1
	don't know	8	5.9

Ethiopia which agrees with the current finding. However, this finding was higher when compared with 68.7% in a survey of knowledge, attitudes and practices about animal bite and rabies in community in India (Ichhupujani et al., 2006), 83% in Addis Ababa residents (Ali et al., 2013).

In the current study, 40% respondents were found to have misunderstanding of the cause of rabies which was starvation and thirst. Similar to this finding, Guadu et al. (2014) reported similar misunderstanding from Bahir Dar town. However, this result is lower as compared to the result of study in and around Dessie town, Ethiopia which was 49.6% (Serebe et al., 2016) and in Debark District, North Gondar, Ethiopia (63.5%) (Yalemebrat et al.,

2016). This could be due to awareness difference of the study area and this misperception could be explained by the opinion of asymptomatic rabies carrier dogs in which stressors like starvation and thirst might induce development of clinical rabies in the carrier dogs. But the idea of asymptomatic rabies carrier dogs by itself is a controversial issue (Wilde et al., 2009), and the association of stressors to the development of clinical rabies might be an implausible claim.

In this study, 57.0% respondents knew that rabies could affect human and other domestic animals which is in line with Yalemebrat et al. (2016). However, Guadu et al. (2014) reported a lower result (21.4%) from Bahir Dar town and higher result (71.9%) was also reported in the

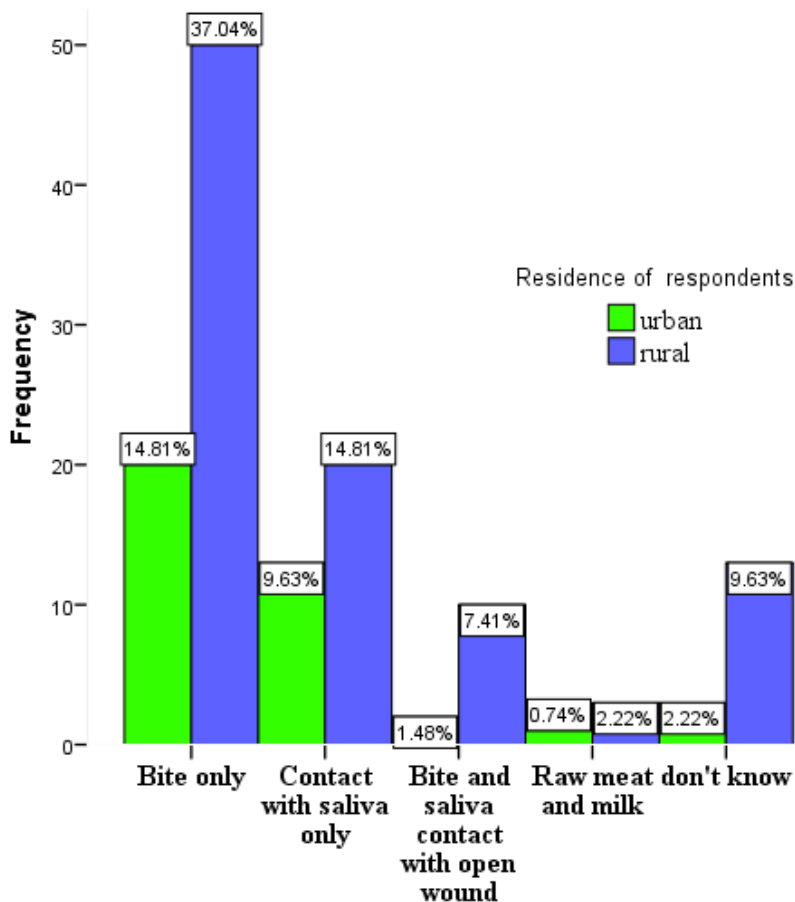


Figure 2. Modes of rabies virus transmission.

