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Editorial

When will the COVID-19 pandemic be over?

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In late 2019 after the first report of COVID-19 cases from China, the disease spread rapidly worldwide. The World Health Organization (WHO) declared a pandemic on March 11, 2020. Globally, over 541 million cases and 6.32 million deaths, and in Ethiopia, over 485 000 confirmed infections and 7500 deaths were reported. The global and national figures are underestimates, and World Health Organization recently indicated that the actual number might be four times higher. Underreporting and access to testing are the principal reasons. It is enigmatic that Africa is the least affected compared to other continents when we analyze the number of deaths. The devil remains in the details.

The damage that the COVID-19 caused to our world is beyond our comprehension. The economic, social, and political implications will stay for years. The health adversaries in individuals recovering from the disease with and without symptoms extend from long COVID-19 (estimated one out of five) to new-onset diabetes mellitus, hypertension, and worsening of preexisting comorbidities. Despite the success of vaccine discovery, and around 55% of individuals vaccinated approaching herd immunity globally, we now see another wave emerging worldwide. The messages portrayed by the global and national media are controversial. We note complacency in non-pharmaceutical prevention strategies, despite the roaring pandemic. Face mask use and hand hygiene are left optional, and the restrictions are almost removed. In Ethiopia, the figures show the number of vaccinated is lower than the global average, and we face colossal vaccine hesitancy that includes health professionals.

The current wave of COVID-19 cases worldwide is due to waning humoral immunity and the rapid emergence of variants. Fortunately, the number of patients requiring hospitalization and subsequently dying is recognizably low. In recent months, multiple lineages of the omicron variant (B.1.1. 529) have emerged. Five subvariants occurred less than a year after the omicron variant was reported (BA 1, BA 2, BA 2.12.1, BA 4, and 5). It is very unusual for a single variant to mutate so often to cause these many subvariants with enhanced infectiousness. As a result, we question ourselves when the pandemic is over? The answer is not yet. At present, the ones most affected are unvaccinated and the unboosted. Still, the vaccines help alleviate the disease severity and boosters, too. Then we ask, how many booster doses are required? No one knows the answer, but for now, we say the third dose with a heterologous vaccine is vital. COVID-19 will stay with us. Thus we should adapt ourselves to live with it.

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Original Article

Association of microalbuminuria with metabolic indicators of atherosclerosis and inflammation in type 1 diabetic patients

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Abstract

Background: *Microalbuminuria is usually accompanied by undiagnosed dyslipidemia. We aimed to assess the correlation between microalbuminuria and early atherosclerotic changes in type 1 diabetics by comparing two groups of patients according to their UAER (Urinary Albumin Excretion Rate) status.*

Methods: *We conducted a retrospective study including 167 patients with confirmed type 1 diabetes segregated into the following groups (patients with normoalbuminuria vs. patients with microalbuminuria).*

Results: *Our study revealed a definite preponderance of males (52.10%). The mean age was 29.55±11.36 years, whereas the diabetes duration was 12.73±8.14 years. The prevalence of microalbuminuria was 39.5%. Significant correlation was observed between lipid profiles such as TG and LDL (r=0.227; r=0.166, respectively) and lipid ratios TC/HDL, LDL/HDL, and TG/HDL (r=0.322; r=0.351; r=0.386, respectively) with UAER. The findings showed that the last quartile of TC/HDL ratio (cOR=6.89[2.61-18.14]; p<10^{-3}) and LDL/HDL ratio (cOR=5.48 [2.10-14.30]; p=0.001) were higher in microalbuminuric patients. Similarly, we noticed higher values in the last two quartiles (3rd and 4th) of the TG/HDL ratio with p values of 0.05 and 10^{-3}, respectively. TG/HDL ratio was a strong indicator for atherosclerotic disease (sensitivity of 82.1%, specificity of 84.2%, and diagnostic accuracy of 0.775). In contrast to females who developed microalbuminuria, lipid ratios and lipid profiles were significantly greater in male patients.*

Conclusions: *Patients who develop microalbuminuria are characterized by dyslipidemia and a higher risk of atherosclerotic cardiovascular disease. Hence, early detection of microalbuminuria associated with dyslipidemia is crucial for the effective prevention of atherosclerotic cardiovascular diseases.*

Keywords: *Atherosclerotic cardiovascular disease; dyslipidemia; microalbuminuria; type 1 diabetes; UAER.*

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Introduction

Diabetic kidney disease (DKD) is the most severe type 1 diabetes (T1D) complication resulting from immune processes related to different proinflammatory signaling pathways, genetic predispositions, and some epigenetic mechanisms which were reported to be implicated in the inflammatory disease and the onset and progression of DKD (1). Past epidemiological studies demonstrated that 25% to 40% of subjects with T1D and 5% to 40% of individuals with type 2 diabetes (T2D) eventually develop DKD (2).

Microalbuminuria has been conventionally established as the primary predictive marker of the risk for progression to the advanced stages of DKD (3).

It has been defined by an increased urinary albumin excretion rate (UAER) approximately in the range of 30–300 micrograms per minute (4). In addition, 20% to 30% of subjects with T1D present with microalbuminuria after an average of 5-10 years of diabetes (2).

Current recommendations from the American Diabetes Association concerning screening for microalbuminuria in patients with T1D suggest annual testing after 10 years of age and after an average of 5 years with diabetes (5). Furthermore, these patients are at higher risk of cardiovascular diseases (CVDs) compared with diabetic patients without microalbuminuria which represents a significant burden to health care systems (6).

Preliminary studies have shown that microalbuminuria is associated with markers of endothelial dysfunction and arterial stiffness (7). Hence, it can be used as a strong predictor of progressive trans-capillary leakage of lipoproteins that increases the risk of atherosclerotic cardiovascular diseases in selected populations, such as type 1 diabetic middle-aged and elderly patients with DKD (8). Additionally, there is compelling evidence that points to major mechanisms of diabetic atherosclerosis and vascular remodeling promoting each other and are part of a vicious combination leading to the advancement of the pathological process of microalbuminuria and CVD (9).

It has been found also that relation between microalbuminuria and some atherosclerotic cardiovascular risk factors, such as hypertension, glycosylated hemoglobin, and serum lipids were higher in type 1 diabetic patients compared with patients without T1D (10). Therefore, microalbuminuria can assume an important role as an index of atherosclerotic vascular disease.

In this regard, we aimed in this study to examine the prevalence of microalbuminuria among patients with T1D to establish whether there is any relationship between a decline in renal function and atherosclerotic cardiovascular disease risk, by assessing blood lipid ratios in type 1 diabetic Algerian patients where similar studies are still lacking.

Patients and Methods

Study design, area, and period:

This was a retrospective study reporting data from January 1, 2009, to December 30, 2019, on type 1 diabetics who visited the Diabetes Center in Sidi-Bel-Abbes, Northwestern Algeria.

Population:

Our study included 167 type 1 diabetics (87 males and 80 females) diagnosed in their pubertal period (according to the WHO guideline) that were over 13 years old during this study (11). All medical records of the participants were revised for the following: status of the diabetic disease, biochemical parameters, and other associated diseases such as low visual acuity, diabetic retinopathy, hypothyroidism, and diabetic foot.

Selection of study subjects:

All patients with previously diagnosed T1D, aged more than 13 years, and who visited the Diabetes Center at least two times a year with no history of any cardiovascular disease were enrolled in the analysis. Although, patients with T2D or T1D aged less than 13 years, missing medical records of the disease, and missing informed consent were excluded.

Data collection process:

For all patients, the anthropometric measurements including body height, weight, waist circumference, and body mass index (BMI) were taken from the patient's medical record. BMI was calculated as weight (kg)/ Height^2 (m), and the patients were classified into under weight, normal, overweight, and obese classes based on BMIs of <18.5, 18.5–24.9, 25–29.9, and ≥30 kg/m^2, respectively.

A sphygmomanometer was used to measure blood pressure in the supine position, followed by a standing measurement (after a few minutes). A systolic blood pressure (SBP) of 140 mmHg and diastolic blood pressure (DBP) of 90 mmHg or more were considered as elevated blood pressure (12). The latest biochemical test results including fasting blood glucose; glycated hemoglobin (HbA1c); high-sensitivity C-reactive protein (hs-CRP); urea; serum creatinine; lipid parameters - total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and triglycerides (TG); and thyroid-stimulating hormone (TSH) were collected from patients' medical records. Microalbuminuria was defined as a urinary albumin excretion rate (UAER) in at least two samples obtained within 3-6 months. Microalbuminuria observed for two or more consecutive years with UAER > 30 mg/24h was regarded as persistent (4). Furthermore, lipid ratios (TC/HDL, LDL/HDL, and TG/HDL) were measured as indicators of atherogenic risk.

Statistical analysis:

Data are shown as mean±SD with its respective 95% CI for continuous variables and as percentage (%) with its relative frequency for categorical variables. The differences between groups with and without microalbuminuria were analyzed by the Chi-square test for qualitative variables and the Student t-test for quantitative variables. A partial correlation test was applied to evaluate the correlation between lipid profile and lipid ratio with UAER levels. Receiver operator characteristic (ROC) curves were applied to identify the predictive values of lipid ratios for atherosclerosis. Statistically significant differences were maintained when the p-value was less than or equal to 0.05 ($p \leq 0.05$).

After adjusting for quartiles of lipid ratios, multivariate logistic regression analysis was utilized to measure crude odds ratios (cOR) and 95% CI for lipid ratios to examine the correlation between microalbuminuria and atherosclerotic disease. All data were computed and analyzed using SPSS software (SPSS 22, IBM Corporation; Chicago, IL. August 2013).

Ethics:

The Ethics Committee of Diabetic Center approved this research and since its retrospective research, ethical approval was acquired from the center in which the research was conducted.

Result

Socio-demographic profile:

The summarized characteristics of the enrolled participants are shown in Table 1. A total of 167 type 1 diabetic patient (52.10% males and 47.90% females) were included in this study. Arbitrarily, patients were segregated into two groups according to the level of urinary albumin excretion rate (UAER) whether below or above 30 mg/24h (patients with normoalbuminuria "UAER<30 mg/24h" vs. patients with microalbuminuria "UAER>30 mg/24h").

Of the 167 diabetic patients, 101 (60.48%) patients were normoalbuminuric while 66 (39.52%) patients were microalbuminuric (Table 1).

Table 1: Summarized characteristics of study participants.

Variables	All Patients n=167 Number (%)	Normoalbuminuria n=101 Number (%)	Microalbuminuria n=66 Number (%)	P-value
Gender (%)				
Male	87 (52.10)	43 (42.60)	44 (66.70)	
Female	80 (47.90)	58 (57.40)	22 (33.30)	0.002
Age groups (years)				
[13-19]	34 (20.40)	28 (27.70)	6 (9.10)	
[20-29]	60 (35.90)	41 (40.60)	19 (28.80)	
[30-39]	40 (24.00)	19 (18.80)	21 (31.80)	$<10^{-3}$
[40-49]	22 (13.20)	11 (10.90)	11 (16.70)	
[50-59]	9 (5.40)	2 (2.0)	7 (10.60)	
≥ 60	2 (1.20)	0 (0.00)	2 (3.0)	
Smoking history (%)				
Male	21 (12.60)	5 (5.00)	16 (24.20)	$<10^{-3}$
Prevalence of weight categories (%)				
Underweight, BMI <18.5 (Kg/m²)	41 (24.60)	22 (21.8)	19 (28.70)	
Normal weight, BMI=18.5-25.0'(Kg/m²)	100 (59.90)	63 (62.40)	37 (56.10)	0.69
Overweight, BMI=25.0–29.9(Kg/m²)	22 (13.20)	15 (14.90)	7 (10.60)	
Obesity, BMI ≥30 (Kg/m²)	4 (2.40)	1 (1.00)	3 (4.50)	
Other associated diseases (%)	58 (34.70)	27 (26.70)	31 (47.00)	0.007
Low visual acuity	38 (22.80)	16 (15.90)	22 (33.30)	0.004
Diabetic retinopathy	29 (17.40)	0 (0.00)	29 (43.90)	$<10^{-3}$
Diabetic nephropathy	26 (15.60)	1 (1.00)	25 (37.90)	$<10^{-3}$
Hypertension	28 (10.20)	5 (5.00)	12 (18.20)	0.37
Hypothyroidism	5 (3.00)	0 (0.00)	5 (7.60)	0.005
Dyslipidemia	29 (17.40)	6 (6.00)	23 (34.90)	$<10^{-3}$
Diabetic foot				

Percentages (%) were compared with Chi-square test, p≤0.05 was considered as statistically significant. BMI: body mass index.

The mean age of the patients was 29.55±11.36 years, whereas the mean diabetes duration was 12.73±8.14 years. The mean age of patients with microalbuminuria was greater than in those with normoalbuminuria (p<0.001). Likewise, the mean duration of T1D was statistically greater among those who had microalbuminuria ((p<0.001) (Table 2).

Clinical profile:

The clinic characteristics and laboratory indexes are described in Table 2. Regarding the anthropometric measurements on admission, significant differences were highlighted in body height (p=0.003) in the microalbuminuria group. Furthermore, concerning blood pressure, significant differences were observed in diabetics who developed microalbuminuria (SBP, p<0.001; DBP, p<0.001) (Table 2).

Remarkably, fasting plasma glucose and HbA1c levels were statistically increased in patients with microalbuminuria (p<0.001 for both cases). A significant difference was observed in terms of hs-CRP (p=0.02).

Regarding lipid levels, HDL-c and triglyceride values statistically differed between the two groups (p<0.001). Moreover, it was revealed that lipid ratios (TC/HDL-c, LDL/HDL-c, and TG/HDL-c) were statistically higher in patients with microalbuminuria (p<0.01).

Regarding thyroid function, serum TSH levels were higher in patients with microalbuminuria than those with normoalbuminuria (p= 0.06).

Table 2: Comparison of clinical characteristics between Normoalbuminuric and Microalbuminuric type 1 diabetic patients.

Variables	All Patients n=167		Normoalbuminuria n=101		Microalbuminuria n=66		P-value
	Mean±SD	95% CI	Mean±SD	95% CI	Mean±SD	95% CI	
Mean age (years)	29.55 ± 11.36	27.81-31.29	26.22 ± 9.66	24.31-28.13	34.64 ± 11.92	31.72-37.58	$<10^{-3}$
Diabetes duration (years)	12.73 ± 8.14	11.49-13.97	10.62 ± 6.81	9.28-11.97	15.96 ± 8.96	13.75-18.16	$<10^{-3}$
Age at 1st diagnosis (years)	16.84 ± 9.51	15.39-18.30	15.61 ± 9.40	13.76-17.47	18.73 ± 9.45	16.40-21.05	0.03
Body height (m)	1.67 ± 0.07	1.65-1.68	1.65 ± 0.07	1.64-1.67	1.69 ± 0.07	1.67-1.71	0.003
Body weight (kg)	59.18 ± 11.19	57.47-60.89	58.40 ± 10.84	56.26-60.54	60.36 ± 11.69	57.48-63.23	0.27
BMI (kg/m²)	21.16 ± 3.52	20.62-21.70	21.24 ± 3.44	20.56-21.92	21.03 ± 3.67	20.13-21.94	0.71
Waist circumference (cm)	80.90 ± 9.33	78.25-83.55	79.04 ± 9.36	75.41-82.67	83.27 ± 8.94	79.31-87.24	0.11
SBP (mmHg)	114.2 ± 13.8	112.0-116.3	109.1 ± 9.2	107.3-110.9	122.0 ± 15.9	118.1-125.9	$<10^{-3}$
DBP (mmHg)	66.3 ± 9.16	64.9-67.7	63.7 ± 8.1	62.1-65.3	70.5 ± 9.2	68.2-72.8	$<10^{-3}$
Fasting plasma glucose (g/l)	2.76 ± 1.28	2.56-2.96	2.50 ± 1.20	2.26-2.73	3.16 ± 1.28	2.85-3.48	0.001
HbA1c (%)	9.47 ± 2.17	9.13-9.81	8.51 ± 1.57	8.19-8.84	10.87 ± 2.19	10.33-11.42	$<10^{-3}$
Hs-CRP (mg/dl)	51.57 ± 68.70	17.40-85.73	7.20 ± 8.53	0.68-15.09	79.80 ± 75.66	28.96-130.63	0.02
Total cholesterol (g/l)	1.60 ± 0.36	1.55-1.66	1.58 ± 0.29	1.52-1.64	1.64 ± 0.45	1.53-1.75	0.26
HDL-c (g/l)	0.44 ± 0.10	0.42-0.46	0.47 ± 0.09	0.45-0.49	0.39 ± 0.10	0.37-0.42	$<10^{-3}$
LDL-c (g/l)	0.88 ± 0.26	0.84-0.92	0.85 ± 0.20	0.81-0.89	0.93 ± 0.33	0.85-1.01	0.06
Triglycerides (g/l)	0.93 ± 0.59	0.83-1.02	0.78 ± 0.39	0.70-0.85	1.15 ± 0.76	0.97-1.34	$<10^{-3}$
TC/HDL-c	3.81 ± 1.19	3.62-3.99	3.45 ± 0.85	3.28-3.62	4.35 ± 1.42	4.00-4.70	$<10^{-3}$
LDL/HDL-c	2.12 ± 0.82	2.00-2.25	1.89 ± 0.64	1.77-2.02	2.47 ± 0.94	2.24-2.71	$<10^{-3}$
TG/HDL-c	2.33 ± 2.02	2.03-2.64	1.79 ± 1.47	1.50-2.08	3.17 ± 2.43	2.57-3.76	$<10^{-3}$
Creatinine (g/l)	13.78 ± 19.04	10.74-16.82	7.28 ± 1.17	6.94-7.62	26.04 ± 28.64	18.15-33.94	$<10^{-3}$
Urea (g/l)	0.42 ± 0.41	0.35-0.48	0.23 ± 0.07	0.21-0.24	0.77 ± 0.54	0.62-0.92	$<10^{-3}$
Microalbuminuria (mg/24h)	133.30 ± 356.87	78.78-187.83	8.01-6.83	6.66-9.35	325.04-513.28	198.86-451.22	$<10^{-3}$
TSH (µIU/ml)	6.84 ± 15.37	2.83-10.85	4.97 ± 5.73	3.29-6.65	14.15 ± 32.19	6.30-34.60	0.06

Means were compared with independent sample Student's t-test, $p<0.05$ was considered as significant. SD: standard deviation, CI: confidence interval, HbA1c: glycosylated hemoglobin, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, hs-CRP: high-sensitivity C-reactive protein, TC: total cholesterol, HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol, TG: triglycerides, TSH: thyroid-stimulating hormone.

Partial correlation was applied to establish the correlation of lipid profiles and lipid ratio to UAER values in patients with T1D by controlling for age, gender, disease duration, and HbA1c levels. There was a significant positive correlation between TG (r=0.227), LDL (r=0.166), TC/HDL ratio (r=0.322), LDL/HDL ratio (r=0.351), and TG/HDL ratio (r=0.386) with the UAER levels. These results elucidate that an increase in lipid values is correlated with an increase in the UAER levels. Conversely, there was a negative correlation between HDL and UAER levels ($r=-0.400$; $p<0.001$), which means that there was an inverse correlation between HDL and UAER (Table 3).

Table 3: Partial correlation between lipid profile and lipid ratio with UAER level after adjustment for age, gender, disease duration, and HbA1c

Variables	UAER (mg/24h)		
	N	r (correlation coefficient)	P-value
Single lipid measures	167		
TC, g/L		0.077	0.321
TG, g/L		0.227	0.003
HDL, g/L		-0.400	$<10^{-3}$
LDL, g/L		0.166	0.032
Lipid ratios			
TC/HDL		0.322	$<10^{-3}$
LDL/HDL		0.351	$<10^{-3}$
TG/HDL		0.386	$<10^{-3}$

The partial correlation was significant at $p\leq0.05$. UAER: urinary albumin excretion rate, TC: total cholesterol, LDL: low-density lipoprotein cholesterol, HDL: high-density lipoprotein cholesterol, TG: triglycerides.

The multivariate regression was utilized to identify the relative risk of atherosclerosis according to lipid ratio quartiles. The results showed that the fourth quartile of the TC/HDL ratio was statistically greater in the microalbuminuria group ($p<0.001$).

Similarly, the last quartile (4^{th}) of the LDL/HDL ratio was significantly higher in the microalbuminuria group ($p=0.001$). Likewise, the ($3^{rd}$and 4^{th}) quartiles of the TG/HDL ratio were significantly higher in patients with microalbuminuria ($p=0.05$, $p<10^{-3}$; respectively) (Table 4).

Table 4: Multivariate analysis of the relationship between lipid ratio quartiles and albuminuria status in type 1 diabetic patients.

Variables	Normoalbuminuria, n=101 Number (%)	Microalbuminuria, n=66 Number (%)	cOR (95% CI)	P-value
TC/HDL ratio				
1st quartile (2.09-3.00)	28 (27.7)	13 (19.7)	Reference	---
2nd quartile (3.01-3.63)	34 (33.7)	10 (15.2)	0.63 [0.24-1.66]	0.35
3rd quartile (3.64-4.34)	29 (28.7)	11 (16.7)	0.81 [0.31-2.12]	0.67
4th quartile (4.35-8.10)	10 (9.9)	32 (48.5)	6.89 [2.61-18.14]	$<10^{-3}$
LDL/HDL ratio				---
1st quartile (0.73-1.74)	28 (27.7)	11 (16.7)	Reference	0.23
2nd quartile (1.85-2.13)	38 (37.6)	8 (12.1)	0.53 [0.19-1.50]	0.09
3rd quartile (2.14-2.74)	22 (21.8)	19 (28.8)	2.19 [0.86-5.56]	0.001
4th quartile (2.75-5.14)	13 (12.9)	28 (42.4)	5.48 [2.10-14.30]	
TG/HDL ratio				
1st quartile (0.45-1.27)	34 (33.7)	7 (10.6)	Reference	---
2nd quartile (1.28-1.85)	31 (30.7)	12 (18.2)	1.88 [0.65-5.38]	0.23
3rd quartile (1.86-2.63)	28 (25.7)	15 (22.7)	2.80 [0.99-7.86]	0.05
4th quartile (2.64-14.95)	10 (9.9)	32 (48.5)	15.54 [5.27-45.75]	$<10^{-3}$

Multivariate logistic regression significant at $p\leq0.05$. CI: confidence interval, cOR: Crude odd ratio, TC: total cholesterol, LDL: low-density lipoprotein cholesterol, HDL: high-density lipoprotein cholesterol, TG: triglycerides.

ROC curve for lipid ratios showed strong discriminatory power for detecting atherosclerotic disease. The TG/HDL ratio was a strongly indicator for atherosclerosis. The optimum cut-off value was ≥3.0, with a sensitivity of 82.1%, specificity of 84.2%, positive predictive value of 78.3%, and negative predictive value of 67.6% with a diagnostic accuracy of 0.775. (Figure 1B, D).

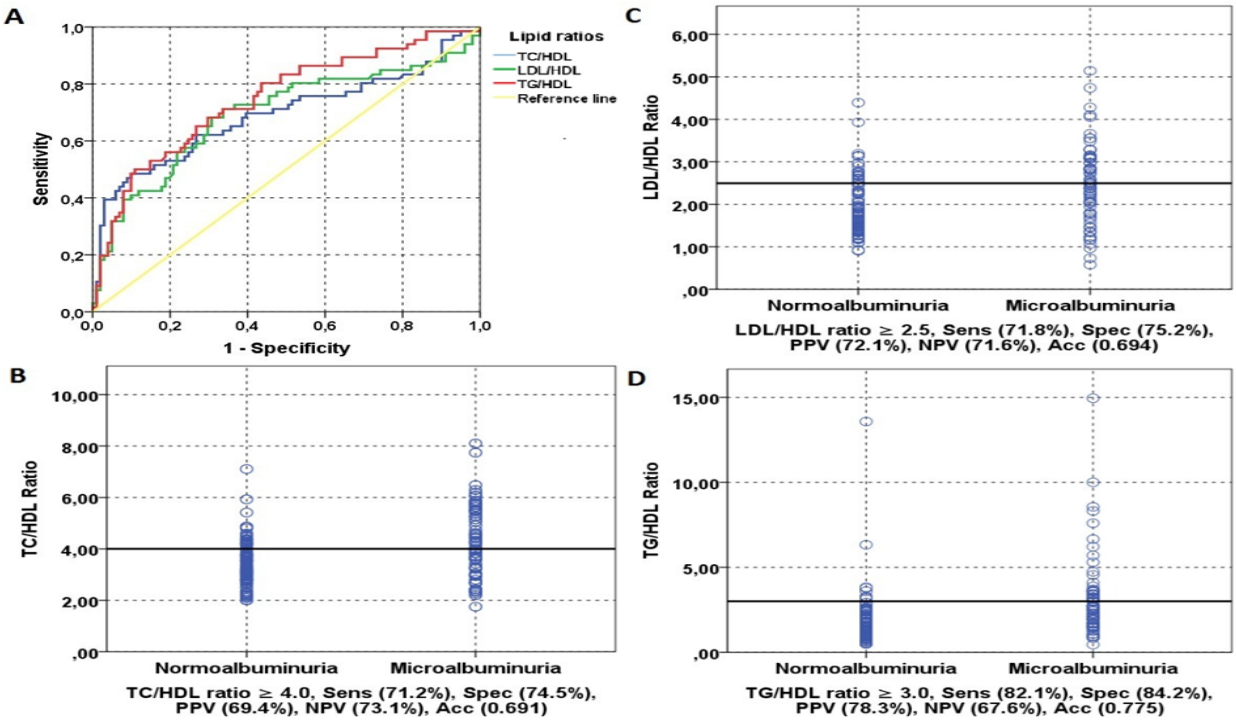

Figure 1: Receiver operating characteristic curve to define the best cut-off lipid ratios to detect atherosclerosis. Sens: sensitivity, Spec: specificity, PPV: negative predictive value, NPV: positive predictive value, Acc: accuracy, TC: total cholesterol, LDL: low-density lipoprotein cholesterol, HDL: high-density lipoprotein cholesterol, TG: triglycerides.

As displayed in Figure 2, in contrast to females who developed microalbuminuria, when comparing all lipid parameters (lipid ratios and conventional lipid parameters) between the two genders, higher values were found in male patients with microalbuminuria.

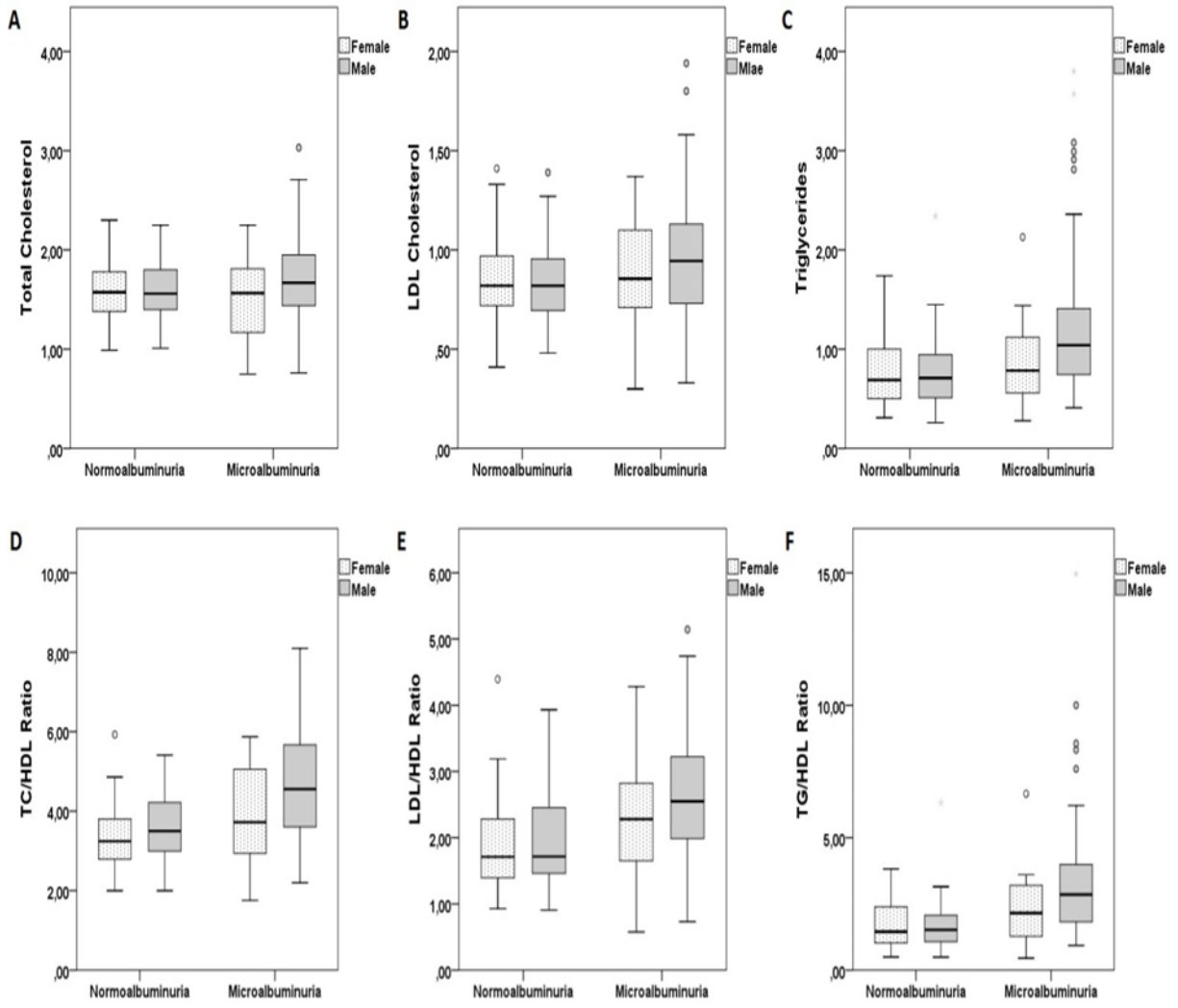

Figure 2: Comparison of lipid ratio levels between patients with and without microalbuminuria according to patients' gender. TC: total cholesterol, HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol, TG: triglycerides.

Discussion

The current study assessed the potential role of biological and clinical features in diabetics with and without elevated urinary albumin excretion rate (UAER) to establish the factors related to the existence of microalbuminuria in this population and to determine whether the association of this clinical finding with atherosclerosis risk could be confirmed by assessing blood lipid ratios in type 1 diabetic patients.We revealed that the distribution of diabetics by gender was inequitable, emphasizing a clear male preponderance over females (52.10% - 47.90%) with a male to female ratio of 1.09. Compared to microalbuminuric patients with UAER>30 mg/24h, the proportion of men was significantly higher than that of women among type 1 diabetic patients. A longitudinal study conducted by Jenkins and colleagues (13) after a mean follow-up time of 14.5 years showed that 824 patients with T1D had an AER < 40 mg/24h; of those, 448 (54.4%) had AER < 40 mg/24h were men, whereas, in the microalbuminuria (40–299 mg/24h) group and the macroalbuminuria (≥300 mg/24h) group, 30 (59.8%) and 30 (71.4%) were men. The results also are consistent with previous studies in the literature (14-16).

Our study displayed significant impacts of age and duration of diabetes on the variance between UAER. The findings concord with other studies' conclusions where the most prominent risk factors for microalbuminuria were age at onset of diabetes and longer diabetes duration (14,15,17).

Another noteworthy finding of the present inquiry was that smoking was associated with increased albuminuria. Numerous studies have shown that cigarette consumption promotes the onset and progression at all stages of DKD in diabetes (T1D and T2D) (17,18). Furthermore, exposure to cigarette smoke could influence vascular phenotypes of early atherosclerosis in patients with T1D (19).

Interestingly, we found that retinopathy was associated with an increased incidence of microalbuminuria, and this was following a previous follow-up study of adults with T1D (20). In the current study group, hypertension was detected in 26 cases, and 25 (37.90%) of cases with microalbuminuria were hypertensive. Our findings are in agreement with the previous study which proves that the prevalence of hypertension increases with advanced diabetic nephropathy and defines hypertension as the most significant risk factor for microalbuminuria (14).
Additionally, consistent with other studies (14,15), we found that with an incremental rise in hbA1c, the risk of microalbuminuria is imminent in these patients. Similarly, Wadén *et al.* demonstrated that higher levels of hbA1c predict not only incident microalbuminuria and progression of established DKD but also CVD events in patients with T1D (16).

Our results showed that there is a significant increase in hs-CRP levels among patients with microalbuminuria. Comparable findings regarding increased plasma markers of inflammation such as hs-CRP were associated with the progression of kidney dysfunction in T1D during both short-term and long-term follow-up (21). In addition, other studies have confirmed that soluble molecules involved in inflammation and endothelial damage including hs-CRP are recognized as potential cardiovascular risk markers through the development and progression of the atherosclerotic inflammation process (22).
We also identified that patients with increased UAER and nephropathy accompanying T1D had an atherogenic lipoprotein profile, which was characterized by elevated plasma levels of LDL-cholesterol and triglyceride-rich lipoprotein subclasses, and lower HDL-cholesterol subclasses, these results concord with the literature (14-16).
An important observation of our study was the association of an increased UAER with high serum TSH levels in patients with T1D. Our findings echo with the observations made by Das *et al.* who ascertained a similar correlation between increased prevalence of microalbuminuria and elevated TSH quartiles (23). Meanwhile, our previous study confirmed that elevated serum TSH levels are characterized by a higher atherogenic index, which implies to be a risk factor for atherosclerosis (24).

Taking into account the strong correlation between UAER and elevated levels of TC/HDL-C, LDL/HDL-C, and TG/HDL-C in our study we can assume that higher UAER in diabetic type 1 patients may indicate an increased cardiovascular risk. Gender difference is one of the utmost outstanding characteristics of CVD. Several studies have reported the impact of gender dissimilarity in the prevalence of CVD risk factors (25,26). In the present study, we found that male subjects with microalbuminuria had hyperlipidaemia (higher LDL, TC, and TG) and higher values of lipid ratios, indicators of atherosclerosis and stroke (TC/HDL, LDL/HDL, and TG/HDL). In recent epidemiological studies, higher levels of proteinuria and microalbuminuria have been associated with an increased risk of cardiovascular mortality, heart failure, coronary disease, and stroke (27). Based on the results of the present study, the association of TG/HDL-C with increased UAER and DKD was stronger in patients with microalbuminuria. It is thus possible that TG/HDL-C could represent the progression of kidney insufficiency even in the early stage of lipid metabolism abnormity. There are several reasons why the level of TG/HDL-C may be superior to that of other lipid parameters in increased UAER and DKD identification. Elevated TG levels and decreased HDL-C levels have been most strongly associated with an increased risk of atherosclerosis and renal dysfunction (28).

Like any research, there are some limitations in the current study. First, the sample size contained a low prevalence of microalbuminuria, which limited our ability to establish the full outcome of diabetic nephropathy. Second, the retrospective nature of the present study and how we have collected the data does not allow making a clear, conclusive decision about the atherosclerosis disease in T1D concerning microalbuminuria. Nevertheless, regardless of these limitations, we believe that our conclusions would remain reliable and valid. Our study also has multiple strengths, including a significant correlation of lipid profiles and lipid ratios with UAER levels, which after adjustment for age, gender, disease duration, and HbA1c, reinforces the importance of our investigation. This study also showed epidemiological evidence from a specific population-based study using nationally representative data reflecting a single ethnicity. To the best of our knowledge, this is the first study establishing the relationship between microalbuminuria and dyslipidemia in type 1 diabetic subjects with an atherogenic lipid profile in Algerian individuals.

Conclusion

In summary, our results indicate that an increase in UAER is associated with an increased risk of atherosclerosis and dyslipidemia. This shows the importance of controlling the lipid index as a method of preventing atherosclerotic cardiovascular disease.

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Competing interests

The authors declared no conflicts of interest.

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Original Article

Evaluation of culture of Mycobacterium tuberculosis on blood agar in resource limited setting in Addis Ababa, Ethiopia

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Abstract

Background: *Tuberculosis is one of the major public health problems in Ethiopia. A number of rapid techniques for the isolation and identification of Mycobacterium tuberculosis has been approved by World Health Organization. However, the available laboratories, which have financial constraints, require culture media or techniques that are rapid and inexpensive .*

Objective*: To evaluate the efficacy of 7% sheep blood agar medium for primary isolation of tuberculois from pulmonary tuberculosis suspected patients.*

Methods: *A comparative cross-sectional study was conducted among 212 tuberculosis suspected individuals from five selected health facilities in Addis Ababa, Ethiopia from November 2013 to March 2014. Sputum specimens were collected and examined using AFB smear microscopy, cultured on 7% sheep blood agar and Lowenstein Jensen medium. Molecular characterization using RD9 deletion was done for AFB confirmed isolates*

Results: *The sensitivity, specificity, positive and negative predictive value of 7% sheep blood agar compared with the golden standard of Lowenstein Jensen medium was 96.4%, 98.1%, 94.7% and 98.7%, respectively. The performance of 7% sheep blood agar and sediment smear microscopy was comparable to Lowenstein Jensen. No significant difference in the rate of contamination (p>0.05) where the rate of contamination was 4.7% (20/424) on 7% sheep blood agar tubes and 5.2% (22/424) on Lowenstein Jensen tubes.*

Conclusions: *Mycobacterial growth time was less on sheep blood agar as compared to Lowenstein Jensen, and 7% sheep blood agar medium may be a good alternative of Lowenstein Jensen medium for rapid detection of Mycobacterium tuberculosis from sputum in resource limited settings.*

Keywords: *Sensitivity, Specificity, Mycobacterium tuberculosis, sheep blood agar, Lowenstein Jensen medium*
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Introduction

Nearly one third of the world's population is infected with *Mycobacterium tuberculosis* (MTB) and still accounts for the highest mortality from any infectious diseases worldwide, even surpassing HIV/AIDs, causing 1·5 million deaths in 2018 (1). It is the oldest infectious agent known to humankind and remains a major public health issue. Increased incidence of multidrug-resistant (MDR) isolates has resulted in treatment failures and, these isolates were able to spread in the community (2). The slow growth of mycobacterial cultures is a major problem in order to easily diagnose MTB, which later explains the slow process of evaluating the susceptibility of MTB to antibiotics (3). Thus, the scarcity of accurate and rapid diagnostic tests for *M. tuberculosis* is an important impediment to global TB control (4), It has been defined by an

increased urinary albumin excretion rate (UAER) approximately in the range of 30–300 micrograms per minute (4). In addition, 20% to 30% of subjects with T1D present with microalbuminuria after an average of 5-10 years of diabetes (2).
Current recommendations from the American Diabetes Association concerning screening for microalbuminuria in patients with T1D suggest annual testing after 10 years of age and after an average of 5 years with diabetes (5). Furthermore, these patients are at higher risk of cardiovascular diseases (CVDs) compared with diabetic patients without microalbuminuria which represents a significant burden to health care systems (6).

susceptibility of MTB to the anti-tuberculosis drug (8).

however small improvements in the coverage of testing, detection, and treatment of MDR and rifampicin-resistant tuberculosis were noted with an increase of bacteriologically confirmed tuberculosis rising 10% from 2017. Nevertheless, people with drug-resistant tuberculosis accounted for more than half a million new cases in 2018, and only 1/3 of these were enrolled for treatment (1, 5, 6). Diagnostic algorithms in current use, most in developing countries, are based on tests that have been in clinical use for many years. Moreover, the two greatest challenges to TB control, the TB/HIV epidemic and the growing problem of multidrug-resistant MTB, cannot adequately be addressed solely by sputum smear microscopy. Thus, lack of accurate and rapid diagnostic tests for TB is an important impediment to global TB control (7). Specific media, such as egg-based media, agar-based media, and liquid media are recommended for culturing Mycobacterium species. Such requirements pose logistic and economic problems, especially in resource-limited settings where bacteriological culture facilities are few, and the prevalence of mycobacterial infections, remarkable tuberculosis is high (8). The study conducted in 2020 in Ethiopia indicated high TB burden in countries like Ethiopia; there are also opportunities to identify children with presumptive TB in the framework of integrated community case management. Even though most health facilities prioritize the identification and management of acute childhood diseases; e. g malnutrition, diarrhea, fever, and pneumonia, TB is likely affecting several children evaluated in these clinics accordingly, Ethiopia was the first African country to develop a national childhood TB road map following the development of the global childhood TB road map in 2015 (9).

Currently few papers have reported that primary isolation of MTB on Sheep Blood Agar (SBA) from extra pulmonary site (lymph node) clinical sample after two weeks or more incubation and to determine of This study aimed to evaluate the diagnostic efficacy of 7% SBA media for the primary isolation of MTB from pulmonary sputum for pulmonary tuberculosis (PTB) suspected individuals under routine diagnosis conditions in resource-limited settings in comparison with conventional Lowenstein Jensen.

Methods

Study Design and Study Population

A comparative cross-sectional health facility-based study was conducted from November 2013 to March 2014 among PTB suspected patients in Addis Ababa, Ethiopia. Patients who were clinically suspected for PTB according to the Ethiopia TB and Leprosy National Guideline was included in the study. The protocol was approved by Armauer Hansen Research Institute/All Africa Leprosy, Tuberculosis, Rehabilitation and Research.Training Center (AHRI/ALERT), Ethics Committee before conducting the study. All participants were signed informed consent forms prior to participating in the study.

Sample Collection and Processing

PTB suspected individuals who consented to participate were properly instructed on how to produce productive sputum and submitted an adequate morning-spot-morning sputum specimen to respective health facilities. A minimum of 3-5ml of sputum was collected from each patient in a leak-proof, wide-mouthed plastic container with the cap and stored at the refrigerator before being transported to the AHRI TB laboratory in accordance with laws and guidelines for transport of biohazard material and laboratory specimens.

Laboratory Procedures Smear Examination

Direct and sediment smears were prepared by transferring two drops from unprocessed and processed sputum of the centrifuged specimens on different glass slides. Zihel Neelsen staining was done for direct (before processing), sediments and from culture suspension of all culture-positive specimens to confirm Acid Fast Bacilli (10). The International Union against Tuberculosis and Lung Disease guideline proposed scale of five groups was used for reporting the average number of Acid -Fast Bacilli observed in sputum specimens (11).

Sample Preparation On 7% Sheep Blood Agar and Lowenstein Jensen Medium

Having equal volumes of clinical sputum specimens and 4% NaOH solution was mixed in 50 ml Falcon tube, vortexes until it became homogeneous followed by decontamination, and incubated for 15 minutes at room temperature. At the end of incubation, the 50 ml mark of Falcon tubes were filled with 6.8 pH phosphate buffer saline (PBS) (Merck, Darmstadt, Germany) and then mixed by gentle inversion to neutralize NaOH and stop decontamination. Then they were centrifuged for 15 minutes at 3000 g. Finally, the supernatants were decanted immediately into a splash proof vessel containing a disinfectant, and the sediments were neutralized with Bromo-cresol purple indicator by adding one drop at a time while swirling until the indicator turned yellow from Blue. Immediately 4-5 drops of sediment (80- 100 ul) of this inoculum were dispensed onto culture medium on the LJ slants, as well as 7% SBA slants in duplicate using a Pasteur pipette to transfer the pellet to the media and then incubated at 37^0_C. McCartney bottles of 7% SBA was sealed with Para film in order to prevent desiccation of 7% SBA media (12, 13).

Blood Agar Media Preparation for Primary Isolation of Mycobacterium tuberculosis

Briefly, 7% SBA was prepared as instructed by manufacturers with the basic modifications (8, 13). The blood agar base contains Lab-Lemco' powder, Peptone, Sodium Chloride and Agar which were from Oxoid LTD Oxoid LTD Basingstoke, and Hampshire, England). Crystal violet, Polymyxin-B (Sigma Aldrich, Denmark), Nystatin (Sigma Aldrich, Romania), Nalidixic acid (Sigma Aldrich, Italy),

(and Trimethoprim Lactate Salt powder form drugs were included in the media of 7% SBA. 60 ml of blood was collected from Jugular vein of sheep and defibrinated with sterile glass beads in sterile Erlenmeyer flask and separated from glass beads by pipet boy. Five hundred ml (500 ml) of distilled water with 20 g of blood agar base and 5 ml of crystal violet solution (0.01%) were mixed well and boiled to dissolve the medium completely. It was sterilized by autoclaving at 121°C for 15 minutes and then waits to cool to 45-50 °C in a water bath.

Nystatin solution (3.150 ml), (18 mg Nystatin dissolved in 9 ml Methanol) and antibiotic solution (1.750 ml), (44.4 mg) Polymyxin-B, (5 mg) Trimethoprim and (20 mg) Nalidixic acid in (10 ml) of sterile distilled water were added to the sterilized blood agar base media in order to avoid growth of contaminants when inoculated processed sample. Antibiotics Solution were added in to the base and mixed well. Then 35 ml sheep blood was aseptically added in to the base and mixed well.
Finally, all contents were mixed well by vortexing for a minute, and approximately 7-8 ml was quickly dispensed to each of 14 ml, flat bottom McCartney bottle tubes. The components of PNNT and their final concentrations in 7% SBA were as follows: Polymyxin B, 50 U/ml (88.8 ug/ml); Nystatin, 5 mg/ml; Nalidixic acid, 40 ug/ml; Trimethoprim, 10 ug/ml.
We used a McCarty tube (tightly capped), incubating the culture tubes with distilled water in beakers surrounding the incubated tubes and sealed using para film in order to prevent drying the media.

Detection of Growth, Identification and Reading of Cultures

WHO quantitation scale was used to interpret the colonies growth result on media where a growth index of 10 or more colonies was considered positive (14). All positive growth was in time confirmed by performing smears and staining for AFB. Time taken to growth of colonies on 7% SBA and LJ was monitored daily up to 8 weeks. Isolates were confirmed for AFB by ZN technique and then species identification by PCR based RD9 deletion typing (Huard *et al.*, 2003) and *Mycobacterium* genus typing analysis (15).

Quality Control

Quality control was done by recording the temperature of incubator, fridge up to run positive (H37Rv) and negative control (plain media) in parallel with processed specimen on both media. All of the slopes of medium 7% SBA and LJ prepared in the day were incubated for 48 hours at 37°C to check sterility of the media, and 2 slopes were randomly selected from each batch of medium and incubated at 37°c for 14 days. Prior to interpreting the test we assessed both control media. If there was contamination or growth on the slants of incubated tubes during sterility check and if negative control tubes had growth, the results were invalid and re-prepare the media and re-run the tests. *M. bovis* and *H37Rv* MTB were used as positive control and Qiagen H_2O as negative control in molecular typing of RD9 deletion typing. *M. avium,* H37Rv MTB*, M. bovis were* used as positive control and Qiagen water as negative control in *Mycobacterium* genus typing, respectively.

Statistical Analysis

Data were analyzed using the SPSS version 21.0 for windows (Statistical Package for the Social Sciences Inc, Chicago, IL, USA). Wilcoxon signed-rank test was used to detect Statistically significant differences in time for uneven distribution for culture positivity. Contingency tables were used to compare the diagnostic test versus conventional, with which sensitivity, specificity, PPV and NPV were obtained with their 95% CI for 7% SBA media. P values less than 0.05 were considered statistically significant. Sensitivity and specificity with a fixed false positive rate of 5%, prevalence of smear positive PTB 14.02% from suspected cases in Eastern part of Ethiopia, (16) sensitivity of the SBA in population and 95% confidence interval were used to calculate the sample size

Ethical consideration

The patient's written consent was obtained before the conduct of the study.

Result

Demographic and Clinical Characteristic of Study Subjects

Out of 222 study participants, 10 participants were excluded from analysis due to insufficient volume and poor quality of sputum. Among 212 participants suspected of PTB, 46 (21.7%) were AFB smear positive from the primary sample (i.e., sediment smear), 59 (27.8%) were culture positive on SBA 56 (26.4%) were a culture positive on LJ and 153 (72.2%) were smear and culture negative. The prevalence of TB among suspected individuals was 59/212 (27.8%). 96 (45.3%) were female showing an overall female to male ratio of 1:1.2. The mean age of the participants was 35.1±SD years (ranging from 16 to 80). Of the 212 participants, 39 (18.4%) were HIV positive (Table 1).

Diagnostic Efficacy of Sheep Blood Agar for Primary Isolation of Mycobacterium Species

Diagnostic efficacy of 7% SBA media was done for the primary isolation of mycobacteria considering LJ media a gold standard method. The specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) of 7% SBA media were 96.43, 98.08, 94.74 and 98.71%, respectively (Table 2). Seven percent SBA had similar outcomes in diagnostic performance of results with a smear from sediment microscopy but which had a better yield in the examination of sputum in microscopy than direct smear microscopy.

Table 1: Demographic and Clinical Characteristic of participants in Addis Ababa, Ethiopia.

	Variables	Frequency	%
Sex	Female	96	45.3%
	Male	116	54.7%
HIV	Positive	39	18.4%
	Negative	173	81.6%
Sputum appear-ance	Bloody	7	3.3%
	Mucoid	26	12.3%
	Mucopurulent	44	20.8%
	Purulent	87	41.0%
	Salivary	48	22.6%
Sputum grade from direct smear	Negative	166	78.3%
	Scanty	5	2.4%
	1+	9	4.2%
	2+	17	8.0%
	3+	15	7.1%
Direct smear result	Positive	59	27.8%
	Negative	166	78.3%

Table 2: Diagnostic efficacy of SBA over the Gold standard method (1) for 212 participants and (2) for 39 HIV study participants from 5 public's Health institutes Addis Ababa, Ethiopia.

	Variables	Frequency	%
Sex	Female	96	45.3%
	Male	116	54.7%
HIV	Positive	39	18.4%
	Negative	173	81.6%
Sputum ap-pearance	Bloody	7	3.3%
	Mucoid	26	12.3%
	Mucopurulent	44	20.8%
	Purulent	87	41.0%
	Salivary	48	22.6%
Sputum grade from direct smear	Negative	166	78.3%
	Scanty	5	2.4%
	1+	9	4.2%
	2+	17	8.0%
	3+	15	7.1%
Direct smear result	Positive	59	27.8%
	Negative	166	78.3%

Time to Detection of Mycobacterial Isolates

There was significant difference in the detection day of macroscopic colonies in 7% SBA and LJ. The median days to detect macroscopic colonies on 7% SBA and LJ were 14 days (ranging from 9 to 28) and 20 days (ranging from 12 to 39) days, respectively (P<0.0001). From direct smear grades 1+ on 7% SBA, mean detection days were 14.8 ± 3.1 days but on LJ the mean detection days were 23.4±6.6 days. The smear positive grade of 3+AFB was detected in 13.8 days on 7% SBA, but on LJ were 15.3 days. The rate of contamination was a little lower in SBA 20/424 (4.7%) than LJ 22/424 (5.2%).

Molecular Characteristics of Mycobacterium spp.