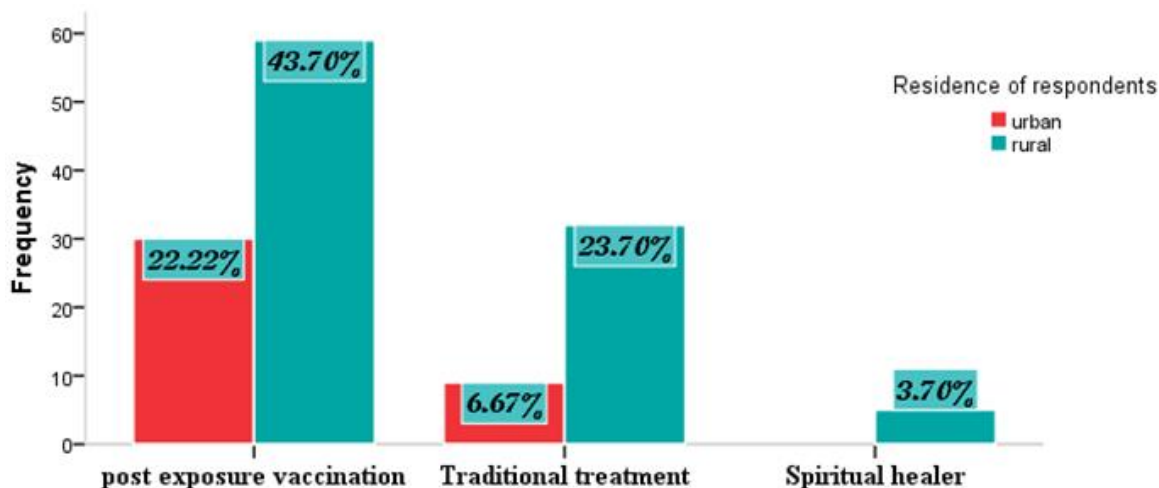


Figure 3. Preferred action taken for bitten human.

city of New York, USA (Eidson et al., 2004). The possible reason for this could be due to the availability of different

host range, level of awareness and educational status of community.

Table 3. Community attitudes and practices regarding rabies.

Variable	Category	Frequency	Percentage
Feeling if infected by rabies	Fear	63	46.7
	Shame	12	8.9
	Surprise	7	5.2
	Sadness	25	18.5
	Nothing	28	20.7
Is rabies fatal	Yes	108	80.0
	No	11	8.1
	don't know	16	11.9
Observation of rabid human	Yes	66	48.9
	No	69	51.1
Observation of rabid animal(s)	Yes	99	73.3
	No	36	26.7
Action for rabid animal	Tie	36	26.7
	Killing	96	71.1
	Nothing	3	2.2
Ever bitten by dog	Yes	33	24.4
	No	102	75.6
Incubation period of rabies	Immediate	23	17.0
	<40 days	63	46.7
	< 90 days	5	3.7
	don't know	44	32.6
More risky population	Children	100	74.1
	old people	32	23.7
	No difference	3	2.2
Preferred action taken for bitten human	post exposure vaccination	89	65.9
	Tradition treatment	41	30.4
	Spiritual healer	5	3.7
First aid for bitten human	wash with water and soap	67	49.6
	Tying with cloth	25	18.5
	herbal extract	25	18.5
	Nothing	18	13.3
Anti-rabies vaccine in human after exposure	Immediate	88	65.2
	Later	10	7.4
	any time	8	5.9
	don't know	29	21.5
Easily treatable after onset of clinical signs	Yes	65	48.1
	No	28	20.7
	don't know	42	31.1
Rabies is preventable by vaccination of dog	Yes	45	33.3
	No	90	66.6
Eliminating stray or Confining dogs helps to prevent rabies	Yes	42	31.1

Table 4. The multivariable logistic regression analysis output of community knowledge attitude and practice scores (KAP) in relation to socio-demographic characteristics.