Isolates out of the 59 positive cultures in both 7% SBA and LJ, 58 (98.3%) were MTB by RD9 deletion typing. While among 13 smear negative culture positive cases of *Mycobacterium* spp, 7% SBA supports growth of MTB in 10 smear negative culture positive cases and *Mycobacterium* spp in one case whereas LJ media supports growth of MTB in nine smear negative culture positive cases and *Mycobacterium* spp in one case. Out of 59 isolates from both media, 57 isolates were grown on 7% SBA; whereas 56 isolates were grown on LJ. Out of three discordant results, which were growing only on 7% SBA, one isolate was member of *Mycobacterium* spp. Fifty-five isolates from LJ were MTB and one isolate was *Mycobacterium* spp. Two isolates were grown only on LJ

Discussion

In most of clinical microbiology laboratories, standard blood agar is commonly used for isolating bacteria from clinical samples because it is cheap, simple to prepare, and many species can be grown (13). Recent studies reported that SBA could be used for isolation of MTB within 1-2 weeks, and the mean number of colonies was significantly larger on blood agar than on the egg-based medium (13, 15). In our study, the specificity, positive predictive value, and negative predictive value of culture on blood agar were observed to be 98.08, 94.74 and 98.71%, respectively. These values were in agreement with those obtained in a study by Palange i.e., 99.59%, 93.75%, and 98.02%, respectively from India (5).Drancourt and his colleagues illustrated that blood agar has at least equivalent to an egg-based medium for the isolation of MTB from respiratory and lymph node specimens (7). In the present study we evaluated the diagnostic efficacy of 7% SBA in ison with LJ media for primary isolates of MTB from sputum specimens under routine diagnosis. Our finding on primary isolation of MTB on 7% SBA has similar sensitivity with other studies done in Turkey reported 97.5% (17), 94.2% (17), 98.9% (8), 94.2% (13) and 89.3% (5), but disagree with 27.3% sensitivity with other study done in India (18) which may be due to small sample size (only20 smear positives) and difference in drug ingredients concentration. Studies conducted before six decades showed a similar finding in the performance in comparison to LJ media (94.2%), but they used human blood instead of sheep blood (18) reported that sensitivity of direct and concentrated smear microscopy was not show significantly different (15). In this study we have found that sedimentation prior to ZN staining significantly increased sensitivity for TB over direct smear microscopy which is comparable with the results of three previous studies that found higher sensitivity in HIV endemic populations after sputum concentration (19-21). In a study conducted in France they reported that MTB isolates easily grown on blood agar with in average of 1-2 weeks to the diagnosis of TB (11). Blood agar slants are a good substitute of LJ medium for rapid detection of MTB from sputum in resource-limited settings by saving up to one-third of the time, this agrees with that of the previous study (16). So, it is the time to evaluate the new media for their capability to stand alone or with the help of smear microscopy at least as an acceptable culture method. The present study has shown that 7% SBA took short detection time in comparison to LJ. The time to detection (TTD) for macroscopic colonies in 7% SBA was significantly different (P<0.0001) where the median days to detect macro -colonies on 7% SBA and LJ were 14 (range 9 to 28) days and 20 (range 12 to 39) days, respectively. Various studies on SBA TTD from smear positive and negative pulmonary sample revealed in the range of 1-3 weeks (13) Center for Disease Control and Prevention (CDC) recommends that the reports of isolation and identification of MTB complex species should be available within 10 to 14 days or 21 days of specimen collection (10). In our study we showed that growth of *M. tuberculosis* in 7% SBA saves 1/4 time in comparison with LJ and other study showed that the detection time of MTB saves 1/3 time in comparison with LJ and 1/2 time save (13, 16). *Mycobacterium tuberculosis* needs to be enriched and selective media to minimize contamination A contamination rate of 2 -5% is acceptable in laboratories, as a general rule when receiving fresh specimens. Laboratory which experiences no contamination probably uses a method that kills too many of the tubercle bacilli (16) The addition of PNNT to the SBA media performance was excellent and managed to reduce the contamination rate to 4.7% in this study, which is within acceptable range for culture from non-sterile site. This study suggests that isolation of MTB on blood agar will not be an anecdotal unscientific and that contamination of culture will not be a frequent problem if we follow decontamination procedure strictly and use antibiotics solutions to inhibit contaminants (10). Based on CLSI and WHO recommendation, our 7% SBA finding on contamination rate revealed difference from 1.5% in 5% SBA; 1.6 % in 7% SBA versus to 7.8% in LJ medium.

This finding has similarity with that of Shidiki and Pokhrel in which 2% contamination rate on both BA and LJ media (11) and s 1.6% on blood agar, 1.5% on blood agar (8) and 7.8% on LJ medium (16).
Even though our protocol and Mathur *et al*. was the same on media preparation but both of us were used different decontamination procedures. The difference may be due to decontamination because they could not get growth from 4 grade 1+ smear sputum specimens after decontamination using NALC-NaOH method. But our media allowed growth even from scanty smear grade specimens by using 4% NaOH. Other reasons for low contamination rate maybe they used high concentration antibiotics and increase the decontamination time to inhibit contaminants. In previous studies (22) LJ contamination varied from 7.8 to 21.1%. Conclusion Based on this finding 7% SBA may be good alternative of LJ in recourse-limited setting. TB Laboratory without culture facility, sediment microscopy may have equal diagnostic efficacy as 7% SBA, and sediment microscopy should be done at the same time to urgent treatment of the patient. The main advantages of blood agar medium are simplicity of preparation and ability to grow MTB from inoculum easily and recognizable in a short time. The ingredients are easily obtainable, and fresh media can be prepared within a few hours. All these factors should encourage a wider use of cultural methods for the bacteriologic diagnosis of tuberculosis.

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Original Article

Identification of bacterial profile, common associated risk factors, and antimicrobial susceptibility patterns, of bacterial keratitis in community hospitals of Asmara, Eritrea

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Abstract

Background: *Bacterial keratitis causes infection and inflammation of the cornea resulting in pain, blurred vision, photosensitivity, lacrimation, eye discharge, and loss of vision in severe cases. The present study was designed to investigate the bacterial profile, associated risk factors, and Antimicrobial Susceptibility Patterns (AST) of bacterial keratitis in Berhan Aini hospital, Godaif, and Biet Mekae community hospitals of Asmara Eritrea.*

Methods: *A cross-sectional study was designed to assess the incidence of bacterial keratitis among suspected keratitis patients who visited Berhan Aini hospital and community hospitals of Godaif and Biet Mekae over the past year. The study subjects were diagnosed by an ophthalmic officer and sample was collected.*

Results: *A total of 330 suspected bacterial keratitis patients (330 eyes) were examined during the study period of more than one year. The total 220 (66.66%) cases were culture positive, the most common isolated bacteria was Staphylococcus aureus, 110 (50%); followed by Coagulase-negative Staphylococcus aureus CoNS 66 (30%), Streptococcus pneumoniae, 33(15%); and Streptococcus viridans 11 (5%). S.aureus isolate showed 99 (90%) sensitivity to ciprofloxacin, rifampin, gentamycin, vancomycin, and nitrofurantoin, 88 (80%) to chloramphenicol, 77 (70%) to clindamycin, 66 (60%) to erythromycin, and 55 (50%) to tetracycline, whereas it was 110 (100%) resistant to oxacillin and Penicillin. The most predisposing factor among the cases was trauma.*

Conclusion: *The most common bacteria causing bacterial keratitis in Asmara Ophthalmic Hospitals were Gram-positive bacteria. Trauma was found to be the most common exposing factor for bacterial keratitis which was not statistically significant associated with the culture-positive result ($p>0.05$). S. aureus was found to be highly sensitive to ciprofloxacin, gentamycin, vancomycin, rifampin, and nitrofurantoin, and 100% resistant to oxacillin and penicillin upon evaluation of antimicrobial activity of several antibiotics.*

Keywords: *Keywords: Asmara, Bacterial Keratitis, AST, Risk factors, Eritrea*

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Introduction:

Eyes, which distinguish distant objects from near objects, determine their color and shape and perceive light to see the world and understand how objects relate to each other, are complex organs of vital importance for everyday life.

The cornea protects our eyes from harmful substances with the help of eyelids, eye sockets, tears, and sclera within the white part of the eye [(1)].

The corneal and conjunctival epithelial cells are tightly bound together, providing another barrier to microbial invasion, and epithelial cells themselves can phagocyte and transport microbes [(2)]. The thing which lies between the front of the cornea and our environment is a very thin tear film [(3)].

Acute or subacute pain, conjunctival injection, and corneal ulceration with a stromal inflammatory infiltrate are characteristic signs of microbial keratitis which is an infection of the cornea [(1).]

Microbial keratitis, which can be caused by bacteria, fungi, viruses, or parasites, is a potentially vision-threatening condition and requires prompt diagnosis and treatment to prevent serious implications. The cornea provides natural resistance to infection, so microbial keratitis rarely occurs in the normal eye. However, bacteria can invade the cornea due to exposure of the eye to risk factors such as trauma, contact lens wear, dry eyes, ocular surface disorders, and immunosuppression, these risk factors may alter the defense mechanism of the outer eye, and allow microorganisms to invade the eye and cause infection [4].

Bacterial keratitis, which is an inflammation of the cornea, progresses rapidly, and corneal destruction may be completed in 24 - 48 hours with some of the more virulent bacteria. The main symptoms of bacterial keratitis are pain, redness, watering, mucopurulent or purulent discharge, photophobia, defective vision, foreign body sensation, and loss of vision in severe cases. The underlying condition of the cornea and the pathogenicity of the infected bacteria generally display the severity of corneal infection [5].

In bacterial keratitis, many patients have a poor clinical outcome if aggressive and appropriate therapy is not promptly initiated due to susceptibility of the avascular corneal stroma to bacterial infection. Bacterial keratitis is one of the most visually threatening ocular infections resulting from the high incidence and potential complications. The presence of particularly invasive pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* can lead to corneal perforation in less than 24 hours. When fluoroquinolone antibiotic is used as a mono-therapeutic agent, the emergence of multiresistant strains is also a major concern.

Bacteria which cause keratitis may be Gram-positive or Gram-negative, the wide range of bacteria includes *Staphylococci, Streptococci, Pseudomonas, Enterobacteriaceae, Corynebacterium species, Moraxella species, Serratia species, Haemophilus, and N. gonorrhea* [5]. Eighty percent of bacterial corneal ulcers are caused by *Staphylococcus aureus, Streptococcus pneumoniae, and Pseudomonas species.* The most frequent and most pathogenic ocular pathogen which can cause corneal perforation in just 72 hours is *Pseudomonas aeruginosa.* Bacterial keratitis can be controlled and treated by prompt and meticulous investigation, otherwise, it becomes a serious condition and may progress to endophthalmitis, perforation, and blindness. Contact lens wears followed by a preexisting ocular disease that includes ocular trauma, ocular surgery, laser refractive surgery, and use of topical steroids are some of the predisposing factors for bacterial keratitis.

Finally, geographic and climatic factors can also influence the bacterial pattern of keratitis; rural or city area populations have also seen many differences in keratitis profile. This difference between geographic and climatic factors on the profile of bacterial keratitis can be explained by the high variation of habits, such as antibiotic use, contact lens wear, or even specific rural pathogen exposure [5].

The management of bacterial keratitis should include adequate ocular prevention, knowledge of the microbiological patterns in a given clinical practice, and a correct choice of antibiotics available for treatment, and considering drug toxicity and bacterial resistance, will also be key to success in the effective management of bacterial keratitis [6,7].

Eritrea is geographically located in Sub-Sahara Africa where there is an increased burden of microbial keratitis. Understanding the magnitude of bacterial keratitis problem as a cause of vision loss, fewer or no studies have been reported yet from Eritrea which makes our study novel. There may be many studies from surrounding countries, but with changes in geographic location and changes in the physiology of people the final outcome varies. The current study will be helpful to identify the main risk factors that predispose the Eritrean population to bacterial keratitis and increase their awareness regarding the risk factors. It will help health service providers to give appropriate treatment according to the specific cause of keratitis. It will take a sharp interest to the health service providers to understand the main etiologies. The study will pave the way for a large-scale study on all microbial keratitis in all clinical areas.

Materials and Methods:

Study design:

The current study was a cross-sectional study designed to determine the incidence-associated risk factors of bacterial keratitis with their antibiotic susceptibility pattern.

Study area:

This study was conducted in Berhan Aini National Ophthalmic Hospital and community hospitals of Godaif and Bet Mekai which are found in Asmara. All samples were processed and analyzed in the microbiology laboratories of the National Drug Quality Control Laboratories (NDQCL) and the National Health Laboratory (NHL). The study period was from July 2020 to October 2021.

Study population:

The study population were individuals of all ages who were visiting the eye hospital during the study period with signs and symptoms of infectious keratitis.

Inclusion criteria:

- All patients visiting the outpatient department with corneal infection
- Patients diagnosed with infectious keratitis and admitted to the hospital

Exclusion criterion:

- Patients under antibiotic therapy

Sample size:
Convenient sampling with inclusion and exclusion criteria was used in which the maximum number of participants was included within the time of data collection.

Data Collection Techniques:
A researcher-administered questionnaire was used to collect demographic data and risk factors associated with infectious keratitis. The questionnaire was filled out by the researcher in the form of an interview. Data obtained from the questionnaire were associated with the microbiological laboratory results.

Specimen collection:
The analytical part of the study started with the preparation of all important materials, reagents, and equipment before the process of specimen collection and transportation to the site of analysis. Each specimen was designed to be labeled with a series of numbers that contains the sample collection date and sample code.
Slit-lamp biomicroscope was used by an ophthalmologist for the examination of all patients.
The present study used a corneal swab, as it is the simplest option and is called a "**quick culture**." Corneal scrapings are the gold standard method of corneal sample collection, but due to lack of specialists and risk in the collection, the researchers were advised to go with corneal swabs.

Corneal swab specimens were collected with sterile swabs from the infected cornea after applying 2 drops of local anesthetic drop (Tetracaine 1%). The swab was labeled with the specific name of the participant and sample code after filling out the questionnaire. Then the samples were transported using the swab transport media (Stuart) to the laboratory within one hour of collection.

Specimen processing:
The samples were checked if they were properly labeled, and the media was labeled with respect to the sample code. The swab specimen was then primarily inoculated with three media that passed the quality control, i.e., chocolate agar, mannitol salt agar (MSA), and mcconkey agar (MAC) (Hi-Media, Mumbai, India) by the striking method which was under continuous sterilization. The cultured media were incubated at 37^{o}C for 18-24 hours. After the appropriate incubation time, the culture was examined for the presence of any growth, and the morphology of the growth was carefully described, depending on the size, color, and shape of the colony. This gave some clues to the bacterial identification. For example, the color changes indicated in mannitol fermenters and the possible acid production when the bacteria can utilize the supplement present in the media, the shape and size are also helpful to see if it is mixed or if two bacteria were grown in the media. Mannitol Salt Agar has mannitol and high salt concentration (i.e., 7.5% NaCl), it is selective as it selectively supports the growth of gram-positive staphylococcus SPP and differential as it differentiates mannitol fermenters from non-mannitol fermenters.

Mcconkey agar is used as a selective media which supports the growth of gram-negative bacteria, as it has crystal violet which hinders the growth of gram-positive bacteria, and as a differential media which differentiates lactose fermenters from non-lactose fermenters. Chocolate agar is an enriched media that supports the growth of microaerophilic bacteria.

Primary Isolation and Identification Tests:
Following 18 to 24 hours of incubation, the culture media were read. Gram staining and biochemical tests were done for those media which showed growth.

Gram Staining: This method was used to identify and differentiate Gram-positive and Gram-negative bacteria. A loop full sample of the bacteria was mixed with a drop of normal saline on a microscope slide and allowed to air dry. The smear was covered with crystal violet and allowed to stand for one minute. The stain was briefly washed off using distilled water. The excess water was drained off. Then the smear was covered with Gram's iodine solution for one minute, which was used as a mordant so that the gram-positive bacteria will retain the color of crystal violet. It was then washed off, and the slide was held 45- degree angle and allowed 95% (isopropyl alcohol) to flow down the surface of the slide until the alcohol turned colorless as it flowed from the smear down the surface of the slide. Decolorization was stopped by washing the slide with a gentle smear of water. Then the smear was covered with safranin stain for 1 minute, washed with water, and air-dried. The slides were examined under oil immersion [(1)]. All reagents for Gram staining were purchased from an Indian company. (Hi-Media, Mumbai, India).

Biochemical Test: These tests were performed for detailed identification of bacteria to their species level using enzymatic or color change (PH indicators). The Gram-positive isolates were differentiated using the catalase test, DNase test, and optochin test. Gram-negative isolation is more complex than Gram-positive because they require several tests like oxidase test, citrate test, urea test, indole test, methyl red test (MR test), carbohydrate tests (glucose, mannitol, sorbitol, sucrose, and triple sugar iron (TSI), (Hi-Media, Mumbai, India), etc., to identify the isolates.

Antimicrobial Susceptibility Test:
Antibiotic susceptibility testing of each isolated pathogen was done using routine antibiotic discs according to the disc diffusion technique on muller hinton agar (MHA). The organism, which was primarily inoculated was again sub-cultured for refreshing just before performing antimicrobial susceptibility tests.

A bacterial suspension was prepared from freshly isolated colonies, and a small amount of the suspension was put in a biochemical test tube. Using a sterile swab, the bacterial suspension was inoculated onto muller hinton agar using a sterile swab. The organisms were tested against different types of anti-Gram-positive drugs for susceptibility profiles. The drugs were separated into two groups of 5 and 6 to provide enough space for disc diffusion in the media, and then the discs were set in position in the media. The two media were incubated overnight (24 hours) at 37^0C. Then the results were read and the zone of inhibition was measured using a ruler and the isolates were designated as sensitive, intermediate, and resistant using the standard reference ranges mentioned in Clinical and Laboratory Standards Institute 2019 (CLSI 2019) [8].

Quality control:

To assure correct results, the working situation should appropriately be quality controlled. Quality control was started by cleaning the laboratory. Regarding the quality control of materials and equipment, the equipment which was necessary to perform the research was checked for their performance capacity.

As part of the quality assessment function of the incubator, autoclave, oven, and refrigerator were checked. The incubator was set to a temperature of 37- 38^0C using a thermometer daily. The temperature of the refrigerator was also maintained at 2 – 6^0C, and this was controlled using a thermometer each day. Then, the autoclave was checked for the presence of water and the sterility was controlled using a color tape indicator. For prevention of contamination, all materials such as test tubes, reagent containers, Petri dishes, media, etc. were autoclaved. The swabs used for sample collection were checked for their potency using a stock organism and by inoculating the swab without any usage of the sample for its sterility. Along with this, to signify the quality of the media, 10% of the prepared media were incubated at 37^0C to control the growth and predict the degree of contamination. Furthermore, the quality of the media was checked by inoculating stock organisms. The working area was disinfected before and after performing any test using 10% bleach or alcohol. Along with all this, to maintain the quality of the specimen and the viability of the microorganisms, the corneal swab was processed within one hour of collection.

Data analysis:

After the samples were processed, the results were conveyed in statistical terms using a statistical computer software version 20 SPSS. The quantitative results are given in terms of percentages, graphs, and tables. The chi-square test was used to compare the independent variables, and a p-value < 0.05 was considered significant in analyzing the relationship between the variables.

Ethical consideration:

The study was conducted according to the declaration of Helsinki; Asmara College of Health Sciences and obtained ethical approval by the ethical committee of the Research Centre of the Ministry of Health. Furthermore, it also got permission from the medical director of Berhan Aini Hospital and ophthalmic officers of Godaif and Biet Mekae Community Hospitals. A written consent was obtained from the participants, and proxy consent was obtained from parents or guardians for those participants below 18 years of age.

Results:

General description of the study:

During the study period, a total of 330 keratitis cases who visited the ophthalmic centers of different hospitals in Asmara were included. Out of the 330 participants, 132(40%) were from Berhan Aini Hospital, 110(33.3%) from Biet Mekae community hospital, and the rest 88(26.6%) from Godaif community hospital.

Patient's demographic characteristics:

Out of the 330 study participants, 165 (50%) were males and females each, so the overall male-to-female ratio was 1:1. The study participants' age ranged from 4–88 years with a mean age of 50 years (SD 23.7). Zoba Maekel had the highest number of keratitis cases with 132(40%) followed by Zoba Debub 88(26.6%), Northern Red Sea 55 (16.6%), Southern Red Sea 33(10%), Anseba and Gash Barka 11(3.3%) One hundred eighty-seven (56.6%) of the study participants were living in urban, while the remaining 143(43.3%) were from rural areas. The employment status of the study participants showed that 154(46.6%) were unemployed, 121(36.6%) farmers, 22 (6.6%) mechanics, 11 (3.3%) students, 11 (3.3%) secretaries, and 11 (3.3%) merchants. **Table 1**

Table 1: Study participants' demographic characteristics

Parameter	Frequency	Percentage
	Age	
4-24	33	10%
25-45	121	36.6%
46-66	88	26.6%
67-88	88	26.6%
	Sex	
Female	165	50%
Male	165	50%
	Occupation	
Student	11	3.3%
Mechanic	22	6.6%
Secretary	11	3.3%
Merchant	11	3.3%
Farmer	121	36.6%
Unemployed	154	46.6%
	Residence	
Urban	187	56.6%
Rural	143	43.3%
	Zone	
Maekel	132	40%
Debub	88	26.6%
NRS	55	16.6%
SRS	33	10%
Anseba	11	3.3%
Gash Barka	11	3.3%

Ethnicity distribution:
In terms of ethnicity, the majority were Tigrigna, followed by Tigre, Saho, and Bilen. No study participant was found from the remaining ethnic groups. **Figure 1**

Fig 1: Ethnicity of study participants

Sample analysis of the corneal swab specimens:
Following sample collection, the corneal swab samples were inoculated on mannitol salt agar, mcconkey agar, and chocolate agar. Following 18-24 hours of incubation at 37°C, the samples were read, and the samples which showed growth were further processed using Gram's stain, catalase test, optochin test, and dnase test to identify the isolates. Finally, the isolates were tested against different anti-Gram-positive drugs to test their antimicrobial susceptibility pattern.

Culture results of corneal swab:
Laboratory results were a vital part of this research alongside the clinical information gathered from the questionnaire. In the current study, 330 samples were obtained and processed using culture and sensitivity techniques. The outcome of the culture results indicates that 220(66.6%) of the study participants showed growth, while the remaining 110 (33.3%) showed no growth. **Figure 2**

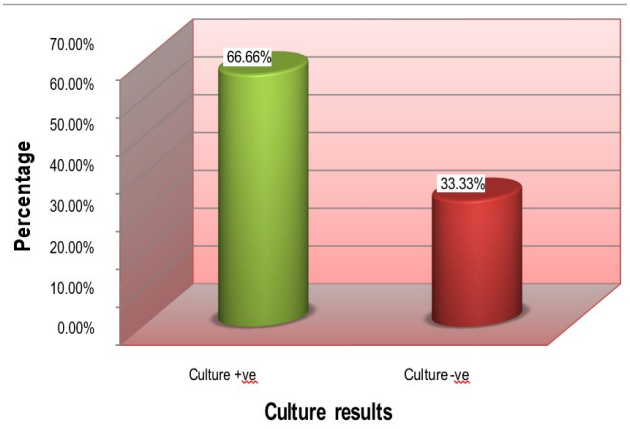

Fig 2: Culture results of the corneal swab

Identification of culture results:
Culture-positive samples were further processed for Gram staining. All isolates were found to be Gram-positive. Catalase, dnase, and optochin tests were performed. The catalase test was performed by taking a few colonies from the culture with the help of an applicator stick and dropping them on 3% hydrogen peroxide (H_2O_2). If the bacteria are catalase-positive, they will break hydrogen peroxide into oxygen and water molecules which are indicated by the production of bubbles. This type of test was used to distinguish *Staphylococcus* from *Streptococcus spp* where the former was catalase +ve, **Figure 5**. For those who were catalase-positive, the dnase test was done to differentiate *S.aureus* from other *Staphylococci spp*. This type of test was done by subculturing on dnase agar media, where a positive test was interpreted when a clear zone was observed around the colony following the flooding of HCl acid. Catalase and dnase-positive bacteria were considered clinically significant, and it was *S.aureus,* otherwise, they were *CoNS.* Based on the alpha-hemolytic pattern on chocolate agar, the optochin test was done to differentiate *S. pneumoniae* from *S.viridans*, as *S. pneumoniae* is sensitive to it. **Table 2.**

Table 2: *Identification of culture results*

	Results		
Identification Tests	Positive n (%)	Negative n (%)	Total
Mannitol Fermentation	176(80%)	44(20%)	220
Gram stain	220(100%)	0(0%)	220
Catalase Test	176(80%)	44 (20%)	220
DNase Test	176(80%)	44(20%)	220
Hemolytic pattern on Chocolate agar	Alpha hemolysis 44(20%)	176 (80%)	220
Optochin disk	33 (15%)	187 (85%)	220

Bacterial Etiologies:
After doing the appropriate culturing, subculturing, and biochemical tests for the 220 culture -positive results, our findings showed that *staphylococcus aureus* was the most dominant 110(50%), followed by *CoNS* 66(30%), *streptococci pneumoniae* 33(15%), and *streptococci viridians* 11 (5%). No gram-negative bacteria were found. **Figure 3, 4 & 6**

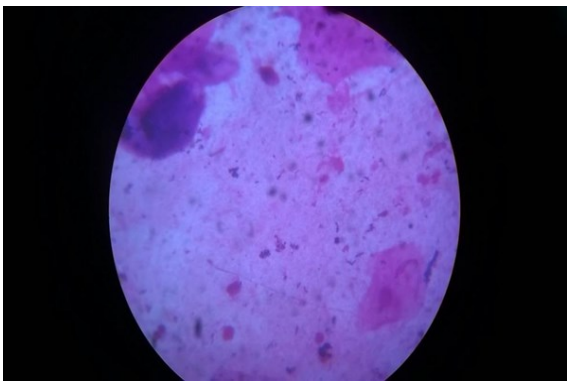

Fig 3: Gram Stain showing Gram-Positive Diplococci *Streptococcus pneumoniae*

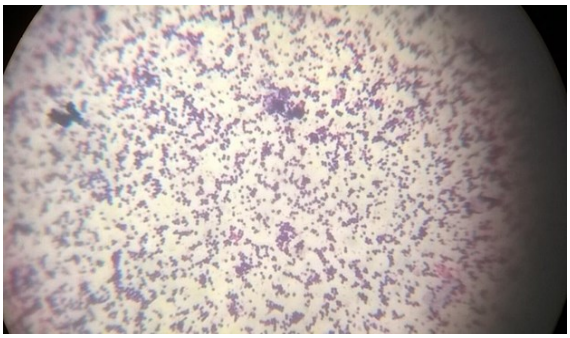

Fig 4: Gram stain showing Gram-Positive cocci in clusters *Staphylococcus aureus*

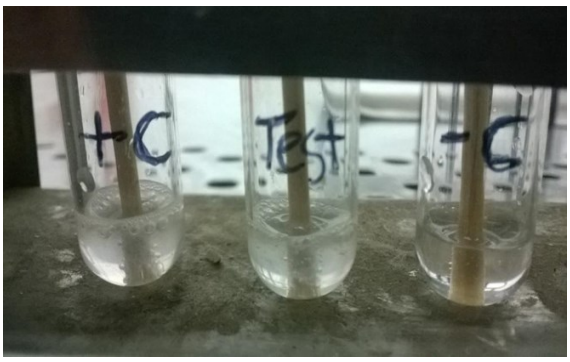

Figure 5: Tube catalase test to distinguish *Staphylococcus* from *Streptococcus spp*

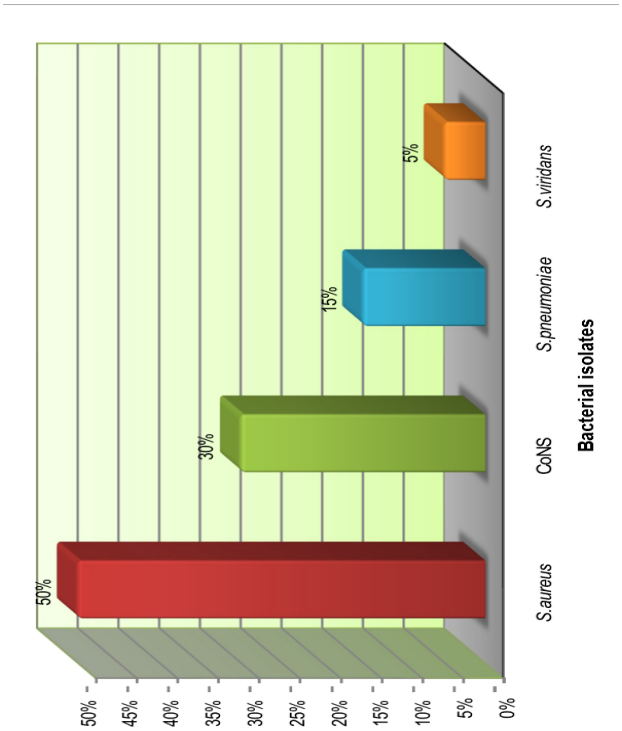

Fig 6: Identified bacterial etiologies:

Culture results in relation to demographic characteristics:

Of the 220 corneal swab samples which were culture positive, 132(60%) were male and 88(40%) female. The study participants 99 (45%) were below 40 years old and 121 (55%) above 40 years old. **Figure 7**

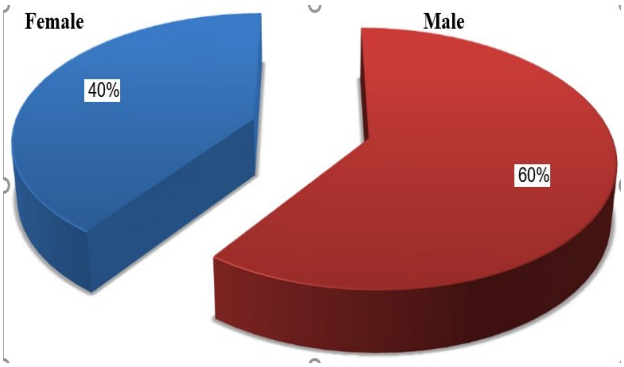

Fig 7: Culture results in relation to sex:

Culture results in relation to occupation:

The employment status of the study participants in relation to culture-positive results, 110 (50%) were unemployed, 77 (35%) farmers, 11(5%) students, 11(5%) secretaries, and 11(5%) merchants.

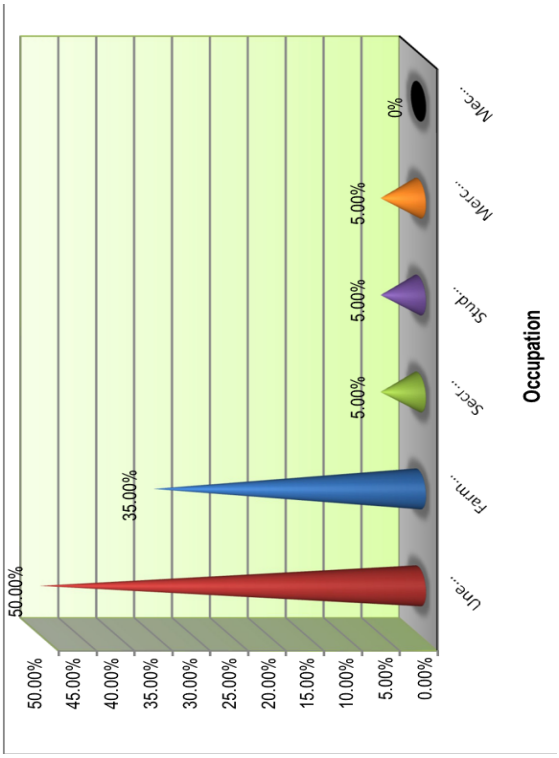

Fig 8: Culture results in relation to occupation

Risk factors predisposing to ocular conditions:

The present study also assessed the most potential risk factors that expose an individual to infectious keratitis. Generally, the possible known risk factors for infectious keratitis are trauma, foreign bodies, wearing contact lenses, ocular and eyelid surgery, other than these ocular surface diseases, corneal epithelial abnormalities and systemic diseases have a contributing factor. During the study time, a questionnaire was used to assess the risk factors of bacterial keratitis, and the questionnaire was filled out by the researchers. Trauma was the most common risk factor which was found in 165(50%) of the total cases, followed by the entrance of foreign bodies, systemic disease, and cases with unknown cause, i.e., 55(16.6%), 45(13.6%), and 65(19.6%), respectively. The participants were also assessed for their smoking status, alcohol consumption, ocular surgery, and any vitamin deficiency, however, no study subject was found with those above conditions. SPSS software version 20 was used to determine the association of risk factors with culture results. Out of the 220 culture-positive results, 132(60%) were due to trauma, 33(15%) due to systemic disease, 22(10%) due to foreign bodies, and 33(15%) due to unknown causes though no significant association was found with a *p*-value less than 0.05. **Table 3**

Table 3: Associated risk factors with culture results

Risk factors	Culture Results			
	Positive n (%)	Negative n (%)	Total n (%)	P-value
Trauma	132(60%)	33(30%)	165(50%)	0.123
Foreign bodies	22(10%)	33(30%)	55(16.6%)	0.551
Systemic disease	33(15%)	11(10%)	44(13.3%)	0.593
Unknown	33(15%)	33(30%)	66(20%)	0.306
Total	220	110	330	

Antimicrobial susceptibility results:

According to the National Health Laboratory, the sensitivities of pathogenic *S. aureus* and *S. pneumoniae* isolates were tested with different drugs for their sensitivity pattern. The panels used in AST varied based on the Gram stain characteristics and the antibiotic mode of action. Except for CoNS and *S. viridans,* all Gram-positive bacteria were subjected to antimicrobial tests because they are normal flora. The antibiotic panel used for Gram-positive bacteria were Ciprofloxacin, Clindamycin, Chloramphenicol, Erythromycin, Gentamycin, Oxacillin, Nitrofurantoin, Penicillin, Rifampin, Tetracycline, and Vancomycin. The selection of antibiotics was based on the treatment guidelines of the country for treating different bacterial infections [9,10]. The AST pattern of Gram-positive bacteria is shown in **Table 4**.

AST patterns for *S.aureus*:

S.aureus isolate showed 100% sensitivity to rifampin, while it was 90% to ciprofloxacin, gentamycin, vancomycin, and nitrofurantoin. 80% to chloramphenicol, 70% to clindamycin, 60% to erythromycin, and 50% to tetracycline, whereas it was 100% resistant to oxacillin and penicillin.

Table 4: AST patterns for *S.aureus*

	Sensitive n (%)	Intermediate n (%)	Resistance n (%)	Total
Ciprofloxacin	99(90%)	0(0%)	11(10%)	110
Clindamycin	77(70%)	11(10%)	22(20%)	110
Chloramphenicol	88(80%)	22(20%)	0(0%)	110
Erythromycin	66(60%)	0(0%)	44(40%)	110
Gentamycin	99(90%)	0(0%)	11(10%)	110
Oxacilin	0(0%)	0(0%)	110(100%)	110
Nitrofurontin	99(90%)	11(10%)	0(0%)	110
Penicillin	0(0%)	0(0%)	110(100%)	110
Rifampin	110(100%)	0(0%)	0(0%)	110
Tetracycline	55(50%)	0(0%)	55(50%)	110
Vancomycin	99(90%)	0(0%)	11(10%)	110

AST patterns for *S.pneumoniae*:

The sensitivity pattern observed for *S.pneumoniae* is displayed in **Table 5**. It showed 100% sensitivity to clindamycin, chloramphenicol, tetracycline, gentamycin, and 33.3% to vancomycin, whereas it was 100% resistant to oxacillin.

Table 5: AST patterns for *S.pneumoniae*

	Sensitive (%)	Intermediate (%)	Resistance (%)
Clindamycin	33(100%)	(0%)	0(0%)
Chloramphenicol	33(100%)	0(0%)	0(0%)
Gentamycin	33(100%)	0(0%)	0(0%)
Oxacillin	0(%)	0(0%)	33(100)
Tetracycline	33(100%)	0(0%)	0(0%)
Vancomycin	11(33.3%)	22(66.7%)	0(0%)

Discussion:

Bacterial keratitis is one of the most visually intimidating eye diseases because of its high frequency and potential problems. Gram-positive bacteria such as Staphylococcus. aureus, Staphylococcus. epidermidis, and several Streptococcus spp, as well as Gram-negative bacteria such as Pseudomonas Aeruginosa, are largely involved in bacterial keratitis infection (11). The current is a cross-sectional study conducted among suspected bacterial keratitis patients. A total of 330 swab samples were collected with the help of one ophthalmologist. Samples collected from the infected cornea were inoculated on mannitol salt agar, mcconkey agar, and chocolate agar for microbiological analysis.

Microbial keratitis is now recognized by World Health Organization (WHO) as a major cause of corneal blindness which leads to visual disability. Microbial keratitis incidence varies from one country to another, and there is regional variation in organism type within the country (12).

In this study, out of the 330 most susceptible participants for bacterial keratitis, 220 (66.7%) were found to be culture-positive and all isolates were Gram-positive. However, the remaining110 (33.3%) were culture negative. All corneal samples collected from keratitis patients were not positive for bacterial cultures, because there is a possibility of other pathogens, and our study is limited to identifying only bacterial keratitis.

The most predominant bacterial isolate was *S. aureus* (50%), followed by *CoNS* (30%), *S. pneumoniae* (15%), and *S. viridans* (5%). A Review study done in the United Kingdom (UK) revealed that *Staphylococci* (40.1%), followed by *Pseudomonas* species (28.5%), were the most common bacteria isolated from bacterial keratitis subjects, while other Gram-negative species (17.2%) and *Streptococci* was (7.1%) [13]. In another related study conducted at the University of Lausanne, Switzerland (2001), the most commonly isolated bacteria were *S. epidermidis*, 40%; *S. aureus*, 22%, and *S. pneumoniae* 8% [14]. A similar study in a tertiary eye center in India reported (64.5%) *Staphylococcus* species in total positive bacterial cultures, followed by (12.3%) *Streptococcus* spp. and (9.7%) *Pseudomonas aeruginosa*. A supporting study conducted in South Africa (2011) reported that the most commonly isolated bacteria were *S. aureus* (29.5%) and *P. aeruginosa* (30%) [15]. In Assiut University, Egypt (2010) reported *Staphylococcus* species in 45 out of 50 cases [16]. In another study of 50 cases, 30 were found positive for *S. aureus* as the most common bacteria, while an unidentified species of *Staphylococcus* was also isolated from 15 cases [17]. Unlike our results, a study conducted in Sudan 2008 showed that *Pseudomonas* was the most predominant 58.8% cases, followed by *Staphylococcus aureus* 2 (11.8%) [18]. There was no presence of Gram-negative bacteria in our study, and this could be due to regional geographical and climatic variation in the occurrence of corneal pathogens. While other studies conducted in other parts of the world showed the prevalence of both Gram-positive and negacteria [17,18].

Predisposing factors are required for bacterial keratitis to happen. Corneal ulceration risk factors are different throughout the world, ocular trauma or ocular surface disease up to recently were associated with microbial keratitis. In developed countries, contact lens wearing has been a major risk factor for bacterial keratitis compared to low-income countries where trauma is the far more common predisposing factor where it accounts for up to 77.5% of cases. [19]. As in our case, the most predisposing factor was trauma as it was seen in 132 (60%), followed by foreign bodies 22(10%), systemic disease (diabetics) 33(15%), and those with unknown cause 33(15%) all with positive culture results. There was no statistically significant association between risk factors and culture-positive results as the p-value was greater than 0.05. A study conducted at Tertiary Eye Centre in India (2013) reported that in (64%) of keratitis patients' trauma was the common predisposing factor [20] and similar figures were reported from studies conducted in Sudan, Iran, South Africa, and Qatar. The other factors which were most frequently reported were Mud, dust, and soil followed by leaf & vegetable matter which was (35.6%) and (31.6%), respectively [21]. During the windy season and the peak season of agricultural work, injury by dust particles and injury by vegetable matter is possibly more common and this injury leads to damage to the eye. Another study conducted in Egypt showed that 63.4% of the 115 cases of keratitis were due to trauma, and they concluded that trauma as the most significant risk factor, other than trauma, unknown factors, foreign bodies, wearing contact lenses, hypertension, diabetes, and cataract surgery all showed lower percentages [22].

While, in Saudi Arabia, contact lens wearing followed by corneal trauma and diabetes mellitus was a risk factor in 20 cases, the study has not reported any significant correlation between systemic risk factors and severity of clinical presentation [23]. The antimicrobial agents available today are mostly microbiostatic, requiring a prolonged course of therapy. Antibiotics susceptibility testing of each isolated pathogen was done using routine antibiotics discs according to the disc diffusion technique on muller hinton agar (MHA). The antimicrobial activities of microorganisms were assessed based on the diameters of the clear inhibition zone of adjacent paper disks. If there is no inhibition zone, it indicates no antimicrobial activity. The antimicrobial activity of *Staphylococcus aureus* and *Streptococcus pneumoniae* with various antibiotics such as tetracycline; clindamycin, ciprofloxacin; oxacillin, erythromycin, chloramphenicol, vancomycin, gentamycin, nitrofurantoin, rifampin, and penicillin were studied. From the results, it was observed that the antibiotics showed their capacity to inhibit the growth of these bacteria and some were quite resistant to antibiotics, as indicated in **Tables 4 and 5**.

The current work has shown that gentamycin, ciprofloxacin, nitrofurantoin, rifampin, and vancomycin are sensitive against *S.aureus* (90%) whereas *S.pneumoniae* is (100%) sensitive to gentamycin, chloramphenicol, and tetracycline. *S.aureus* was (100%) resistant to oxacillin and penicillin, while *S.pneumoniae* was only subjected to oxacillin as it showed (100%) resistance [24]. The results of the current study are in line with other similar studies conducted in Ethiopia that revealed *S.aureus* was sensitive to gentamycin (85.7%), vancomycin (83.3%), and ciprofloxacin (88.1%), however, it was highly resistant to penicillin (100%). In our study, we found that *S.pneumoniae* was sensitive to chloramphenicol (100%), and vancomycin (100%), our finding is in line with the study conducted in India, Iran, and Nigeria [25]. A study conducted in South Africa also showed *S.aureus* and *S.pneumoniae* were highly sensitive to vancomycin and chloramphenicol, while *S.aureus* was resistant to ciprofloxacin [26,27,28,29].

Conclusion :

Bacterial keratitis requires an initial diagnosis to ease its purpose of stopping future visual loss, and after early diagnosis, if the treatment is ineffective, again bacterial keratitis may progress to perforation and blindness. The current study concluded that the incidence of bacterial keratitis in Ophthalmic Hospitals of Asmara was found to be 50% for *S. aureus*, 30% for CoNS, 15% for *S.pneumoniae,* and 5% for *S.viridans*. The most frequent leading feature for bacterial keratitis was found to be trauma, but there was no significant association with positive culture results.

The antimicrobial activity of *S. aureus* was highly sensitive to ciprofloxacin, gentamycin, vancomycin, rifampin, and nitrofurantoin, as it was resistant to oxacillin and penicillin. Similarly, *S.pneumoniae* was found to be sensitive to clindamycin, chloramphenicol, and tetracycline, as it was resistant to oxacillin. The current findings of our study support the use of the above-mentioned antibiotics effectively for the treatment of serious bacterial keratitis.

Limitations of the Study

1. Corneal scraping was not possible because of technical problems and a shortage of expert people.
2. Limited time frame to get sufficient samples.
3. Very limited recorded data was available based on our requirement to conduct a retrospective study; therefore, we conducted a cross-sectional study.
4. Novobiocin disc, which is used to differentiate coagulase-negative Staphylococci, was not available.
5. Due to lack of funding and shortage of well-equipped labs, we focussed only on the identification of bacteria instead of identifying all causative agents of keratitis.

Recommendations:

1. This research will generate baseline data for future studies. Therefore, it is recommended that:
2. Microbiological assay should be done for patients with keratitis before starting their treatment as it will aid in prescribing the most effective antibiotics.
3. For the resistant strain of *S. aureus,* further studies which include molecular level should be conducted to find the exact resistance pattern and administration of the right treatment.
4. Continuous large-scale studies must be conducted in the future that focus on microbial keratitis.

Conflict of Interest: There is no coflict of interest

Funding: There is no external or internal funding for the study conducted

Data Availability: The data collected to support the results are available on different websites.

Authors'Contribution: Dr.Khawaja Shakeel Ahmed: Supervised the whole study and written the manuscript. Mr. Bharat Kumar Bhayal: Provided the necessary materials and literature and co-supervised the study. Mr. Dawit Eman: Helped in collecting samples. Miss. Yordanos Tekeste: Helped in analysing the samples. Mr. Michael Tadelle**:** Helped in analysing the samples**.** Miss. Fitsum Omaha: Analysed the samples. Miss. Freweyni Tesfay: Analysed the samples. Mrs. Kisanet Mebrahtu: Analysed the samples. Miss. Luwam Michael: Analysed the samples. Miss. Silas Amanuel: Analysed the samples.

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Original Article

Chronic heavy katikala addiction on liver enzymes in Chencha town, Southern Ethiopia

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Abstract

Background: *Chronic alcohol consumption damages liver functions causing health problems. Because of the cold-environment, some adults in Chencha town are heavy consumers of strong local-Areki called "Katikala" (34.09% ABV). However, information concerning the impact of heavy" Katikala" intake on liver enzymes has not yet been explored.*

Objective: *To assess the impacts of chronic heavy "Katikala" intake on liver enzymes and secondly to see changes in percent of body fat level on adult subjects living in "Chencha" town and compare it with non-alcoholic controls.*

Methodology: *A group of 34-chronic heavy "Katikala" consumers were compared with 34 abstainers of comparable ages (mean age: 35 years). Information was obtained on the quantity and duration of alcohol consumed. Serum Aspartate transaminase, Alanine transaminase and Gamma Glutamyl transpeptidase levels were measured to standard laboratory procedures. Percent body fat (%BF) was recorded and SPSS (ver. 21software used to analyze data by taking p-value < 0.05 for declaring significance.*

Results: *Compared with abstainer controls, chronic "Katikala" consumers showed significantly higher Aspartate transaminase, Alanine transaminase and Gamma Glutamyl transpeptidase Serum levels with ($p<0.001$). Percent of body fat (%BF) was significantly lower in chronic drinkers than abstainers ($p<0.001$). AST to ALT ratio (> 2) was higher in chronic heavy drinkers than controls. Duration and quantity of "Katikala" consumption were uncorrelated with the concentration of Aspartate transaminase, Alanine transaminase and Gamma Glutamyl transpeptidase ($p>0.05$).*

Conclusion: *Subjects chronically consuming "Katikala" showed significantly raised serum Aspartate transaminase, Alanine transaminase and Gamma Glutamyl transpeptidase as well as lower percent body fat level compared with normal controls. Our data suggests the negative influence of "Katikala" consumption on liver function and as well as body weight affecting health.*

Keywords: *Alcoholism, Katikala (local-areki), body fat, liver enzymes, AST/ALT-ratio, Chencha*

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Introduction

The liquor, alcohol, is an addictive substance when taken for longer durations repetitively [1]. Consequently, chronic alcohol consumption is a threat for health and provokes anti-social behaviors, crimes, loss of interest to work, and accidents that leads to death [1]. Nearly 3.8% of global deaths and 4.6% of worldwide disability occur due to alcohol consumption[2]. In developing countries, it was reported that alcohol consumption is rapidly rising [2, 3]

Ethiopia is one of the countries where traditionally fermented beverages are produced and utilized in a wide scale [4]. Among traditionally produced and fermented beverages in Ethiopia include Katikala (locally brewed Areki with 34% alcohol), Tej (wine made from honey), Tella, Borde, Keribo, Korefe (different forms of beers made from various types of cereals) [5].

Of all locally brewed beverages, Katikala (Areki) is the strongest alcohol accounting to about 34% by volume of pure ethanol [6]. In Chencha and other places of Ethiopia, it is observed that Katikala is consumed by cup known as "Dibulo".

The volume of Dibulo (local cup) amounts to 100 ml and one Dibulo of Katikala (100 ml) is equivalent to 27.62 grams of 100% pure ethanol as calculated by using the density of ethanol as conversion factor [7]. Liver is the primary site for alcohol detoxification [8] and it is, therefore, susceptible for alcohol induced injuries. Liver detoxifies alcohol and results in production of potentially dangerous waste products such as acetaldehydes and other highly reactive free radicals [9]. These byproducts contributes to alcohol induced liver diseases [10]. Moreover, other hematological and biochemical parameters are also affected by chronic alcohol consumption [11]. Commonly used traditional markers for alcohol consumption are liver enzymes Aspartate transaminase (AST), Alanine transaminase (ALT) and Gamma Glutamyl transpeptidase (GGT) [12]. In addition to these liver enzymes body composition, particularity fat level, is commonly examined in chronic alcohol consumers. It was shown that alcoholics exhibit lower percentage of body fat (%BF) than those with normal body weight [13].

The city "Chencha" located in southern part of Ethiopia lies 2,732 meters above sea level and manifests a cold environmental temperature throughout the year (average 15^0C). Adults consume "Katikala (local Areki)" on the assumption that alcohol helps protects them from the cold-environmental temperature. However, some adults get addicted to Katikala and drink every day. Though chronic alcohol consumption is toxic and causes many health problems, no studies have been undertaken in Chencha town, where alcohol in the form of "Katikala" consumption is highly practiced. We, therefore, aimed in assessing the effect of chronic Katikala consumption on liver enzymes on inhabitants of Chencha town. It was hypothesized that chronic Katikala consumption may raise concentrations of liver enzymes compared to non-Katikala consuming subjects.

Material and methods

Study design:

Community based comparative cross-sectional study was conducted in Chencha town from March-to-June, 2019.

Study subjects

Inclusion criteria:

Age between 18- 65 years of age. Chronic Heavy Katikala drinker includes one who drinks $\geq$ 2 ½ "dibulo" of Katikala/day in one occasion for at least 4-days in a week for the last 5 years (exposed). Comparison groups included healthy adults (age 18-65 years), who live in the same community with addicts and who don't consume "Katikala" (non-exposed).

Exclusion Criteria for:

Study subject: Obese, pregnant and "Katikala" drinker with identified cardiac problem, other chronic disease history and the terminally ill were excluded.

Sampling:

Purposive sampling method was used for selection of eligible participants. 5ml of venous blood was collected by a trained nurse from antecubital vein and collected in tubes containing no additives. The tube was allowed to clot for 30-minutes at room temperature and centrifuged at 1500g for 5 minutes. Then serum was extracted and frozen at -20^{o}C until analyses. AST, ALT and GGT serum concentrations were measured to standard laboratory procedures at Arbaminch General Hospital and finally data obtained were recorded.

Percentage of body fat is age and sex specific prediction formula which was analyzed by taking BMI, age and sex (males =1, females =0) into account [14]. In adults the prediction formula was:

BF%= 1.20*BMI+0.23*Age-10.8*Sex-5.4

Sample size determination:

The study utilized two population proportion formula technique to determine the study population sample size. The prevalence value for exposed group was 50% and for control group was 13.4%, taken from previously published article that was done in USA. Assuming a 5% level of significance and 80% power to detect the above difference, a sample of 34 exposed and 34 non-exposed study participants were required.

Ethical clearance:

Our study has been approved by departmental research and ethical committee (DRC) of college of health sciences, Tikur-Anbessa Hospital, Addis Ababa University with reference number of Anat/Phy/253/2018. All participant were informed on the objective of the study and provided a written approval of the study.

Data analysis

SPSS (version 21) was used to analyze the results. Data were described in mean, standard deviation, frequency and percentage. Independent sample test was used to compare serum mean concentration of liver enzymes. Pearson correlation was used for correlations of different variables.