Variables		Poor	Good	OR (95% CI)	P-value
Gender	Male	22(28.5)	55 (71.4%)	37.16 (4.7- 92.1)	0.001
	Female	43(74.1%)	15 (25.8%)		
Age(in year)	15-30	20 (85.1%)	15 (42.8%)	Reference	0.023
	31- 45	34 (54.8)	28 (45.2%)	2.1 (0.5 - 8.7)	
	>46	11(28.9)	27 (71.1%)	8.9 (1.6-47.2)	
Residence	Urban	14 (35.9%)	25(64.1%)	4.35(1.3-14.14)	0.014
	Rural	51 (53.1)	45 (46.9%)		
Religion	Muslim	61(47.6)	67 (52.3%)	2.9 (0.17- 49.19)	0.458
	Orthodox	4 (57.2%)	3 (42.8%)		
Marital status	Married	56 (50%)	56 (50%)	1.3 (0.203- 8.58)	0.772
	Unmarried	9 (39.1)	14 (60.8%)		
Household size	1-3	26 (49.1%)	27(50.9%)	1.7 (0.46-6.8)	0.606
	4-6	19 (44.2%)	24 (55.8%)	1.8 (0.487- 6.9)	
	> 6	20 (51.2%)	19 (48.8)	Reference	
Educational status	Illiterate	44 (63.7%)	25 (36.2%)	Reference	0.040
	Elementary	14(31.1%)	31(68.8%)	4.6 (1.3 - 15.6)	
	Secondary	5(6.25%)	11 (68.75)	7.7(1.05 - 56.64)	
	University	2 (40%)	3(60%)	1.2(0.104 - 14.4)	
Occupation	Employed	3(33.3%)	6(66.6%)	Reference	0.355
	Unemployed	2 (33.3%)	4(66.6%)	0.68(0.01-33.07)	
	Housewife	28(66.6%)	14(33.3%)	4.9(0.27-89.36)	
	Farmer	23(40.35%)	34 (59.6%)	0.8(0.05-12.5)	
	Unskilled worker	3(75%)	1(25%)	0.1(0.001-8.07)	
	Private business	4(50%)	4(50%)	1.7(0.07- 39.6)	
	Student	2(22.2%)	7(77.7%)	28.8(0.282- 2959.8)	
Dog ownership	Yes	23(35.38%)	42(64.6)	2.9 (1.12-7.93)	0.029
	No	42(60%)	28(40%)		
Got training about rabies	Yes	26(37.14%)	44(62.8%)	3.37(1.17-9.69)	0.024
	No	39(60%)	26(40%)		
Average household income per month	No defined income	32(44.4%)	40(55.5%)	Reference	0.540
	Irregular	30(56.6)	23(43.4)	0.6(0.21-1.73)	
	Regular	3(30%)	7(70%)	1.6(0.12-22.52)	

In the current study, 49.6% of the respondents reported that they would wash the wound using soap and water as a first aid for bitten human(s). This result is lower when compared with study in Debark District (76.4%) (Yalemebrat et al., 2016) and higher as compared to the

study conducted in Gondar zuria district (30.7%) (Digafe et al., 2015) and in a rural community of Gujarat, India (31.1%) (Singh and Choudhary, 2005). This difference could be associated with awareness level of the community. This treatment is cheap, readily available and

feasible for all to apply. Washing of rabies-infected wounds with soap and water can increase survival by 50% (Radostits et al., 2007). In the current study, 65.9% participants prefer post exposure vaccination for bitten human(s) and 65.2% of the respondent replied that it should be given immediately after exposure. Similarly, the study in Bahar Dar town reported 55.7% response for immediate post exposure vaccination (Guadu et al., 2014). The World Health Organization (WHO) also recommends wound washing and vaccination immediately after contact with a suspected rabid animal which can prevent almost 100% of rabies deaths (WHO, 2013). Regarding incubation period of rabies, the most frequently mentioned incubation period was less than 40 days (46.7%). This misconception about the incubation period indicates that those who believe this incubation period do not seek post exposure prophylaxis once the perceived incubation period has passed. This is serious for rabies-infected individuals where mortality is 100% after development of clinical signs. The incubation period varies from 5 days to several years (usually 2 to 3 months; rarely more than 1 year) depending on the amount of virus in the inoculum, the density of motor endplates at the wound site and the proximity of virus entry to the central nervous system (WHO, 2013). Furthermore, children were mentioned by 100 (74.1%) of the respondents to be at higher risk than old people. This could be due to the fact that children are closely playing with dog at home and even in streets. In addition, elders are well aware of the danger of rabies and look for medical care than children (Aworth et al., 2011). The result of current study revealed that 66.6% participants do not believe in rabies prevention by vaccinating dog and 68.8% of them replied that rabies cannot be prevented by eliminating stray or confining dogs. This is serious knowledge deficiency and community deserves public awareness.