Results:

Table 1: Sociodemographic characteristics and basic health information of our study participants

Variable		Drinkers (n=34)		Non-alcoholics (34)	
		Frequency	Percent	Frequency	Percent
Gender	Male	34	100%	34	100%
	Female	0	0%	0	0%
Religion	Orthodox	30	88.2%	14	41.18%
	Protestant	2	5.88%	19	55.88%
	Muslims	0	0%	1	2.94%
	Other	2	5.88%	0	0%
Marital Status	Married	17	50%	17	50%
	Not Married	14	41.2%	17	50%
	Divorced	3	8.82%	0	0%
Age	21-27	4	11.7%	7	20.58%
	28-35	15	44.1%	13	38.2%
	36-42	9	26.4%	11	32.4%
	43-50	2	5.88%	2	5.88%
	50+	4	11.7%	1	2.94%
Educational level	An illiterates	0	0%	0	0%
	Read and write only	8	23.52%	1	2.94%
	Formal school	6	17.64%	8	23.52%
	College	12	35.3%	10	29.41%
	University	8	23.52%	15	44.1%
Occupation	Employed	16	47.05%	17	50%
	Unemployed	14	41.18%	10	29.41%
	Trade	4	11.76%	3	8.82%
	Student			4	11.76%
AST to ALT ratio	AST/ALT<1	5	14.7%	24	70.6%
	1≤AST/ALT<2	13	38.2%	10	29.4%
	AST/ALT≥ 2	16	47.1%	0	0%

A total of 68 male adults were interviewed and serum Liver enzymes particularly AST, ALT and GGT test was done, of which 68 participants had completed both the interview and measurements making the response rate of 100%. Among the 68 study participants, 50% were chronic heavy "Areki/Katikala" drinkers and 50% were non- drinkers. Of the drinkers, 100% were males, 50% were married, and 30 (88.2%) were Orthodox religion followers.

While the two groups were comparable in most socio-demographic characteristics, the non-drinkers were mostly (55.8%) Protestants. About 47.1% of exposed group individuals (drinkers) showed AST/ALT greater than or equal to two. Majority (70.6%) of non-exposed group individuals showed AST/ALT <1(as shown in Table 1).

Table 2: Comparison of anthropometric parameters of chronic "Katikala/Areki" drinkers and abstainers (i.e., controls).

Parameters	Mean ± SD		P value
	Abstainers (Controls)	Chronic Katikala drinkers	
Age (yrs.)	35.68 ± 8.79	34.47 ± 6.24	0.052*
Height (cm)	166.20 ± 8.06	169.00 ± 8.12	0.452
Weight (Kg)	63.21 ± 9.17	59.80 ± 9.63	0.046*
BMI (Kg/M^2)	22.69 ± 1.17	20.94 ± 1.50	0.000*
%BF	18.82 ± 2.80	16.86 ± 1.83	0.001*

Based on the above anthropometric result there was no significant difference in mean age of drinkers and non-drinkers with mean of 34.47± 6.24 and 35.68± 8.79 respectively. Comparing of the mean BMI between the two groups showed chronic Katikala drinkers (exposed groups) had lower BMI. There was no significant difference in mean height between the groups. The average (mean ± SD) of %BF among chronic drinking group was lower (16.86 ± 1.83) as compared to non-alcoholic abstainers (18.82 ± 2.80).

Table 3: Mean ± SD liver enzymes (IU/L) of chronic heavy "Katikala" drinkers and non-alcoholic controls.

Parameters	Abstainers (n=34)	Katikala Addicts (n=34)	Derangement by fold	P-value
AST	20.41 ± 6.1	178.18 ± 82	8.7	0.000*
ALT	23.26 ± 5.8	128.03 ± 59	5.5	0.000*
GGT	26.5 ± 6.5	162.4 ± 91.5	6.1	0.000*

As the above table showed chronic heavy Katikala drinkers' average (mean ± SD) of serum aspartate aminotransferase, alanine aminotransferase, and Glutamyl transaminase was 178.18 ± 82, 128.03 ± 59 and 162.4 ± 91.5 respectively. And those of non -exposed group had average (mean ± SD) 20.41 ± 6.1, 23.26 ± 5.8 and 26.5 ± 6.5 of AST, ALT and GGT respectively. Among chronic drinkers 8-fold rise for AST, 5-fold rise for ALT, and 6-fold rise for GGT was observed as compared with non-alcoholic controls (non-exposed)

Table 4: Pearson's correlation coefficient of liver enzymes, quantity and duration of alcohol consumption and % Body fat level

	ALT	AST	GGT	%BF
Quantity	0.804(r =0.044)	0.584(r = 0.097)	0.277(r =0.192)	0.052(r = -0.336)
Duration	0.260(r = 0.199)	0.77(r = 0.051)	0.31(r = 0.179)	0.870(r = -0.280)
ALT	-	0.01(r =0.434)	0.354(r = 0.164)	0.922(r = -0.017)
AST	0.01(r =0.434)	-	0.00(r =0.769)	0.001(r = -0.529)
GGT	0.354(r = 0.164)	0.00(r =0.769)	-	0.00(r = -0.723)

Pearson's correlation test for %BF with quantity, duration, ALT, AST and GGT was (-0.336), (0.28), (-0.017), (-0.529) and (-0.723) with p value of 0.052, 0.87, 0.922, 0.001 and 0.00 respectively. Which was negatively correlated. On the other hand Pearson's correlation test for ALT with quantity and duration of alcohol intake was 0.044 and 0.199 with p value of 0.804 and 0.260 respectively.

Pearson's correlation test for AST with quantity and duration of alcohol intake was 0.097 and 0.051 with p value of 0.584 and 0.77 respectively. Pearson's correlation test for GGT with quantity and duration of alcohol intake was 0.192 and 0.179 with p value of 0.277 and 0.31 respectively.

Table 5: Association between Habit of drinking Katikala and AST to ALT ratio

Groups	AST to ALT ratio category			Total	X^2	p-value
	AST/ ALT≤ 1	1<AST/ ALT<2	AST/ ALT≥ 2			
Drinkers	5	13	16	34	27.9	0.000**
Abstainers	24	10	0	34		

About 47.1% of exposed group individuals (drinkers) showed AST/ALT greater than or equal to two. Majority (70.6%) of non-exposed group individuals showed AST/ ALT <1. About 85% of exposed group individuals (drinkers) showed AST/ALT greater than 1.

Discussion:

The comparative study performed in our investigation has shown that the local alcohol "Katikala" addiction demonstrated a significant rise in liver enzymes including AST, ALT, and GGT compared with abstainers (as shown in Table 3)As the liver is an organ where different metabolic reaction takes place, normal circulating levels of serum liver enzymes commonly considered as an indicator of good health status [8]. The metabolic chart for normal ranges in serum levels for AST is (0-35 U/L), for Alt is (7-56 U/L) for GGT is (9-85U/L) [15]. In our present study a significant 8-fold rise for AST, 5-fold rise for ALT, and 6-fold rise for GGT was observed as compared with non-alcoholic controls (Table 3) clearly indicates that heavy Katikala consumption has a higher risk of developing liver abnormalities.

Our findings are in line with Marghoob et al [16], Alatalo et al [17] and Katherine M. Conigrave, et al. [12], who also registered a significant increase in liver enzymes after consuming different alcohols of high strengths similar to locally fermented alcohol "Katikala", >20% by volume alcohol.

ALT: Elevated level of serum could be caused by many different substances like Alcohol, medications, fats, heavy metals, or even excessive amount of meat intake [18]. Small amount of ALT may be obtained in normal range of 18-85U/L in our blood. Clinically high level of ALT in serum have been associated with stroke risk and cardiovascular mortality [19]. Our current study showed a significant correlation between levels of serum ALT in alcoholics (128.03 ± 59) when compared with the abstainer controls (23.26 ± 5.8) with $p < 0.05$ (as shown in Table 3). Similar to the current study, Marghoob et al [16] and Alatalo et al [17] have reported significantly increased ALT concentration in alcohol addicts than abstainers controls.

AST: Present study showed a significant rise of serum level of AST in alcoholics (178.18 ± 82) when compared with the abstainer controls (20.41 ± 6.1) with $p < 0.05$ (as shown in Table 3). Similar to the current study, Jang et al. [20], Alatalo et al. [21]and Walter A, and Mohammed Ashraf [3] have reported significantly increased serum AST concentration in alcohol addicts than abstainer controls. When both ALT and AST enzymes are elevated, a comparison of patterns of elevation can provide information about the specific liver disease and its causes. When AST elevated more than ALT, this commonly shows that the cause liver condition is alcohol related [22]. The ALT and AST enzymes pattern of elevation could be diagnostically helpful to differentiate the causes of liver disorders [22]. Most acute hepatocellular disorders shows higher level of ALT rather than AST; whereas Alcoholic liver diseases shows AST: ALT ratio of greater than or equal to 2 [23]. This may due to both enzymes require vitamin B6 (pyridoxal -5'-phospate) to function well [23]. In heavy drinkers vitamin B6 is much lower due to their poor appetite and this has relatively much higher effect on ALT production than AST production with correspond changes in serum concentration causing AST/ALT to be greater than other liver diseases [23].

Ratio of AST to ALT: Although the normal range of AST and ALT values varied all over the world, ratio of AST to ALT is key for diagnosing liver diseases [22]. In health individuals ratio of AST to ALT would be around 1.15 U/L and if AST/ALT greater than 2 this denotes alcoholic liver disease [24]. In our study, ratio of AST to ALT >2 was higher in chronic heavy "Katikala" drinking group as compared with control non-Katikala consuming subjects (as shown in Table

Our study results support the previous studies involved in assessing the relation between severe alcohol intake and ratio values by Subir Kumar Das and D.M.Vasudevan[25], H. Nyblom et al. [22] and Walter A, and Mohammed Ashraf [3].

Gamma Glutamyl transpeptidase: most sensitive and commonly employed biochemical marker of alcohol ingestion [26]. Consequently, increased levels of GGT indicate antioxidant deficiency and several community based studies have shown that in addition to alcoholic liver diseases, GGT associated is associated with cardiovascular mortality[19, 27]. Current study revealed chronic heavy alcohol consumption as a significant independent factor for high level of serum GGT in alcoholics (162.4 ± 91.5) when compared with the abstainer controls (26.5 ± 6.5) with $p < 0.05$ as (shown in Table 3). Similar result had been reported by Alatalo et al, Sara A & Mahmoud Omer and Marghoob et al [16, 17, 26].

Body Mass: In this study Chronic heavy Areki/ Katikala drinking group had shown significantly lower Body mass index and percent of body fat as compared with non-alcoholic group (Table 2). Reduced adipose tissue in chronic drinker may occur due to simultaneous decreased intake of nutrition with alcohol [28]. In general alteration in body composition occur in alcoholics as ethanol supply more than 50% of dietary energy that cannot be stored [28]. Our current study shows Pearson correlation test for %BF with quantity and duration of alcohol consumption; which was (r= -0.336) and (r= -0.280) with p-value of 0.052 and 0.870 respectively (as shown in Table 4).

This negatively correlated association is consistent with another studies in which it was reported that the amount of alcohol consumed by an individual was found to an independent factor to for lower percent of body fat [28-30]. The current study result was unlikely with study report from Wannamethee et al. [31] in which it was reported that men who consumed high alcohol showed higher levels of central adiposity than non-drinkers and lighter drinkers.

Limitations of the study

Female participants were not obtained during data collection and the total participants were males. So, this result may not represent chronic Katikala (local-alcohol) addicted females. On the other hand some other biochemical parameters like ALP and lipid profiles are not assessed due budgetary issues.

Conclusion

Subjects chronically consuming Katikala/Areki showed significantly raised serum AST, ALT, and GGT that may cause abnormal liver function. Chronic "Katikala/Areki" drinking group showed significantly lower %BF compared with non-alcoholic controls, implying that fat metabolism is negatively affected with Katikala addiction. Not only long-term (>1 year), even short-term intake of local alcohol (Katikala) consumption is bad for health.

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Original Article

Bitter kola and kola nut use and their effect on treatment outcome on People Living with HIV at a Military Hospital in Benue state Nigeria

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Abstract

Introduction *Bitter Kola (Garcinia Kola) and kola nut, contains substances attributed to numerous effects on humans including anti-inflammatory, anti-allergic properties, anti-infective and caffeinism. This study explores Bitter kola and kola nut use and its impact on treatment outcome on People Living with HIV (PLWHIV).*

Methods: *The study was conducted at a Military Hospital in Nigeria. An analytical cross-sectional study was done using questionnaire among 700 HIV-positive clients selected using simple random sampling. Data were collected by researcher and three trained assistants. Chi-square test and binary logistic regression were used for identifying associations and predictors, respectively. The level of significance was set at $p < 0.05$.*

Results: *Findings show that 260 (63.6%) and 179 (25.6%) have ever and currently used Bitter kola/Kola nut, respectively. Also, 14 (7.8%) used Bitter kola/Kola nut alone while 165 (92.2%) used it in addition to other substances, especially with alcohol 123 (68.7%). Bitter kola use was associated with age ($p = 0.037$), gender ($p < 0.001$), occupation ($p = 0.001$), and number of children ($p < 0.011$). Identified predictors were being a female (AOR 0.79; 95% CI 0.08-0.92) and earning <18,000 Naira (AOR 2.91; 95% CI 2.03-21.54). There was no association of Bitter kola/kola nut use with CD4 count and viral load suppression.*

Conclusion: *Though Kola nut and Bitter kola use was high as in the general population we have not found any effect on treatment outcome among PLWHIV. This calls for more research to ascertain if there are other possible beneficial effects on PLWHIV.*

Keywords: *Kola nut/Bitter kola use, HIV clients, treatment outcome, Nigeria*

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Introduction

Bitter Kola (Garcinia Kola), a popular nut consumed in virtually all parts of West Africa contains substances that are attributed to its numerous effects on humans. It contains carbohydrates, protein, water and crude fibers as nutritional components [1]. Also Tannin, oxalate, phytate, Trypsin inhibitor and phytochemicals referred to as anti-nutrient contents have protective or disease preventive properties. These phytochemicals are phenol, saponin, alkaloid, Flavonoid, and Glycoside [2].

Bitter Kola contains a high level of cardiac glycosides and kolaviron which are known to have anti-inflammatory [2,3] and anti-allergic properties [4]. Kolaviron blocks the signaling pathway in lipopolysaccharides induced inflammatory gene expression in macrophages and decreases secretion of Interleukin-6 (IL-6) [3].

has antioxidant properties by scavenging free radicals [5] inhibits stress response proteins and has metal chelating properties [6]. It is also known to have hepatoprotective properties [7,8] and reduces liver enzyme makers [9].Other effects of bitter cola extracts include antibacterial [10,11] and anti-parasitic properties [12] reduction of plasma glucose [13,14] inhibition of cancer cell proliferation [6]. reduction of plasma cholesterol [15] and may delay neurodegenerative disorders associated with disturbed cholinergic neurotransmitter system [16]. These mentioned properties of bitter kola are suggestive that it may be of value to PLWHIV who may require agents that may helpprevent the emergence of opportunistic infections and reducing pill burden. But on the other hand, bitter kola reduces the bioavailability of some drugs when administered concomitantly possibly by induction of liver

enzymes [17] and this may have implication on Anti-Retrovital Therapy (ART).

Kola nut has an important place in virtually all parts of Nigeria and West- Africa where it is consumed commonly for social, religious, and medicinal reasons like in the treatment of whooping cough and other obstructive airway diseases [18]. It contains a high quantity of caffeine1 which causes increased alertness [19] and is said to have antioxidant properties [20] Caffeine (kola nut) administered concomitantly with Halofantrine reduces its absorption and plasma maximum concentration (Cmax) [21,22]. This may have implications on other drug's plasma concentrations as well.

Chronic Bitter kola/kola nut consumption is also associated with increased plasma concentration of low-density lipoproteins (LDL), uric acid, triglycerides, and total cholesterol [23]. Daily consumption of caffeine >500mg daily is associated with caffeinism (anxiety, restlessness, irritability, agitation, muscle tremor, insomnia, headache, diuresis, sensory disturbances, tachycardia, arrhythmia, and nausea) [24]. High Kola nut consumption may, therefore, have implications on the general well-being of PLWHIV who are on treatment. On the other hand, kola nut is said to increase the programmed cell death (apoptosis) of breast cancer cells [25]. This may be of value in preventing AIDS-defining cancers among HIV patients such as Kaposi sarcoma.

There is also a dearth of literature on Bitter kola/Kola nut use among HIV-positive patients in Nigeria. Presently, there is a gap in our understanding of why some of the clients fail on their ART even when they claim to have good drug adherence to their medication. Exploring psychoactive substance use like Kola nut among PLWHIV and their effect on treatment outcome is expected to throw more light on these issues.

Methods

Study design and setting

An analytical cross-sectional study was done at the Centre for Infectious Diseases Control (CIDC), 161 Nigerian Air Force Hospital, Makurdi of Benue state, Nigeria. The Centre provides comprehensive HIV care services for over seven thousand (7,000) clients. These include military personnel, their relatives, and other civilian clients from Benue state and other bordering states.

Study participants

HIV-positive clients ≥ 18 years who have accessed care for ≥12 months preceding the study at the center or transferred in with a record of being on treatment for ≥ 12 months and who gave consent were studied. The sample size was determined using the single population proportion formula [n=Z2pq/d2] taking 50% as the prevalence of Bitter kola/Kola nut use [26]. A total of 410 clients were needed, however, 700 clients were studied.

A simple random sampling method was adopted. On each day, each client was given a card bearing a number, from 1 to the maximum attendance number usually about 120. Then random numbers were used to select 15 clients from the daily pull. Each selected client was checked to confirm if he/she meets the inclusion criteria and if nor was replaced.

Data collection tools and procedures

Data were collected using a pretested, semi-structured, interviewer administered questionnaire. It has five (5) sections. The first section was on clients specific ID. The second section captured the participant's socio-demographic variables; while the section three (3) captured the participants' HIV infection related data. Section four (4) was the alcohol, smoking and substance use (Bitter kola and kola nut) screening section; while the 5th section captured the client CD4 and Viral load results. The questionnaires were coded from numbers 001 to 700. Each selected clients client's unique ID in the facility was matched to a questionnaire code by the principal investigator and recorded in a codebook. This was to ensure the linking of the questionnaire with the treatment record. Data was collected by researchers and three trained research assistants. They were trained for 6 hours on the contents of the questionnaire and procedures on data collection.

Data analysis

Data analysis was done using IBM Statistical package for social sciences (SPSS) version 23.0. The tool elicits ever used, current use (in the last 3 months), and the frequency of Bitter kola/Kola nut use. The CD4 increase was categorized as a good increase which is an increase of CD4 of at least 50cells/ ml per year from the baseline CD4 when the client was commenced on ART or a poor CD4 increase if the increase per annum was less than 50cells/ml, or if CD4 started dropping to baseline CD4 after the initial increase or less than 50% of pick ever attained since the commencement of ART. Also, good suppression is viral load of less than 1000 copies/ml after six months of commencement.

of ART, while poor suppression was viral load greater than or equal to 1000 copies after six months on Highly Active Anti-Retroviral Therapy (HAART). A Chi-square test was applied to ascertain if there was any significant association between Bitter kola/Kola nut use with characteristics and treatment outcome (CD4 count and Viral load) of clients. The level of significance was set at $p < 0.05$. Logistic regression was used for further analysis to identify predictors at the chi-square test p-value of 0.2 and below.

Ethical Considerations

Ethical clearance was obtained from the Health Research and Ethics Committee of the University of Nigeria Teaching Hospital (UNTH). Permission was obtained from the Commander and management of the institution to embark on the study. Written informed consent was obtained from all those interviewed after the purpose of the study was explained to them. Information obtained from clients were kept confidential. The client's freedom to withdraw from the study at any point in time in spite of the consent was also respected.

Results:

Table 1 shows that the mean age of the clients was 39.3 (±10.1) years. Majority 618 (88.3%) were aged 30 to 60 years, females 441 (63.0%), Tivs 688 (98.3%), had tertiary education 304 (43.5%), married and living with their spouse 326 (46.6%) and Christians 693 (99.0%). Also higher proportion earn 18100 – 50000 naira 314 (44.9%) followed by <18,000 naira 302 (43.1%). Equally, they majorly engage in trading 255 (36.5%) followed by farming 201 (28.7%). Most have 1-3 children 317 (45.5%) followed by those with ≥4 children 354 (36.3%).

Table 1: Sociodemographic Socio-demographic characteristics of respondents

Variables	Frequency (n=700)	Percent %)
Age Groups (years)		
<30	65	9.3
30-60	618	88.3
>60	17	2.4
Mean ± SD (years)	39.3 ± 10.1	
Gender		
Male	259	37.0
Female	441	63.0
Education		
Tertiary Education	304	43.5
Secondary Education	232	33.1
Primary Education	164	23.4
Marital Status		
Married and Living With Spouse	326	46.6
Widowed/Divorced	140	20.0
Married not Living with Spouse	133	19.0
Single	101	14.4
Religion		
Christianity	693	99.0
Others (Islam, traditional religion)	7	1.0
Income (₦)		
< 18000	302	43.1
18100 – 50000	314	44.9
>50,000	84	12.0
Tribe		
Tiv	688	98.3
Others*	12	1.7
Occupation		
Business	255	36.5
Farming	201	28.7
Public Servant	173	24.7
Student/applicants	71	10.1
No of children		
None	129	18.4
1-3	317	45.3
≥ 4	254	36.3

*Others includes all non-Tiv participants

Table 2 shows 260 (63.6%) have used Bitter kola/Kola nut before and 179 (25.6%) of the participants currently used Bitter kola/Kola nut in the past 3 months. A higher proportion of the clients 150 (83.8%) used Bitter kola/ Kola nut mildly and 29 (16.2%) used moderately. Also, 89 (49.7%) used it one once/twice and 6 (3.4%) daily in the past 3 months. Concisely, 14 (7.8%) used Bitter kola/Kola nut alone while 165 (92.2%) used in addition to other substances. The substances used together with Bitter kola/Kola nut were alcohol 123 (68.7%), tobacco 82 (45.8%), cannabis 6 (3.4%), and others 12 (6.8%).

Table 2 : Use of Bitter kola/Kola nut

Variables	Frequency (n=700)	Percent (%)
Ever Used Bitter kola/Kola nut		
Yes	260	37.1
No	440	62.9
Currently used Bitter kola/Kola nut in past 3 months		
No	521	74.4
Yes	179	25.6
Level of Bitter kola/Kola nut use in 3 months* (n=179)		
Mild	150	83.8
Moderate	29	16.2
Frequency of usage in past 3 months (n=179)		
once/twice	89	49.7
Monthly	51	28.5
Weekly	33	18.4
Daily	6	3.4
Use of Bitter kola/Kola nut (n= 179)		
Bitter kola/Kola nut alone	14	7.8
Bitter kola/Kola nut and other substance	165	92.2
Use of Bitter kola/Kola nut and other substance (n =179)$		
Bitter kola/Kola nut and alcohol	123	68.7
Bitter kola/Kola nut and t obacco	82	45.8
Bitter kola/Kola nut and cannabis	6	3.4
Bitter kola/Kola nut and others#	12	6.8

#Others includes cocaine, sedatives, inhalants, opioids, hallucinogens
$ - mutually exclusive/multi choice

Table 3 shows that there were statistically significant associations between current use of Bitter kola/Kola nut with gender ($p < 0.001$), educational level ($p= 0.040$), and number of children ($p = 0.003$). There were no statistically significant associations between current use of Bitter kola/Kola nut with age group ($p = 0.053$), marital status ($p = 0.119$), religion ($p= 0.685$), tribe $p = 0.514$), (occupation ($p= 0.075$) and income ($p= 0.057$). Females were about 80% more (AOR 0.83; 95% CI 0.06-0.97) likely to never use Bitter kola/Kola nut than males.

Table 3: Socio-demographic characteristics influencing current use of Bitter kola/Kola nut

Current use of Bitter kola/Kola nut				
Variables	**Yes**	**No**	**χ^2 (p value)**	**AOR[#] (95% CI[$])**
	Freq[*](%)	**Freq*(%)**		
Age cat				
18-25	12(18.5)	53(81.5)		3.62 (0.23-14.55)
26-60	159(25.7)	459(74.3)	5.86(0.053)	1.77 (0.09-8.63)
>60	8(47.1)	9(52.9)		1
Sex				
Female	86(33.2)	173(66.8)	12.59 (<0.001)	0.83 (0.06-0.97)[*]
Male	93(21.1)	348(78.9)		1
Education				
Primary	30(18.3)	134(81.7)		1.68 (0.46-10.35)
Secondary	61(26.3)	171(73.7)	6.45(0.040)	1.39 (0.67 -5.31)
Tertiary	88(28.9)	216(71.1)		1
Marital Status				
Married (With Spouse)	74(22.7)	252(77.3)		2.27 (0.62-7.47)
Married (not with Spouse)	35(34.7)	66(65.3)	5.85 (0.119)	4.41 (0.78-11.55)
Single	35 (26.3)	98(73.7)		3.69 (0.82-16.08)
Widowed/Separated/ Divorced	35(25.0)	105(75.0)		1
Religion				
Christianity	178(25.7)	515(74.3)	0.47(0.685)	NA
Others	1(14.3)	6(85.7)		
Tribe				
Tiv	175(25.4)	513(74.6)	0.39 (0.514)	NA
Others	4(33.3)	8(66.7)		
Occupation				
Farming	38(18.9)	163(81.1)		1.52 (0.72-3.45)
Business	71(27.8)	184(72.2)	6.91 (0.075)	1.34 (0.67-4.53)
Civil/Public Servant	48(27.7)	125(72.3)		1.67 (0.12-12.44)
Student/none	22(31.0)	49(69.0)		1
Income				
< 18000	67(22.2)	235(77.8)		3.67(0.03-11.74)
18100 – 50000	94(29.9)	220(70.1)	5.72 (0.057)	2.36 (0.93-12.32)
>50,000	18(21.4)	66(78.6)		1
No of Children				
None	48(37.2)	81(62.8)	11.45(0.003)	2.44(0.16-10.66)
1-3	75(23.7)	242(76.3)		3.10 (0.77-9.25)
≥ 4	56(22.0)	198(78.0)		1

NB: Freq[*] – Frequency, AOR[#] – Adjusted Odd Ratio, CI[$] –Confidence Interval, Level of significance + p value < 0.05

Table 4 shows that there was no statistically significant association with the CD4 count increase of those that currently use Bitter kola/Kola nut (p = 1.000) and severity of use (p = 0.453). Also, there was no statistically significant association in the viral load suppression of those that current use (p=0.682) and severity of use (p = 0.873) of Bitter kola/Kola nut among the clients.

Table 4: : Bitter kola/Kola nut use influence on HIV related factors among clients

CD4 COUNT				
Variables	**Good n (%)**	**Poor n (%)**	**BIVARIATE χ2(p value)**	**MULTIVARIATE AOR (95% CI)**
Current use of Bitter kola/Kola				
Yes	214(92.6)	17(7.4)	0.00(1.000)	NA
No	378(92.6)	30(7.4)		
Level of Bitter kola/Kola use				
Mild	472(93.1)	35(6.9)	0.74(0.453)	NA
Moderate	120(90.9)	12(9.1)		
Severe	-	-		
VIRAL LOAD				
	Good n(%)	**Poor n(%)**		
Current use of Bitter kola/Kola				
Yes	229 (90.2)	25 (9.8)	0.22(0.682)	NA
No	395 (91.2)	38 (8.8)		
Level of Bitter kola/Kola use				
Mild	490(90.9)	49(9.1)	0.02(0.873)	NA
Moderate	134(90.5)	14(9.5)		
Severe	-	-		

NB: AOR[#] – Adjusted Odd Ratio, CI[$] – Confidence Interval, Level of significance + p value < 0.05

Discussion

The prevalence of bitter kola and kola nut in this study was high with over one-fourth (1/4) of the clients using one form of Kola or the other. Generally bitter cola and kola nut are socializing agents/ingredients in the country and is shared in many traditional social events. This high prevalence of kola nut and bitter cola in our society has been shown in various studies conducted among the Nigerian population 27,28]. Bitter cola is also used locally as a treatment for cough and catarrh. On the other hand, the prevalence of kola nut and bitter cola noted in this study was less than the prevalence of Kola nut use among PLWHIV at Usmanu Danfodiyo University Teaching Hospital Sokoto state, Nigeria [26]. This is expected since kola nut use is commoner among Hausas and other tribes in the northern part of Nigeria where kola nut is consumed as a socializing agent unlike in Benue where alcohol is regarded as a more socializing agent than kola nut..

Males have a higher tendency than females to use kola nut and bitter cola. This higher prevalence of use just like other psychoactive substances among males than female counterparts is in agreement with various studies locally in Jos [29] and Abuja [30]. This is also in agreement with other studies carried out among other populations in Nigeria [28,31].

Therefore, gender is a strong predictive factor to psychoactive substance use among PLWHIV who are accessing care at 161 NAF hospital Makurdi similar to what is obtainable in the general population.

Bitter kola (Garcinia) and kola nut (Cola nitida and Cola acuminate) are commonly used substances by the respondents but there was no significant difference in the CD4 count or viral load of clients who use them and those who do not use them. A probable explanation for the finding may be the low quantity and frequency of kola nut use as reported in this work where higher proportion of the clients (over 80%) used bitter kola/kola only mildly. The level of consumption may be below threshold to have clinical significance including impact on treatment outcome.

However, Bitter Kola contains a high level kolaviron which are known to have anti-inflammatory effect [2,3], decreases secretion of Interleukin-6 (IL-6) [3]. has antioxidant properties by scavenging free radicals [5] and have hepatoprotective properties [7,8] These mentioned properties of bitter kola are suggestive that it may be of value to PLWHIV who may require agents that may help prevent the emergence of opportunistic infections and reducing pill burden. On the other hand, it is said to increase the programmed cell death (apoptosis) of breast cancer cells [25]. This may be of value in preventing AIDS-defining cancers among HIV patients such as Kaposi sarcoma. One study demonstrated that baicalin, a flavonoid isolated from Scutellaria baicalensis (Lamiaceae), inhibits HIV-1 infection and replication. Baicalein and other flavonoids such as robustaflavone and hinokiflavone have been shown to inhibit HIV-1 and reverse transcriptase.[32https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6454915/ - ref22]. High Biterkola/Kola nut consumption may, therefore, have implications on the general well-being of PLWHIV who are on treatment.

Although there were not much studies regarding their effect on viral load or CD4 count of clients on HAART, some studies have indicated that they may influence the plasma concentration of some medication medications [33,34] For instance kola nut taken concomitantly with Halofantrine reduces its absorption and plasma maximum concentration (Cmax) [21,22]. possibly by induction of liver enzymes [17]. This is likely to have implication on other drugs' plasma concentrations as well including ART which is not the case in this study.Therefore, it may be necessary that further studies be done to see other potential effects of either bitter kola or cola nut on the treatment outcome of clients on HAART apart from CD4 count and viral load assay.

Conclusion

The use of kola nut and Bitter kola use was high as in in the general population. Socio- demographic characteristics influences use. It has no effect on treatment outcomes. More research is necessary to see if there are possible beneficial effects on such patients .

Conflict of interest

The authors have declared that they have no conflict of interest, financial or non-financial.

Authors contributions

GE and EA conceptualized and designed the work. GE wrote the literature review. EA did data analysis. Both EA and GE wrote the whole work, reviewed it and agreed on its publication.

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Lema et al

Original Article

Knowledge, attitude and practice of health workers towards leprosy at a high burden rural site in Ethiopia

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Abstract

Background: *Leprosy is a chronic mycobacterial disease of public health importance. The role of the health workers in leprosy diagnosis and management of leprosy is crucial. Hence, in this study, the knowledge, attitude and practice of the health workers was assessed at one of the leprosy high burden pocket areas (Kokosa) in the Oromia Regional State.*

Methods: *A cross-sectional study was conducted at Kokosa public health centers at 7 health facilities and 86 health workers included.. Upon informed consent, data were collected from health workers through a self-administered structured questionnaire in July 25-26, 2015. On-site observation was used to assess individuals and group performance. Bloom's cut off point was used to describe the knowledge and practical skills whereas Likert's scale was used to describe the attitude of the respondents.*

Results: *Data obtained from 86 health workers were included to the final analysis of knowledge and attitude. Among the participants, 72.1% of the health workers had poor knowledge of leprosy. A quarter of respondents (25.6%) had unfavorable attitude towards leprosy. Among 62 health workers assessed for practical skills, only 4 (6.5 %) diagnosed leprosy correctly. Forty percent of the health workers had less than 4 years of service whereas 48% of them had 5-14 years of service.*

Conclusion: *Leprosy tailored training program should be implemented to improve knowledge and skills of health workers on leprosy diagnosis and treatment.*

Key words: *Leprosy, Knowledge, Attitude and Practice, Health workers, Ethiopia*
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BACKGROUND

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae.* It affects the skin, nerves and mucosa of the upper respiratory tract (1). With the introduction of multidrug therapy (MDT) , the prevalence of leprosy decreased globally (2). Ethiopia is one of the 23 countries identified as "global priority countries" for leprosy reporting > 1000 cases annually and ranked 1st in Africa in 2019 (3).In the Ethiopian health care system, leprosy care was a vertical program until 2001managed by leprosy specialized personnel at leprosy specialized hospitals.

The leprosy control program was fully integrated into the General Health Service (GHS) by the end of 2001 to ensure early patient diagnosis and deliver MDT to prevent disability (4). However, this integration meant patients were seen by general health workers during outpatient visits rather than by leprosy specialized personnel in leprosy dedicated clinics, exposing patients to the risk of misdiagnosis and inappropriate treatment (5).

Health centers diagnose and initiate MDT for leprosy patients who seek health care of their own accord (passively) and present at health facilities.

These health facilities are also expected to manage mild reactions and refer severe reactions and complications to a hospital. Leprosy control is strongly dependent on the knowledge, attitude and skills of health workers in the recognition of the signs and symptoms of leprosy at an early stage of the disease (6-9).
Ethiopia established the Health Extension Program (HEP) in 2003; an innovative community based program and a strategy to move towards Universal Health Coverage (UHC) which helped the country achieve Millennium Development Goals (MDGs) (10)**.** The main actors for the implementation of the program were Health Extension Workers (HEWs), young women assigned to work within the community they came from facilitating easy communication. They implement the 17 essential health packages developed by the FMoH (11). The roles of the HEWs in leprosy is visiting each household and screen all household members for signs and symptoms of leprosy and record and present the suspects to the health centers for confirmation by clinical nurses and health officers. They were given two days training at Kokosa Woreda.

This study was carried out to evaluate the Knowledge, Attitude and Practice (KAP) of the health workers in the health facilities of one of the Woredas with a relatively high burden of leprosy affected rural communities in Ethiopia, Kokosa Woreda.

Methods

Study site and study Settings
Kokosa Woreda is one of the five Woredas (district) in West Arsi Zone of the Oromia Regional State. The population of the Woreda is 175,184 with 36,495 households (HHs). There are 22 Kebeles and 22 health posts. Kokosa has 7 health centers namely: Kokosa, Boro, Bokore, Hogiso, Gerbe Hurufa, Hebano and Ararso. In this study, all (n=86) health workers were included: 12 health officers (HOs), 50 clinical nurses and 24 HEWs working in the study area.

Study design:
A cross sectional study was used and the KAP of health workers at Kokosa Woreda health centers was assessed at the start of the study. The design of the tool was adapted from Abeje's KAP work (8). Questionnaire from the previous study done by Abeje *et al* was adapted and the purpose of the study was explained to the study participants before they were asked to fill the questionnaires. After discussion with the leprosy experts and the research team, some of the questions were customized to our context. The practical skills of the health workers were assessed using a structured and standardized checklist by two experienced nurses who had worked on leprosy for more than 35 years in a leprosy referral hospital and in the field in the vertical programs. Active case detection pattern was assessed for five years period.

Active new case detection assessments' started in June 16/2016 and was completed in August 31/2017.

The new case detection was taken as an indicator of improvement of the KAP of the health workers for the two rounds of training given to the health workers in the area. The training focused on the three cardinal signs of leprosy (1)Hypopigmented or reddish lesions with loss of sensation, 2) enlarged peripheral nerves and 3) acid fast bacilli (AFB) in slit skin smear) that are basic in the diagnosis of leprosy; the differential diagnosis, how to do the sensory testing and voluntary muscle testing and manage leprosy complications that includes leprosy reactions and ulcers, how to classify the leprosy patients into multibacillary and paucibacilliary using WHO classification since their treatment is different. Besides they were trained in how to prescribe the three drugs (Rifampicin, Dapsone and Clofazamine) and also how to treat uncomplicated leprosy reactions with steroids and refer severe cases to hospitals. The HEWs were trained in the major signs and symptoms of leprosy and presenting them to health centers.

Sample size:
Eighty six health workers present during the study period were included for knowledge and attitude assessment and the health officers and the clinical nurses (72.1%) were further assessed for their skill in diagnosing leprosy.

Assessment of Knowledge and attitude:
Seventeen and 8 questions were used to assess knowledge and attitude of the health workers, respectively. Bloom's cut off point was used to measure knowledge of the respondents whereas their attitude was measured using Likert's scale (6) .

Those who couldn't correctly answer at least 8 out of the 17 knowledge questions were graded as having "low" knowledge about leprosy. Those who responded to 10-14 questions correctly were graded as "medium" and those who correctly answered >14 questions as having "high" knowledge of leprosy. For the 8 attitude questions asked, those who answered 3 or less questions related with attitude were considered as having "unfavorable" attitude and those who correctly answered to 4-5 as "intermediate" and ≥6 considered as "positive" attitude towards leprosy.

Assessment of skills:
Bloom's cut off point was also used to measure practice of HOs and clinical nurses when they examined a patient suspected for leprosy whereas simulation was used in the absence of leprosy suspects. Taking the relevant history in relation to leprosy, doing physical examination to reach to a diagnosis of leprosy, grading the disability,

classifying the disease and initiating treatment, prescribing the right drugs at the right dose for the right duration and explaining to the patient, and preparing patient treatment card and recording on the unit leprosy register was observed in the practical assessment. In the physical examination, they were observed when they performed skin examination for touch sensation, Sensory Testing (ST) for peripheral nerves and Voluntary Muscle Testing (VMT).

The assessment tool used had previously been tested in our previous KAP study done in Amhara and Oromia regions (8) and the grading was done by leprosy experts and researchers excluding the principal investigator (PI). (6) Among the thirty-three skill tests used to measure practical skills; those who performed ≤17 practical procedures correctly were graded as "unsatisfactory";18-25 as "satisfactory" and ≥26 as having "excellent" skill to diagnose leprosy.

Data analysis:
Data was entered on Open Clinical database and analyzed using R statistical software version 3.4.0. Proportions were calculated and the Chi-square test was used to examine associations between response and exposure (explanatory) variables. The level of significance was set at $p < 0.05$.

Operational Definitions:-
Grading knowledge, attitude and practice using Blooms cut off and Likert's Scale (8)
Seventeen knowledge questions were asked and percent of correct response grading was done:

Below 60 % (≤ 9/17), as low
≥60-80 % (10-14/17) as medium and
Above 80 % (>14/17) as high knowledge of leprosy

Eight attitude questions were asked and Likert's scale was used to measure their response

Less than 39 % (≤ 3/8) as unfavorable
40-60 % (4-5/8) as intermediate
Above 60 % (≥6/8) as positive attitude towards leprosy

Thirty-three skill tests were used to measure practical skills using Blooms cut off values:

Below 59 % (≤17/33) as unsatisfactory
60-80 % (18-25/33) as satisfactory
Above 80 % (≥26/33) as excellent skill to diagnose leprosy

Results

Socio-demographic characteristics
Eighty-six health workers were included in the study. The female to male ratio of the respondents was nearly 1:1 with the median age of 24(IQR 2) years. Male study participants comprised 51.2 % and the duration of service years ranged from 5-14 years. The proportions of health workers by education status were 14% with first degree (BSc), 58.1% with diploma and 27.9% with 10 +1. Regarding training in TB/leprosy, only 32.1% had training of less than 4 weeks either as a formal course or as a refresher course whereas the majority has not taken any training, although 60.5% of them were involved in activities of the leprosy control program (Table 1).

Table 1: Socio-demographic characteristics of health workers of Kokosa Woreda, Oromia region, Ethiopia, July 2015

Variables	Characteristics	Number	%
Sex	Male	44	51.2
	Female	42	48.8
Health workers Qualification	BSc degree	12	14.0
	Diploma	50	58.1
	10+1 (HEWs)	24	27.9
*Years of service (experience)	0-4	32	39.5
	5-14	39	48.2
	>15	10	12.3
Training in TB/leprosy	Yes	28	32.6
	No	58	67.4
**Involvement in leprosy control activities	Yes	52	60.5
	No	34	39.5

**Involvement means: HEWs =Screening patients and household contacts, HOs and Clinical nurses=diagnosing, treating and referring patients to hospital

*Five results missing, only 81 samples were included

Knowledge of Health workers

The majority of the participants, 62/86 (72.1%), showed low level of knowledge. Among HEWs, 91.7% had low level of knowledge. 67.4% of the participants had never taken any training in diagnosis, classification and treatment of leprosy. Among the participants, 71/81 (87.7%) had health service experience of below 15 years (Table 2). Short training was conducted in Kokosa for all the health workers following the KAP study findings in their local language, Oromiffa before the main study was started in 2016. Second training was also given to the health workers from the same Woreda and surrounding Woredas by our group before completing the data collection in 2018.

Table 2: Level of knowledge of health workers of Kokosa Woreda, Oromia Region, Ethiopia, July 2015

Variables	Knowledge score (%)				
	Low	**Medium**	**High**	**Total**	**P -value**
Health workers' qualification					
10+1	22(91.7%)	2(8.3%)	0(0%)	**24(27.9%)**	0.01
Diploma	34(68%)	16(32%)	0(0%)	**50(58.1%)**	
BSc.	6(50%)	5(41.7%)	1(8.3%)	**12(14.0%))**	
****Year of experience (years of Service)**					
0-4	21(65.6%)	10(31.3%)	1(3.1%)	**32(39.5%)**	0.47
5-14	32(82.1%)	7(17.9%)	0(0%)	**39(48.2%)**	
>15	7(70%)	3(30%)	0(0%)	**10(12.3%)**	
Training in TB/ leprosy					
Yes	20(71.4%)	7(25%)	1(3.6%)	**28(32.6%)**	0.35
No	42(72.4%)	16(27.6%)	0(0%)	**58(67.4%)**	

**The years of service refers to the years that the health workers had been working as a health practitioner. It is not specific to leprosy work

**Five results were missing, only 81 samples were included

Attitudes of Health workers

Likert's scale was used to describe the attitude of the respondents. Only 22/86 (25.6%) of the respondents had unfavourable attitude towards leprosy while 37/86 (43%) had intermediate attitude and 27/86 had unfavourable attitudes in this study refers to the attitude of health workers who 6 (31.4%) had positive attitude.

(Table 3). considers leprosy as a minor public health problem of the country; there is a high risk of contracting the disease while managing a leprosy patient with or without deformities and considers tracing of leprosy patients who do not come for treatment and tracing their family contacts is not important.

Table 3: Attitude levels of health workers in Kokosa Woreda, Ethiopia, July 2015

Variables	Levels of attitude (%)				
	Unfavorable	**Intermediate**	**Positive**	**Total**	**P value**
Health professional's qualification					
10+1	11(45.8%)	6(25%)	7(29.2%)	**24(27.9%)**	0.01
Diploma	11(22%)	26(52%)	13(26%)	**50(58.1%)**	
BSc	0(0%)	5(41.6%)	7(58.3%)	**12(14.0%)**	
Gender (sex)					
Male	5(11.4%)	22(50.0%)	17(38.6%)	**44(51.2%)**	0.01
Female	17(40.5%)	15(35.7%)	10(23.8%)	**42(48.8%)**	
***Years of experience**					
0-4	6(18.7%)	19(59.4%)	7(21.9%)	**32(39.5%)**	0.03
5-14	14(35.9%)	12(30.8%)	13(33.3%)	**39(48.2%)**	
>15	1(10%)	3(30%)	6(60%)	**10(12.3%)**	
Training in TB/leprosy					
Yes	9(32.1%)	11(39.4%)	8(28.6%)	**28(32.6%)**	0.63
No	13(22.4%)	26(44.8%)	19(32.8%)	**58(67.4%)**	

*Five results were missing, only 81 samples were included

Level of Practice of Health workers

Among the 86 health workers, 62 participated in the practical assessment. Only 4/62 (6.5 %) of them diagnosed leprosy correctly. The remaining 58/62 (93.6%) were found to have substandard level of practice. No health worker 0(0%) showed best practice (excellent) (Table 4).

Table 4: Level of practice of health workers in Kokosa Woreda health facilities, Ethiopia, July 2015

Variables	Level of practice (%)				
	Unsatisfactory	**Satisfactory**	**Excellent**	**Total**	**P- value**
Health worker's qualification					
Diploma	46(92%)	4(8%)	0(0%)	**50(80.6%)**	0.31
BSc	12(100%)	0(0%)	0(0%)	**12(19.4%)**	
Gender (sex)					
Male	39(90.7%)	4(9.3%)	0(0%)	**43(69.3%)**	0.17
Female	19(100%)	0(0%)	0(0%)	**19(30.6%)**	
***Years of service (experience)**					
0-4	27(93.1%)	2(6.9%)	0(0%)	**29(50.0%)**	0.89
5-14	18(94.7%)	1(5.3%)	0(0%)	**19(32.8%)**	
≥15	9(90.0%)	1(10.0%)	0(0%)	**10(17.2%)**	
Training in TB/leprosy					
Yes	13(92.9%)	1(7.1%)	0(0%)	**14(22.6%)**	0.91
No	45(93.7%)	3(6.3%)	0(0%)	**48(77.4%)**	

** Four results are missing among the 62 health workers

New case detection at Kokosa Woreda

We assessed the improvement of case detection before giving the first training (2015). We had given the second round of training at year three. As can be seen clearly from table 5, the trend of case detection in the Woreda remained the same except for the year we had been working on active case detection in the Woreda.

5 years of new leprosy cases (years (G.C.) in Kokosa					
Number of cases detected	**2015/ 2016**	**2016/2017**	**2017/2018**	**2018/2019**	**2019/2020**
	52	**91**	54	21	24

Inadequate recording and reporting practices

Relevant documents (leprosy unit register and reports) of the 7 health centers of Kokosa Woreda were reviewed in order to see if there was proper documentation and if the health providers worked according to FMoH guidelines of data capture. The checklist consisted of 13 questions. None of the health centers 7 (100%) prescribed steroids and nor did they do the monthly assessment of VMT and ST as shown in Table 6.

Table 6: Descriptive result of checklist for review of leprosy record and reports (for MDT clinic health workers +Workshop)

S.no	Checklists	Yes	No	Partial
1	Is all the essential information recorded in the patient treatment card?	1(14.3%)	5(71.4%)	1(14.3%)
2	Is the patient information registered in the unit leprosy register complete, and correct?	1(14.3%)	1(14.3%)	5(71.4%)
3	Are disability grades recorded completely and correctly?	3(42.9%)	3(42.9%)	1(14.3%)
4	Are attendances filled in correctly and completely?	3(42.9%)	1(14.3%)	3(42.9%)
5	Are steroid doses recorded correctly and completely?		7(100%)	
6	Do VMT/ST forms used routinely for leprosy patients on treatment?		7(100%)	
7	Is the information on the VMT/ST form filled out completely and correctly?		7(100%)	
8	Is there a record of collection and distribution list of footwear and appliances for prevention of disabilities?		7(100%)	
9	Is the treatment outcome recorded correctly and completely?	1(14.3%)	6(85.7%)	
10	Is the unit register updated regularly?	3(42.9%)	3(42.9%)	1(14.3%)
11	Does the health worker compile quarterly reports?	6(85.7%)	1(14.3%)	
12	Are there copies of a report of case finding and treatment outcome for the past 1 year?	3(42.9%)	4(57.1 %)	
13	Is the report consistent with the cases registered?	5(71.4%)	2(28.6%)	

Discussion

In this study, all the health workers except one (85/86) were found to have poor knowledge of the early signs and symptoms of leprosy, its treatment, and management of leprosy reactions. Overall, there were 72.1%, 26.7% and 1.2% of health workers with low, medium and high knowledge, respectively. Knowledge score is increased as the level of education increase and it has a statistically significant correlation between knowing leprosy and level of education (p-0.01). A quarter (25.6 %) of health workers had unfavourable attitude to the disease and only a third (31.4%) had positive attitudes. The health workers' attitude improves as their years of experience increase, as shown in table 3, of the workers that have 0-4 years of experience only 21.1% of them has a positive attitude, again when their experience is more than 15 years about 60% of them holds a positive attitude score in regards to leprosy. Only 8 % had sufficient skills to conduct a proper clinical examination and diagnose leprosy correctly.

The new case detection of new leprosy cases in Kokosa in the years prior to our study were recorded as 52 (2014/2013), 21 (2013/2012) and 27 (2012/2011). New leprosy cases from Kokosa have shown a declining trend after our study showing that there was poor leprosy knowledge in the capacity of diagnosing leprosy cases since after our withdrawal the numbers of leprosy cases were decreased. In addition, the HEWs are responsible to deliver 17 health packages to the local community. The workload may not allow them to give attention to leprosy related activities and improve their knowledge in the area (11).

From the observations made in the follow-up, it was noted that the training does not improve the case detection rate which could be attributed to the case detection modalities. Though the health workers took the training; they still practice the passive case detection method on the patients that come to the health facilities. The case detection rate showed improvement when we employed the active case detection. Thus, the method needs reassessment in a large scale and may need to switch to active case detection modalities.

In Ethiopia, the HEWs play the role of connecting the community with the health facilities and are responsible for creating awareness in the community about leprosy, and in screening the households and their contacts. Hence, they need proper training to be able to identify the signs and symptoms of leprosy and bring leprosy suspected individuals to the health facilities for further screening and confirmation (11, 12).

In our study, none of the respondents was capable of carrying out ST and VMT. When patients visit the health facilities for their monthly MDT, they should be assessed for VMT and ST, but this was not done because of lack of expertise or proper practical training (13). The performance of the health workers did not show any significant association with their level of qualification, in-service trainings and years of experience unlike Abeje's study which showed a significant association (8). Nerve damage as one of the leprosy complications can be reliably tested by ST and VMT, the basic diagnostic tests to prevent disabilities and deformities. ST can be used alone in situations where VMT cannot be done though it will be very useful if both methods are used (14, 15).

Low knowledge, lack of practice after training and absence of post training supervisions could be some of the reasons that contributed to the low level of performance in our study. KAP studies conducted among health workers involved in leprosy management in Bangladesh have shown that training played a key role in improving their knowledge. In Sri Lanka, health education had a sound effect on early case detection and contact tracing. Besides inclusion of leprosy in the continuous medical education programs for health workers, refresher training was considered important for improved performance of the leprosy control program. An Indian study also supported the need for refresher training and recommended training of new recruits as key activity to be considered by health planners (16-18).

There was also a major gap in recording treatment outcome in 6 of the 7 health centers which indicates the possibility of improper patient management. As a result, some cases were treated for longer periods and some others were treated below the WHO standard. This may lead to relapses and emergence of drug resistant strains, which will have an impact on the leprosy control program. Additionally, the high staff turnover, rotation of the trained staff on leprosy was also the program challenges. This calls for the attention of the Woreda and Zonal Health Bureau TB and Leprosy Focal Persons for a strict and continuous supervision in order to improve the recording and registration gaps observed in Kokosa Woreda health centres.

Conclusion

In conclusion, the study showed that the majority of health workers had low knowledge of leprosy and lacked the practical skills of physical examination. Very few health workers were able to diagnose leprosy correctly. In order to improve the knowledge and practice of the clinical nurses and the HOs, basic training of leprosy on the cardinal signs of leprosy, differential diagnosis of leprosy, differentiating leprosy reactions and its management, knowing the type of disabilities, classifying and grading has to be included. Taken together, strengthened training on early diagnosis of leprosy is critical that will aid the leprosy control program of the country in line with the WHO strategies.