In this study, the overall knowledge, attitude and practice (KAP) score revealed that 51.9% of the respondents had a good KAP. This finding is lower when compared with the study by Guadu et al. (2014) who reported about 64.1% among the community of Bahir Dar town and Yalamebrat et al. (2016) who reported 60.3% in Debark District, North Gondar. This difference could be due to the difference in sample size and level of awareness of community about rabies in the study area.

During analysis of KAP with independent variables, multivariable analysis revealed that KAP score was significantly ($p= 0.001$) associated with gender being higher in males (71.4%) than females (25.8%). The same proportion of statistical difference on KAP score of male (53.4%) and female (10.75%) was reported in Bahirdar town by Guadu et al. (2014), in Addis Ababa (male, moderate (77.09%) and good (10.55%), female, moderate (73.62%), good (5.08%) (Ali et al., 2013), in Debark District in males (64.6%) in females (54.5%) (Yalamebrat et al., 2016). The statistical significant difference in KAP

score between males and females might be due to increased activity of males in their daily life when compared with females and better chance of acquiring correct information about rabies.

Furthermore, the association of education level with overall knowledge about rabies revealed statically significant difference ($P= 0.040$). The respondent who were at secondary school education level were found to have 7.7 times more good KAP score about rabies than illiterate. This finding was also supported by a study conducted in Bahir Dar (Guadu et al., 2014), in Addis Ababa (Ali et al., 2013) and the studies conducted in Flagstaff, Arizona, USA (Andrea and Jesse, 2012). People with higher education tend to know more about rabies (Palamar et al., 2013), while illiterate persons tend to know less about rabies (Herbert et al., 2012). The possible explanation could be that educated person would have better information access and can easily understand the disease.

Moreover, significantly ($P=0.029$) higher good KAP score was also found in dog owners than those who had no dog. Dog owners were found to have 2.9 times more KAP score than those who had no dog. This finding is in agreement with Guadu et al. (2014) who found higher KAP score in dog owner than those who had no dogs. This may be associated with the adoption of dog owners with dog as it might help to know more about dog and dog disease and better chance of acquiring correct information on rabies.

Raising community awareness level has been mentioned as important tool to control rabies by many scholars (Eshetu et al., 2002; Wudu et al., 2013). In the study area, most of the participant had got training about rabies. In particular, Jimma University has been giving and had given the training through the program called "community based training program (CBTP)." In this study, the participants who got training on rabies were found to have significantly higher KAP score than non-trained participants ($p=0.024$). The odd of higher good KAP score on rabies in trained respondents were 3.37 times more likely than non-trained respondents. This supported the general fact that awareness creation has paramount role in prevention and control of rabies.

Conclusion

Conclusively, rabies was considered as the disease of both a veterinary and public health importance in the study area. All (100%) respondents had heard about rabies previously and 88.9% of respondents knew that rabies can be transmitted from animal to human. This is good knowledge. However, knowledge deficiency was observed regarding cause, mode of transition and incubation period of rabies. Increased knowledge is especially needed regarding rabies prevention by vaccination of dog, confining and elimination of stray dogs. Furthermore, rabies awareness campaigns and

education programs should focus on awareness community especially on first-aid treatments after a dog bite and how rabies is transmitted. Generally, the overall KAP score recorded in this study is an indication of low overall awareness level. Thus, close collaboration and integration of public health, veterinary sector and local authorities is a key element for preventing this fatal incurable disease.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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