Limitations

- The trainings conducted in Kokosa Woreda and the second training at Shashemene was short: each were given for 2 days only.
- Post training KAP assessment was not done due to the high staff turnover; more than 50% of the trained staff has left the Woreda.

Abbreviations:

AHRI: Armauer Hansen Research Institute; FMoH: Federal Ministry of Health; GHS: General Health Service; HEWs: Health Extension Workers; HO: Health Officer; MDT: Multidrug treatment; WHO: World Health Organization.

Competing interests: The authors report no conflicts of interest.

Authors' contributions

TL, KB, TA, and AA designed the study protocol;
TL, KB, and AM conducted the field activities;
TL and KB drafted the manuscript;
SA, TH, EN, YB, TA and LY involved in formulation of the questionnaire and data analysis;
AA, EN, YB and YW, read and commented the manuscript;

All authors contributed to the interpretation of the data and writing of the manuscript and read and approved the final version.

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Ethical considerations

Ethical approval was obtained from AHRI/ALERT Ethical Review Committee (AAERC), PO37/2014; and the National Research Ethics Review Committee (NRERC), A.A, Ethiopia, (3-10/014/2015). The study also had support letter from the Oromia Regional Health Bureau. The participants were informed about the study and all agreed to participate and gave informed verbal consent. All data were anonymized and confidentiality was maintained.

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Daniel et al

Case Report

Gallbladder volvulus

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Abstract

Background: *GB volvulus is rare to happen and the total cases reported range from 300-500. The anatomic risks include a floating GB with elongated mesentery. It is most difficult to diagnose pre-operatively.*

Case presentation: *A 30-year-old male patient presented with a complaint of exacerbation of right upper quadrant pain of three days duration to Arbaminch general Hospital, Arbaminch Ethiopia. Physical examination revealed normal except the temperature which is 36.8^0c. Murphy's sign was positive. Ultrasonography examination revealed echo complex pericholecystic left sub-phrenic and sub-hepatic fluid collection. Gangrenous GB volvulus was diagnosed intra-operatively and cholecystectomy was done. The patient was discharged on the 7^{th}- post-operative day.*

Conclusion: *Although the disease is not common in young male patients, it can be considered one of the differential diagnoses for patients coming with sudden and severe right upper quadrant pain. Early exploration and cholecystectomy can prevent gangrenous GB volvulus.*

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Keywords: *Gallbladder volvulus, acute abdomen, cholecystectomy, right upper quadrant pain*

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Introduction

Gallbladder volvulus is a rare condition first recognized in the late 19th century by Wendel. According to the different reports, the total number of case reports is in the range of 300-500 (1–3). Gallbladder volvulus is defined as the twisting of redundant GB along its mesentery. It usually is diagnosed intra-operatively when the patient presents with (symptoms, signs, and imaging) results that resemble complicated cholecystitis (1,2,4).

Case Report

A 30 years old male patient presented to our emergency room Arbaminch General Hospital, Arbaminch Ethiopia with chief compliant of: - exacerbation of right upper quadrant pain of three days duration. The pain was on and off type for which he took unspecified medication but no improvement. He had also a history of frequent vomiting of ingested matter and low-grade intermittent fever. Vital sign examination revealed normal. Except murphy sign, other abdominal examinations are non-revealing. The whole blood count and liver function test were within the normal limit. His ultrasonography examination revealed that the gall bladder was significantly distended with a length of 11.4cm and a diameter of 4.9cm. It has thickened wall measuring 8mm and there was intra-luminal echo debris. There was echo complex pericholecystic left sub-phrenic and sub-hepatic fluid collection. Sonographic Murphy's sign was positive. The intra and extra-hepatic bile ducts including the cystic duct were free and not dilated. With a working diagnosis of complicated perforated cholecystitis, the patient was taken to the operation room for exploratory laparotomy. The intraoperative finding was a gangrenous gall bladder that was rotated clockwise by about 270^0 (figure 1, 2, 3). Post operatively, the patient was continued with intravenous fluid, broad-spectrum antibiotics, and analgesics and was discharged improved on his seventh postoperative day.

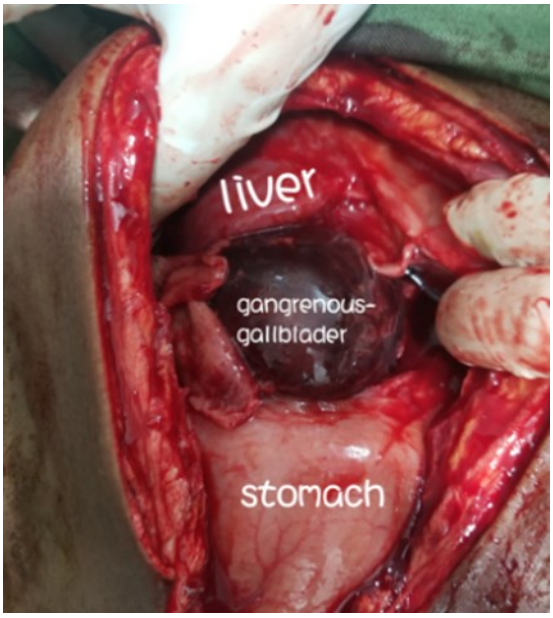

Figure 1: intraoperative finding confirming gangrenous GB

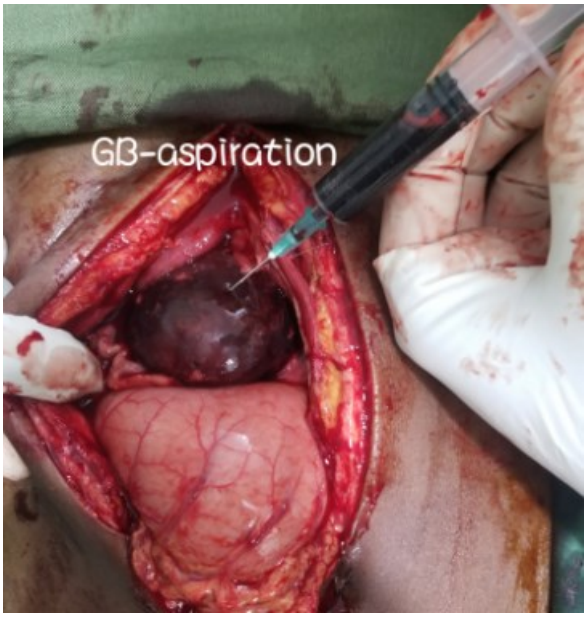

Fig 2: intraoperative decompression

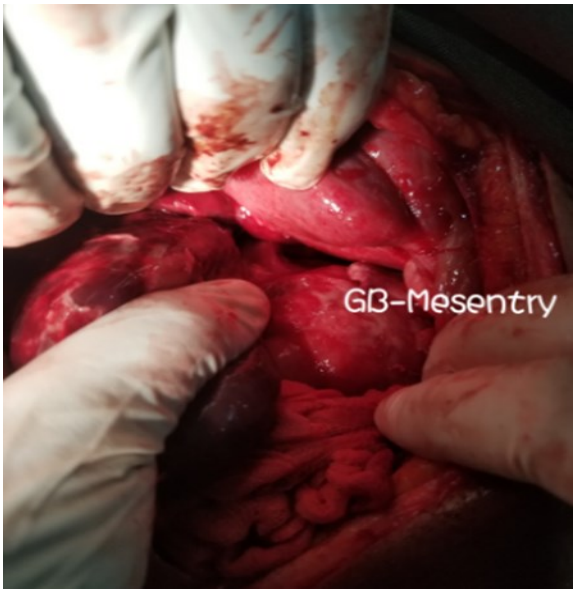

Figure 3: intraoperative de-rotated GB (gangrenous black fundal part).

Discussion

The well-known anatomic risk factors for the development of a gall bladder volvulus include a floating GB with elongated mesentery like our patient. It can then be precipitated by peristalsis of neighboring organs, spinal deformity, loss of visceral fat, and tortuous atherosclerotic cystic artery(1). GB volvulus has overlapping symptoms and signs with acute complicated cholecystitis; hence the diagnosis is usually made intraoperatively (3). A similar situation was identified in our patient. In addition, similar to our patient, GB volvulus is rarely considered a common differential diagnosis of acute RUQ pain hence resulting in gangrenous transformation and delays in the early surgical intervention(1–4). There is no preoperative gold standard imaging for the diagnosis of GB volvulus (3,5). However, modalities such as Ultra Sound and CT-scan can be very supportive(1,2,5). The finding of an anteriorly floating GB without gallstone that has a conical appearance in the neck, and discontinuity of the lumen are specific features of GB volvulus. The nonspecific features include gross wall thickening, GB distention, and cystic duct knot sign (5). The treatment of GB volvulus is always surgical.

Laparoscopic cholecystectomy is feasible, safe, and allows a faster patient recovery and shorter hospital stay than open cholecystectomy (5). In our setting, due to several factors, we prefer to perform open exploration.

The principles of the procedure include: - decompression and evacuation of GB, DE torsion, and cholecystectomy. It is recommended to perform DE torsion first since it avoids tenting and possible injury to the common bile duct, however, early DE torsion in the state of gangrene is criticized for leading to toxin release secondary to reperfusion ultimately leading to systemic effects (2,5). Our patient was treated similarly and was discharged improved.

Conclusion

Although GB volvulus is not a commonly diagnosed condition, we encourage health care providers and surgeons to consider it as a differential diagnosis in patients presenting with right upper quadrant pain. As the symptom, signs, or imaging results are not diagnostic, early exploration and cholecystectomy with a diagnosis of complicated acute cholecystitis are advised and this can prevent further morbidity and mortality.

Informed consent

Written informed publication consent was taken from the patient and hospital.

Abbreviations

BP- blood pressure
CM-centimeter
CT-computed tomography
GB-gallbladder
It's-it is
POD- post operative day
PR-pulse rate
RR- respiratory rate
RUQ- right upper quadrant
T^0-temprature
US-ultrasound

Declarations

Ethical clearance: Ethical clearance to write this case report was obtained from the institutional research ethics review board of Arba Minch University college of Medicine and Health Sciences.

Consent for publication: written informed consent for publication was obtained from the patient himself. We will avail the consent at a time needed.

Availability of data and materials: The data in this case report will be accessed by the contact address of the Authors.

Competing interests: No financial or non-financial competing interests

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Authors 'contribution: all authors actively involved in all parts of the case report.

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Mengistu et al

Case report

Complicated pregnancy in a Neuromyelitis Optica patient at St Paul Hospital Millennium Medical College, Ethiopia: A case report

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Abstract

Neuromyelitis optica (NMO) is an autoimmune mediated demyelinating inflammatory disease that mainly affects the optic nerves and spinal cord. NMO usually occurs in women of reproductive age. NMO and pregnancy have complex relationships with several diagnostic and therapeutic implications. In this report, the first Ethiopian case of NMO in a 28years old woman who presented from rural part of the country mainly with progressive weakness of all extremities and history of complicated pregnancy was described highlighting the diagnostic and therapeutic challenges as well as residual morbidity of NMO in resource limited setups.

Keywords*:* NMO, *Complicated pregnancy, Ethiopia*

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Introduction

Neuromyelitis optica (NMO) is a severe and recurrent autoimmune mediated inflammatory disorder of the CNS. The demyelination of neurons mainly causes a simultaneously or sequentially extensive inflammation of the spinal cord (myelitis) and optic nerve (optic neuritis) [1].Antibodies of the IgG class against Aquaporin-4 (AQP4) are characteristically diagnostic of NMO, present in about 88% of patients with NMOSD and used to distinguish NMO from other demyelinating diseases of the CNS [2]. NMO is a rare disease with the prevalence range of 0.5–4/100,000 and may be slightly higher in certain racial groups[1]. According to the 2006 revised diagnostic criteria[3], the definitive diagnosis of NMO required the following three criteria: A. Optic neuritis, B. Acute myelitis, and C. At least two of three supportive criteria:- i. Contiguous spinal cord MRI lesion extending over ³3 vertebral segments, ii. Brain MRI not meeting the diagnostic criteria for multiple sclerosis, and iii. NMO-IgG seropositive status. NMO is more common in reproductive age women than men, with women comprising over two-third of all NMO patients [4]. Though studies of pregnancy outcomes among NMO patients were challenged by limited sample size as NMO itself is a rare disease entity, there are reports of increased rates of miscarriage and preeclampsia among reproductive age women [4].

Case presentation

A 28 years old female patient presented from rural part of Ethiopia with the complaint of progressive weakness of all extremities of three-month duration. Her body weakness initially started on the left -upper extremity, progressed to left lower extremity after 3-days, extended to right-lower extremity and right-upper extremities in 10-days and 15 days -interval, respectively. She had an associated history of decreased vision of right eye, urinary and fecal incontinences, tingling sensation and numbness of all extremities. She had painless loss of her left eye vision 3 years ago but did not seek any medical attention then. Otherwise, she had no family history of similar illness, history of diabetes or hypertension, and history of arterial or venous thrombosis. She had repeated bad obstetric history in the last three years which included recurrent abortion of three times. The first two miscarriages were claimed to be after 5-months of amenorrhea, and the third was after 6-months of amenorrhea. The first episode of miscarriage occurred about two months after she sustained painless visual loss of the left eye. She was living in rural area and had no antenatal care(ANC) during pregnancies which ended up with miscarriage.

She had ANC follow up for her last pregnancy which she delivered a healthy baby three weeks before the onset of the current illness. However, in this last pregnancy she was also diagnosed to have preeclampsia.

On physical examination, her vital signs were in the normal range. She had complete vision loss of left eye with fundoscopic findings of optic atrophy, and reduced visual acuity of the right eye.

During admission to our hospital, she had flaccid paralysis with quadriplegia, hyperreflexia (brisk with clonus) and bilateral up going plantar reflex. There was no pertinent finding on the rest of the systems. Laboratory investigations have revealed normal whole blood cell count, serum vitamin B-12 level, Anti-cardiolipin antibody, lupus anticoagulant, and anti-neutrophilic antibody(ANA) as shown in Table-1.

Table 1 Laboratory results of an NMO patient with complicated pregnancy diagnosed at St. Paul Hospital Millennium Medical College

CBC	WBC	7,200/mm3 (Neutrophils-54.2%)
	Hemoglobin	14.5gm/dl
	Platelet	190,000/mm3
Cerebrospinal Fluid(CSF) Analysis	White Cell count	15/mm3 (Lymphocytes-55%)
	Protein	20 mg/dl
	Glucose	57 mg/dl
	AFB	No AFB
	Indian Ink	No C. Neoformans
Anti-Aquaporin 4-Antibody	1:1600+++	Normal Reference range<1:10
Anticardiolipin antibody	IgG and IgM isotype	14 GPL
Serum Vitamin-B12	485 pg/mL	Normal
ANA	Negative	
HIV	Negative	
VDRL	Negative	

Her clear appearing CSF-analysis had cell count of 15/mm3, with neutrophil of 45%, and lymphocyte of 55%. The AQP4 antibody titer was done abroad (Germany) after obtaining financial support from charity organization and the result was found to be1:1600+++ titer (Normal Reference range<1:10). Her brain MRI showed bilateral atrophy of optic nerves with edematous signal changes of the right optic nerve. The MRI of spinal cord also showed extensive long segment hyperintensity on T2W and STIR imaging with syrinx (fig-1). Due to financial constraints post contrast MRI could not be done. Pattern reversal visual evoked potential (PRVEP) with monocular stimulation and midline recording was done suggesting bilateral anterior visual pathway dysfunction with mixed demyelination and secondary axonal degeneration predominantly involving the left eye (Table-2).

Table 2 Pattern Reversal Visual Evoked Potential (PRVEP) demonstrating prolonged P100 latency on bilateral eyes and decreased P100 amplitude (mV) of bilateral eyes, more on the Left eye

Stimulation position	P100- **Latency(msec)**	**P100 Amplitude**(μV)
Left Eye	160	1.24
Left Eye	167.6	1.68
Right Eye	182.4	2.54
Right Eye	169.6	3.81

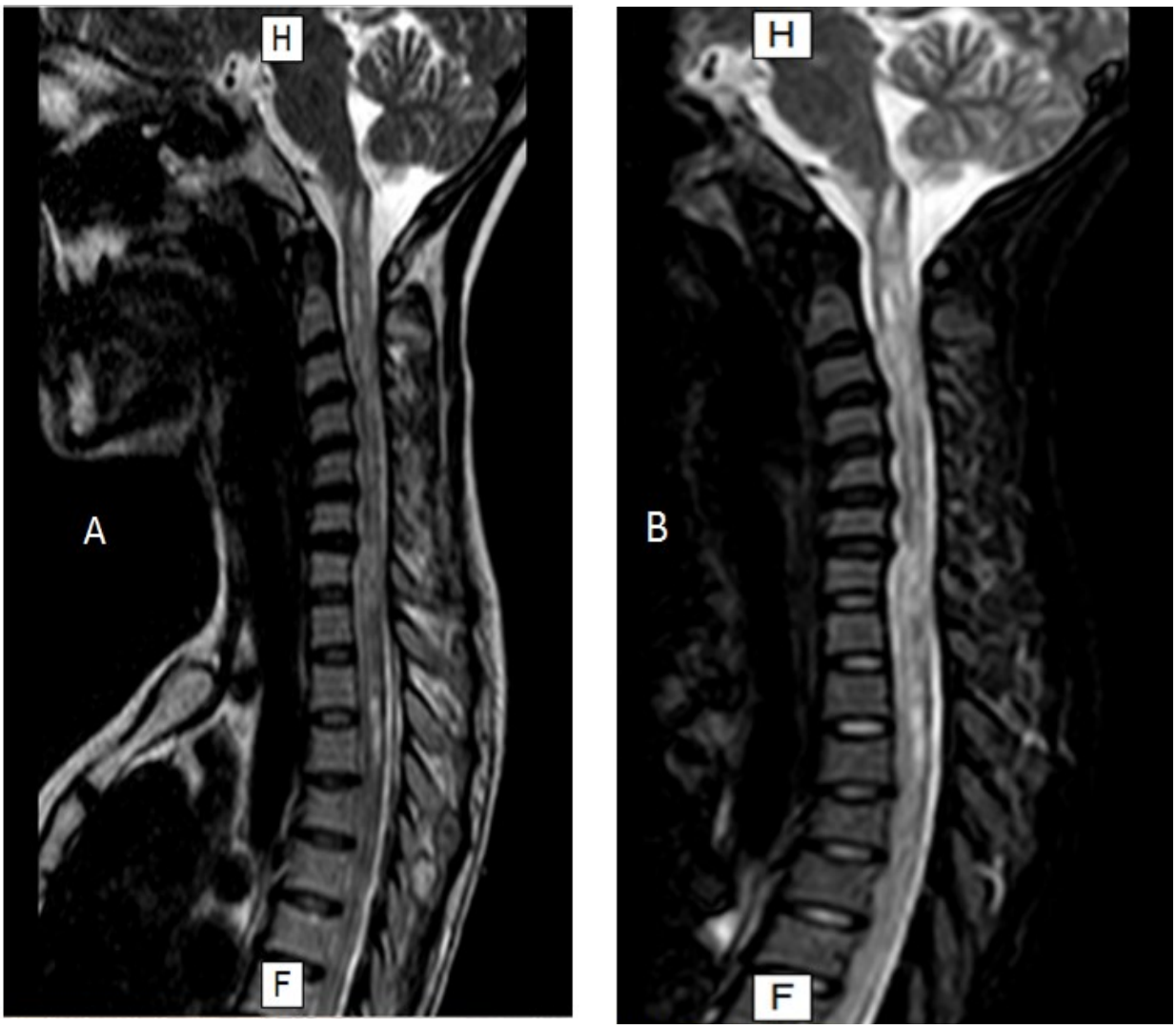

Figure 1. T2W (A) and STIR (B) imaging of the spinal cord from a 28years old Ethiopian women diagnosed to have NMO showing extensive long segment hyperintense lesions (foramen magnum - T6) with syrinx

She fulfilled all the required criteria for definitive diagnosis NMO and managed by multidisciplinary team. She was pulsed with high dose of intravenous Methylpredenisolone for 3-days and then oral prednisolone 1mg/kg daily with subsequent slow tapering. She was put on oral Azathioperin 100mg daily as a long term maintenance immunosupprsive therapy to reduce the risk of further relapse of the disease. In the course of 3-months follow up, she regained her vision of the right eye and also had marked improvement in muscle power of bilateral upper extremities from the initial muscle strength of 0/5 to 4/5 with deep tendon reflex of 2/4. Her condition on the lower extremities was the same(0/5, bilaterally) with hypereflexia and up going plantar reflex. There was also no change of urinary and fecal incontinences for which she was emotionally distressed and discontinued her follow up. It was not possible to restore the follow up despite multiple attempts over the phone.

Discussion

Over the last decade, there has been a tense debate on whether NMO is a distinct entity or a form of MS [1]. Current advances in the clinical manifestations, neuro -imaging features, serologic finding and pathological hallmarks have established that NMO is a distinct demyelinating disease of CNS. Therefore**,** it is important to NMO from MS early, because NMO has a more severe morbidity than MS and standard MS-modifying therapies may not be effective on NMO [5, 6]. The natural history of untreated NMO is significantly worse than that of MS with acquisition of residual disability from initial relapses in the majority of patients. Hence, NMO requires early recognition of cases for early initiation of treatment for acute attacks [6].

The clinical presentation of our patient with myelitis and optic neuritis, extensive long segment spinal cord lesion demonstrated by the spinal MRI, the absence of characteristic lesions of MS in the brain MRI and abnormal serologic titer of anti-AQ4 immunoglobulin fulfilled all the criteria for definitive diagnosis of NMO. She had also frequent pregnancy complications since from the first presumed attack of NMO where she sustained painless visual loss of left eye with no other symptomatic myelitis. There are reports of optic neuritis with asyptomatic myelitis [7] and the rate of optic neuritis with asymptomatic myelitis as initial presentation of NMO reaches as high as 42% [8].

Recently, there are reports of untoward effects of NMO on the pregnancy outcomes [4, 9-10]. AQP4 is expressed on placental syncytiotrophoblasts and the expression is highest around mid-gestation and decreases subsequently [10]. Placental dysfunction, therefore, is associated with miscarriage, intrauterine growth restriction, preeclampsia, and stillbirth, providing a potential mechanism for an increased frequency of these complications in NMO[9-10]. NMO has increased the risk of miscarriage independent of risk of concomitant autoimmunity such as antiphospholipid syndrome(APS) [4, 9]. The risk of preeclampsia is at a rate much higher than in obstetric controls in pregnancies after NMO onset [4, 9]. Therefore, anti-AQP-4 antibody positive NMO better explains the underlying cause of frequent miscarriage and also preeclampsia which our patient experienced. The treatment of NMO constitutes therapies to reverse the acute symptoms of NMO and also to prevent the future relapses. Acute NMO of initial or recurrent episodes are usually treated with pulse of high-dose intravenous methylprednisolone (05gm-1gm daily for three to five consecutive days) as first-line therapy, followed by an oral prednisolone with gradual tapering until Steroid sparing maintenance immunosuppressants take effect [6, 9]. For patients with NMO having severe cervical myelitis, or for those refractory to steroids, rescue therapy with therapeutic plasmapheresis (seven exchanges of approximately 55 ml/kg each, administered every other day for 14 days) should be considered with the possible mechanism of removing autoantibodies, immune complexes and inflammatory mediators from the plasma [11].Despite our patient was pulsed with high of dose methylprednisolone followed by oral prednisone combined with oral Azathioprine, in the course of three-months follow up, she remained incontinent and paraplegic.

She regained her right vision and had marked improvement of upper extremities. Limited availability and financial constraints hindered further therapies including plasmapheresis. She was frustrated by the incontinences as well as the residual weakness of the lower extremities which has kept her bedbound and further impoverished the living of the entire family. She went to her rural dwelling after the three-months of close follow up. However, she discontinued her follow-up altogether and no means of re-communicating her again. Therefore, while addressing the overt neurologic condition of such patients, equal attention should also be paid to the psychosocial and emotional components of patients with residual extremity weakness and incontinences.

In conclusion, this case highlights that in resource limited areas since the diagnosis of NMO as well as its management is challenging and time consuming, high index of clinical suspicion is important to reduce the repercussion of delayed initiation of therapy. The atypical presentation of NMO with obstetric complications should also warrant high index of suspicion. In resource poor counties, patients with neurologic squeal have multiple undisclosed burdens and hence require adequate psychosocial attention in order to optimize the medical therapy.

Declarations

Acknowledgements: We thank our patient for allowing us to share her case.

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Conflict of interests: The authors declare that they have no conflict of interest.

Authors' contributions: Both authors were involved in the management of this patient. MDM written the manuscript, all authors read and approved the final manuscript

Abbreviations: AFB:Acid Fast Bacillus, ANA: Anti-Neutrophilic Antibody, AQP4: Aquaporin 4, CSF-Cerebrospinal Fluid, MRI: Magnetic Resonance Imaging, MS: Multiple Sclerosis, NMO-Neuromyelitis Optica, PRVEP: Pattern Reversal Visual Evoked Potential, STIR: Short-TI Inversion Recovery, VDRL: Venereal Disease Research Laboratory

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Case report

Increased anti-M. Leprae PGL-I igm levels in a child who developed leprosy

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Abstract

As part of a study that aimed at reaching those at risk of developing leprosy through screening of household contacts, a child aged 7 was included as contact of a multibacillary leprosy patient. This study was conducted in Kokosa, hot spot area in Oromia Region, Ethiopia. Compared to other contacts, this child showed increased levels of anti-M. leprae Phenolic glycolipid-I (PGL-I) IgM antibodies as assessed by up-converting lateral flow assay (UCP -LFA) at initial and second-time screening and developed leprosy three years later. Therefore, the anti-PGL-I IgM UCP-LFA can serve as an additional diagnostic field tool in leprosy control programs.

Keywords: *Leprosy, anti-M. Leprae, IgM antibodies*

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Introduction

At the end of 2020, the World Health Organization (WHO) reported 202,185 new cases of leprosy globally, among which 10,813 had new grade-two disability (G2D) cases, and 14,981 were new child leprosy cases. Ethiopia reported 3,201 new cases, standing 6th among the 23 global priority leprosy burden countries and 1st in Africa reporting 507 new child cases and 411 G2D cases (1).

Prolonged contact with untreated newly diagnosed leprosy patients is considered one of the risk factors, which implies the need for regular screening of contacts for possible early case detection (2, 3). Studies showed there is 6 fold relative risks for contacts of all types of leprosy compared to the general population. Contacts of multibacillary (MB) patients even showed 8-fold increased risk compared to paucibacillary (PB) contacts (4). Some studies also rapid detection of leprosy through monitoring antibody titers of household contacts (5, 6). The Anti-PGL-I-IgM UCP-LFA discussed in this paper is a serological test that quantitatively detects anti-M. leprae PGL-I IgM levels. PGL-I is M. leprae-specific glycolipid component of M. leprae found on the cell wall of the bacteria, comprising up to 3% of the total weight of the bacteria.

Antibodies directed against PGL-I can also be used in monitoring treatment outcome, as the levels decrease after MDT due to bacilli destruction which means reduced PGL-I synthesis (5).
This study was designed to reach those at risk of developing leprosy, where the close household contacts (HHCs) should be traced and followed.

Case presentation

A 7-year-old boy from Hebeno Kebele of Kokosa Woreda (HEB-03-018) was screened as one of the household contacts of a multibacillary (MB) leprosy index patient, a 13-year-old child, detected through the house-to-house screening. During contact screening, the 7-year-old boy did not show any clinical signs and symptoms of leprosy. Venous blood was collected from this child for serological analysis. He was screened again after one year when his index case completed leprosy treatment but still did not show any clinical signs and symptoms of leprosy. A second-time point blood was also collected after one year, and all samples were analyzed for the presence of anti-PGL-I IgM antibodies. His anti-PGL-I IgM levels showed a progressive increase from the first to the second-time point (Figure 1).

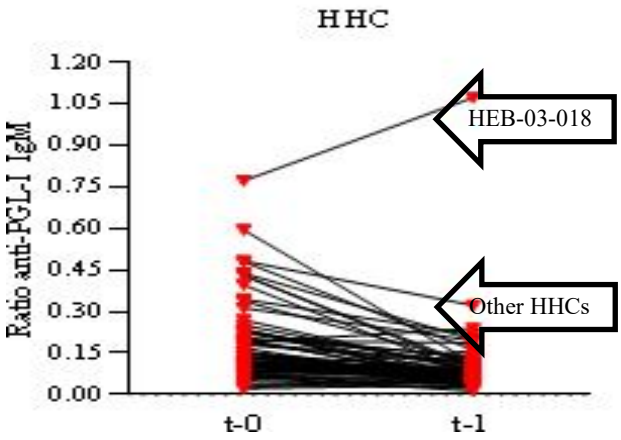

Figure 1: Anti PGL-I IgM antibody levels at two–time points measured by UCP-LFA:

Moreover, the ratio value of the UCP-LFA, which corresponds to the IgM antibody level, was higher in comparison to the other screened contacts. So far, none of the contacts has developed leprosy. Ratio of anti-PGL-I IgM in household contacts at time of first screening (t-0) and at time of second screening performed after one year (t-1).IgM in household contacts at time of first screening (t-0) and at time of second screening performed after one year (t-1).HEB-03-018 is a household contact with an increased anti-PGL-I IgM ratio which later developed leprosy. The child was continuously followed up for three years, and in the fourth year, i.e., after three years of the first sampling; he developed nodular lesions typical of leprosy on his face and hands **(Figure 2).** He was put on MDT-MB and has shown significant improvement.

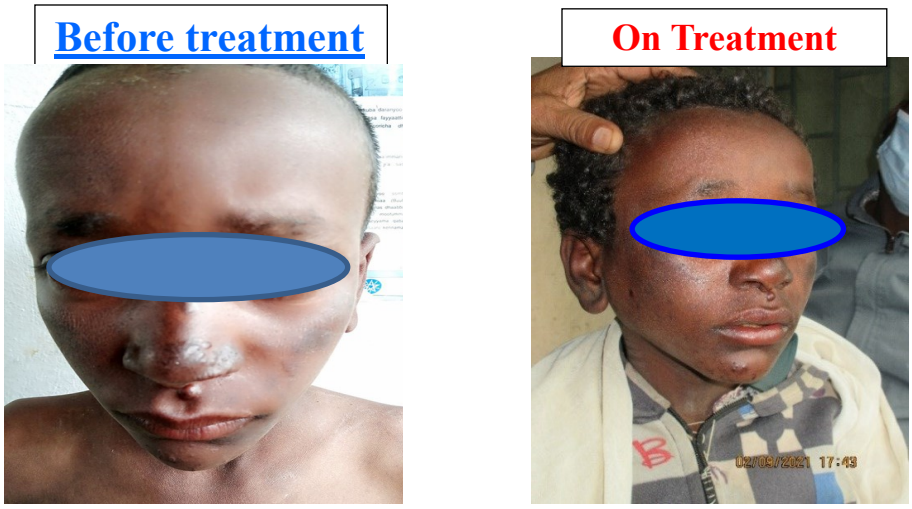

Figure 2 Anti-PGL-1 IgM positive apparently healthy household contact of an index case who developed leprosy 3 years later. The picture was taken after 9 months of MDT.

Discussion

Our study has shown the importance of contact tracing and serological testing to serve as supporting evidence for early detection of leprosy. In line with our findings, Spencer *et al.* previously showed household contacts with progressively elevated antibody titers. Among them, one contact developed borderline lepromatous leprosy (BL) at an early stage after two years routine follow-up.

This demonstrates that repeated measurements of the serum anti-PGL-I antibody levels in HHCs of leprosy patients may be used to evaluate antigen exposure and identify contacts that may progress to disease (6). Moreover, anti-*M. leprae* PGL-I antibody level in young children can indicate the time of infection and be used as a proxy indicator for transmission in an area (7).

However, anti-PGL-I antibody levels alone might not be enough to indicate whether a person develops disease or not as shown in a study in Bangladesh (8).

In fact, a systematic review showed that contact screening for prophylaxis based only on anti-PGL-I antibodies would miss more than half of future leprosy cases. This could be due to inter-individual, or population variations. In recent studies, Anti PGL-I IgM was included in the Multi-Biomarker Test (MBT) as a serological marker to assist in the early diagnosis of leprosy in contacts of leprosy (9, 10). The present study showed that progressively elevated anti-PGL-I IgM antibody level in exposed individuals particularly in children can be used for early detection of those at "high risk" of developing leprosy. Therefore, establishing the method and integrating it as one screening method while implementing Active case detection in high burden areas may facilitate early detection of leprosy that contributes towards reduced transmission, disability and stigma.

Competing interest

The authors declare that this manuscript was approved by all authors in the current form and that no competing interest exists.

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Policy Brief

Emerging COVID-19 virus variants and low vaccination coverage in Ethiopia: The need for tailored vaccination strategy

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Abstract

The world has faced multiple waves of COVID-19 outbreaks, with more than 300 million cases and 5.5 million deaths officially reported globally as of Jan 8, 2022. Within the first year of the pandemic, there was hope that it would soon be under control, yet the pandemic sustains to be the world's priority health agenda. This brief communication provides emerging time-sensitive perspectives on the need for a tailored COVID-19 vaccination strategy in Ethiopia by reviewing studies and expert opinions. As of Jan 8, 2022, Ethiopia has reported 443,339 cases and 7,020 COVID-19-related deaths. Only 9,361,640 people (8%) of the Ethiopian population received at least one dose of the COVID-19 vaccine. While the short supply of vaccines is mentioned as a major bottleneck, the role of vaccine skepticism is largely overlooked, though the vaccine is the primary means to combat the emergence of new variants. Therefore, we recommend vaccine advocacy and awareness creation, planning for vaccine mandate for certain groups of the society, and targeted vaccination and economical use of the vaccines.

***Keywords**: Emerging, Variant, COVID-19, Strategy, Ethiopia*
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Introduction

It has been two years since COVID-19 was officially reported to the World Health Organization as a cluster of cases of pneumonia with an unknown etiology (1). Over this period, our world has faced multiple waves of COVID-19 outbreaks, with more than 300 million cases and 5.5 million deaths officially reported globally as of Jan 8, 2022(2). The authorization of the first COVID-19 within a year of the first reported case gave the hope that the pandemic would soon be under control(3). However, this hope was dashed by inequitable distribution of the vaccines(4)(5), vaccine hesitancy(6)(7)(8)(9), and the arrival of immune escape variants of the virus(10). Currently, although more than 60% of populations in rich countries have been fully vaccinated(11), and despite high rates of Seroprevalence of SARS-CoV-2 in poorer nations(12), the virus is causing more infection than ever(2).

Ethiopia is currently in the fourth wave of the COVID -19 outbreak with a recorded-high number of daily new cases and RT-PCR test positivity rates than ever. As of Jan 8, 2022, Ethiopia has reported 443,339 cases and 7,020 COVID-19-related deaths(13). Oromia, the most populous and largest region, in Ethiopia, has reported 56,338 cases and 1,096 deaths, accounting for 12.7% of the cases and 15.6% of the deaths reported nationally; the second -highest number of cases and death after Addis Ababa(14). However, the real burden of the pandemic remains unknown and likely to be underestimated due to the country's limited testing and surveillance capacity(15).

Large-scale SARS-CoV-2 serosurveys conducted in Oromia and Addis Ababa among healthcare workers (HCWs), urban and rural communities, school children, and patients visiting hospitals indicated a dramatic increment of Seroprevalence between August 2020 and September 2021 (Figure 1). For instance, the Seroprevalence among HCWs increased from around 11% in August 2020 to over 70% in September 2021. in late 2020 to nearly 60% in August 2021(16).

Among community participants, the seroprevalence increased from <30%

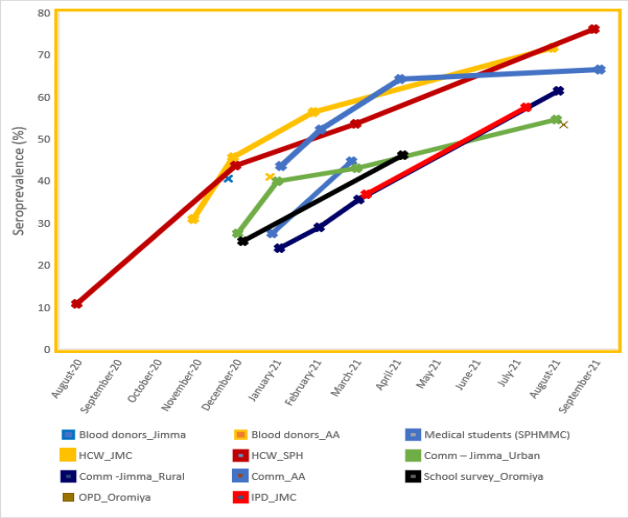

Figure 1: Dramatic increment in SARS-CoV-2 seroprevalence in Ethiopia between August 2020 and September 2021

These findings indicate a high level of virus transmission among HCWs and the community. Putting these results into perspective, nearly three-fourths of the HCWs and about 60% of the general population in Ethiopia have been exposed to the virus. However, the role of detectable antibodies and herd immunity due to natural infection remains not fully understood. Hence, vaccination remains the best means to contain the outbreak risks effectively(17).

Why tailored- vaccination strategy?

Only 9,361,640 people (8%) of the Ethiopian population received at least one dose of the COVID-19 vaccine. While the short supply of vaccines is mentioned as a major bottleneck, the role of vaccine skepticism is largely overlooked(18)(19). Ethiopia rolled out COVID-19 vaccination in March 2021 with initial priority for HCWs. In our survey among 1,314 HCWs in five teaching hospitals just before the arrival of vaccines in Ethiopia, we found that 25.5% (n=332) of the HCWs were hesitant toward the COVID-19 vaccine, and 20.2% hesitated to recommend the COVID-19 vaccination to others (20). After five months of vaccine arrival (August and September 2021), though the Ministry of Health of Ethiopia (MoH-Ethiopia) and the hospitals reported to have enough vaccine doses, we found that only 42.1% took one dose of AstraZeneca Covishield vaccine, while the rate for two doses was only 29.2% among 662 HCWs at Jimma Medical Center and St. Paul Hospital. This indicates profound vaccine skepticism among HCWs. Usually, few deviants among even well-informed individuals may always exist. However, most health workers refuse vaccination against a deadly disease, which is a double burden to any country. First, HCWs are the high-risk group of getting infected and spreading the infection to patients, family members, and the community at large. Secondly, though HCWs are expected to advocate for vaccination, they could spread misinformation to the community, leading to profound vaccine hesitancy. Thus, Ethiopia has to deal with this matter as a priority agenda to avoid further setbacks due to the pandemic.

Low vaccination rates contribute to immune escape variants such as Delta and Omicron(21). With the arrival of such variants and the low vaccination rate in Ethiopia, the outbreak's next wave, or even waves, is inevitable. Ethiopia thus needs to take a proactive approach to mitigate the impact of the outbreak's next wave(s). Vaccination helps reduce the spread of SARS-COV-2, including the Delta and Omicron variants, albeit with reduced efficacy (22)(23). More importantly, vaccines have proven efficacy in preventing severe disease and death (24). Hence, accelerating vaccination and increasing vaccination coverage remains a critical and urgent matter. It is the most effective strategy to end the pandemic. Besides non-pharmacological interventions, MoH-Ethiopia should improve vaccine coverage by enhancing public awareness and economic and contextualized use of the vaccines.

There are vital pieces of evidence that a single dose of COVID-19 vaccine of a conventionally two-dose regimen may sufficiently protect previously SARS-CoV-2 infected individuals(25) (26). With the existing evidence of high SARS-CoV-2 Seroprevalence(16) (27), Ethiopia benefits from a single vaccination strategy to facilitate and expedite immunization campaigns for most of the population. However, the country should follow the conventional approach of two regimen vaccines for the segment of the people at risk for severe disease (those with comorbidities and older individuals). Furthermore, booster dose vaccination – the third dose for conventionally two regimens and the second dose for one dose vaccines, is vital to prevent future surges in selected populations(28). Thus, Ethiopia should design a proactive and contextualized strategy to effectively and economically use available scarce vaccines. The country can save enough vaccine doses for the most at risk by providing a single dose for most of the population and following the conventional strategy for high-risk groups and booster doses for selected groups.

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Summary and recommendations

The threat posed by COVID-19 remains dire despite great success with the vaccine. Inequitable distributions of vaccines, vaccine hesitancy, and the emergence of new variants have put more strain on global and national endeavors. Putting the existing shreds of evidence and the local contexts into perspective, we recommend the country to take the following actions as priority areas for interventions:

1. **Vaccine awareness creation and advocacy.** As stated above, there is a high rate of vaccine hesitancy among HCWs working in tertiary hospitals and the community in Ethiopia (18)(19)(29) in Ethiopia. A community study also revealed that around half of the participants' knowledge regarding COVID-19 was not good, and they also had a negative attitude towards the vaccine. This urges the need to increase community awareness of COVID-19 through health education. Besides, Ethiopia should enhance the effort in COVID-19 vaccine advocacy by promoting the best scientific knowledge, moral attitudes, and public health practice to respond to vaccine concerns of HCWs and communities and reduce vaccine hesitancy.
2. **Vaccine mandate for certain groups of the society.** Vaccine mandates for other diseases exist in some settings like schools and HCWS. Such a vaccine mandate improved the vaccination rate in high-income countries(30). Concerning COVID-19 vaccination, some countries have mandated either full country vaccination to achieve herd immunity or for certain professions or settings like schools(31). Besides, mandatory proof of vaccination requirement for travel has also been implemented globally to control COVID-19 (31). It is thus important if Ethiopia plans to implement a workplace vaccination mandate for all frontline healthcare workers, schools, and universities. MoH-Ethiopia should also identify other high-risk frontline workers in this category.
3. **Targeted vaccination and economical use of the vaccines**. Over a year since it became available worldwide, the COVID-19 vaccine is still in short supply throughout Africa(11). On the other hand, as a recent serosurvey has indicated, nearly two-thirds of the population has already been exposed to SARS0CoV-2 and have detectable antibodies against the virus(16). Thus, countries with high SARS-CoV-2 Seroprevalence and limited vaccine supply benefit from prioritizing vaccines for the high-risk groups (e.g., healthcare workers) and the most vulnerable to severe disease (older people and those with comorbidities) (Targeted vaccination and the speed of SARS-COV2 adaptation). Such a targeted vaccination approach is more economical in its implementation and saving lives than blanket immunization which is not realistic in the current Ethiopian context and is not a priority due to the reasons explained above. Based on these facts, we recommend the country to use one dose vaccine (for conventionally two regimen vaccines such as Pfizer/Biontech and Aztrazeneca/Covishield) to increase vaccination coverage (**Table 1**). However, high-risk groups (frontline workers) should follow internationally recommended vaccination strategies, including frontline healthcare workers and other high-risk professionals. Furthermore, people who are at high risk for severe diseases, such as those with debilitating comorbidities (DM, HIV, cancer, cardiorespiratory diseases, CKD/ CLD), and older people (≥ 65-year) should also follow the conventional approach of vaccination. Booster vaccination for such groups of society should also be considered.

Table 1: Targeted Covid-19 vaccination strategy for Ethiopia

Category	Vaccination strategy	Indications*
A	One dose	12-50 years of age **PLUS** No known comorbidity **PLUS** Apparently healthy
B	Two doses	≥50 years ≥12 years **PLUS** comorbidity
C	Booster dose†	≥65 years ≥50 years **PLUS** comorbidity

*In individuals with confirmed prior SARS-CoV-2 infection as evidenced by positive RT-PCR or detection of SARS-CoV-2 antibody, one dose of the vaccine is enough for category A and B indications. Booster dose vaccination may also be exempted for such groups in category C.

† Booster (third dose) should be given six months after the second dose (the first dose of the Johnson & Johnson vaccine)

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GUIDELINES FOR AUTHORS

The *Ethiopian Medical Journal (EMJ)* is the official Journal of the Ethiopian Medical Association (EMA) devoted to the advancement and dissemination of knowledge pertaining to the broad field of medicine in Ethiopia and other developing countries. Prospective contributors to the Journal should take note of the instructions of Manuscript preparation and submission to EMJ as outlined below.

Article types acceptable by EMJ

Original Articles (*vide infra*) on experimental and observational studies with clinical relevance
Brief Communications
Case Series
Case Reports
Editorials, Review or Teaching Articles: by invitation of the Editorial Board.
Correspondences/Letters to the Editor
Monographs or set of articles on specific themes appearing in a Special Issues of the Journal
Book reviews
Perspectives,
Viewpoints
Hypothesis or discussion of an issue important to medical practice
Letter to the Editor
Commentaries
Advertisements
Obituaries

N.B. Articles are not acceptable if previously published or submitted elsewhere in print or electronic format, except in the form of abstracts in proceedings of conferences.

Content and format of articles:

Title: The title should be on a separate page. It should not have acronyms or abbreviations. The title should be descriptive and should `not exceed 20 words or 120 characters including space. The title page should include the name(s) and qualification of the author(s); the department or Institution to which the study/research is attributed and address of the corresponding Author. If the author has multiple affiliations only use the most preferred one.

1. Original Articles

2,500 words, excluding Abstracts, References, Figures and Tables. The manuscript of the Article, should appear under the following headings:

a) Abstract: The abstract of the Article is prepared on a separate paper, a maximum of 250 words; it should be structured under the titles: a) Background; b) Methods; c) Results; d) Conclusions. Briefly summarize the essential features of the article under above headings, respectively. Mention the problem being addressed in the study; how the study was conducted; the results and what the author(s) concluded from the results. Statistical method used can appear under Methods paragraph of the Abstract, but do not insert abbreviations or references in the Abstract section.

Keywords: Provide three to six key words, or short phrases at the end of abstract page. Use terms from medical subject heading of Index Medicus to assist in cross indexing the Article.

b) Introduction : Should provide a short background and context of the study and provide the rationale for doing the study. It should not be a detailed review of the subject and should not include conclusions from the paper.

c) Patients or (Materials) and Methods: should contain details to enable reproducibility of the study by others. This section must include a clear statement specifying that a free and informed consent of the subjects or their legal guardians was obtained. Corresponding author should submit a copy of institution review Board (IRB) clearance or letter of permission from the hospital or department (if IRB exempt)

with the manuscript. For manuscripts on clinical trials, a copy of ethical approval letter from the concerned body should be submitted with the Manuscript. If confidential data is being used for publication (such as student grades, medical board data, or federal ethics board data), then appropriate support/ agreement letter should be included. Photos of patients should disguise the identity or must have obtained their written consent. Reference number for ethical approval given by ethics committee should be stated. In general, the section should include only information that was available at the time the plan or protocol for the study was being written; all information obtained during the study belongs in the Results section.

d) Results: This section should present the experimental or observational data in text, tables or figures. The data in Tables and Figures should not be described extensively in the text.

e) Discussion: The first paragraph should provide a summary of key finding that will then be discussed one by one in the paragraphs to follow. The discussion should focus on the interpretation and significance of the results of the study with comments that compare and describe their relation to the work of others (with references) to the topic. Do not repeat information of Results in this section. Make sure the limitations of the study are clearly stated.

f) Tables and Figures: These should not be more than six. Tables should be typed in triplicate on separate sheets and given serial Arabic numbers. Titles should be clearly place underneath Tables and above Figures. Unnecessary and lengthy tables and figures are discouraged. Same results should not be presented in more than one form (choose either figure or table). Units should appear in parentheses in captions but not in the body of the table. Statistical procedures, if not in common use, should be detailed in the METHODS section or supported by references. Legends for figures should be typed on separate sheets, not stapled to the figures. Three dimensional histograms are discouraged. Recognizable photographs of patients should be disguised. Authors should submit editable soft versions of the tables and figures.

g) Acknowledgement: Appropriate recognition of contributors to the research, not included under Authors should be mentioned here; also add a note about source of the financial support or research funding, when applicable.

h) References:

- The titles of journals should be abbreviated according to the style used for MEDLINE (www.ncbi.nlm.nih.gov/nlmcatalog/journals).
- References should be numbered consecutively in the order in which they are first mentioned in the text and identify references in text, tables, and legends by Arabic numerals in parentheses.
- Type the References on a separate sheet, double spaced and keyed to the text.
- Personal communications should be placed NOT in the list of references but in the text in parentheses, giving name, date and place where the information was gathered or the work carried out (e.g. personal communication, Alasebu Berhanu, MD, 1984, Gondar College of Medical Sciences). Unpublished data should also be referred to in the text.
- References with six or less authors should all be listed. If more than six names, list the first three, followed by et al.
- Listing of a reference to a journal should be according to the guidelines of the International Committee of Medical Journal Editors ("Vancouver Style') and should include authors' name(s) and initial(s) separated by commas, full title of the article, correctly abbreviated name of the journal, year, volume number and first and last page numbers.
- Reference to a book should contain author's or authors' name(s) and initials, title of chapter, names of editors, title or book, city and name of publisher, year, first and last page numbers.

The following examples demonstrate the acceptable reference styles.

Articles:

- Gilbert C, Foster A. Childhood blindness in the context of Vision 2020: the right to sight. *Bull World Health Org 2001;79:227-32*
- Teklu B. Disease patterns amongst civil servants in Addis Ababa: an analysis of outpatient visits to a Bank employee's clinic. *Ethiop Med J 1980;18:1-6*

- Tsega E, Mengesha B, Nordenfelt E, Hansen B-G; Lindberg J. Serological survey of human immunodeficiency virus infection in Ethiopia. *Ethiop Med J 1988; 26(4): 179-84*
- Laird M, Deen M, Brooks S, et al. Telemedicine diagnosis of diabetic retinopathy and glaucoma by direct ophthalmoscopy (Abstract). *Invest Ophthalmol Vis Sci 1996; 37:104-5*

Books and chapters from books:

- Henderson JW. Orbital Tumors, 3rd ed. Raven Press New York, 1994. Pp 125-136.
- Clipard JP. Dry Eye disorders. In Albert DM, Jakobiec FA (Eds). Principles and Practice of Ophthalmology. W.B Saunders: Philadelphia, PA 1994 pp257-76.

Website:

- David K Lynch; laser History: Masers and lasers. http://home.achilles.net/jtalbot/history/massers.htmAccessed 19/04/2001

2. Brief Communication

Short versions of Research and Applications articles, often describing focused approaches to solve a health problem, or prelnary evaluation of a novel system or methodology

- Word count: up to 2000 words
- Abstract up to 200 words; excluding: Abstract, Title, Tables/Figures and References
- Tables and Figures up to 5
- References (vide supra – Original Article)

3. Case Series

Minimum of three and maximum of 20 cases

- Up to 1,000 words; excluding: Abstract, Title, Tables/Figures and References
- Abstract of up to 200 words; structured; (vide supra)
- Statistical statements here are expressed as 5/8 (62.5%)
- Tables and Figures: no more than three
- References: maximum of 20

4. Case Report

Report on a rare case or uncommon manifestation of a disease of academic or practical significance

- Up to 750 words; excluding: Abstract, Title, Tables/Figures and References
- Abstract of up to 100 words; unstructured;
- Tables and Figures: no more than three
- References: maximum of 10

5. Systematic review

Review of the literature on topics of broad scientific interest and relevant to EMJ readers

- Abstract structured with headings as for an Original Article (*vide supra*)
- Text should follow the same format as what is required of an Original Article
- Word count: up to 8,000 words, excluding abstract, tables/Figures and references
- Structured abstract up to 250 words
- Tables and Figures up to 8

6. Teaching Article

A comprehensive treatise of a specific topic/subject, considered as relevant to clinical medicine and public health targeting EMJ readers

- By invitation of the Editorial Board; but an outline of proposal can be submitted
- Word limit of 8,000; excluding abstract, tables/Figures and references
- Unstructured Abstract up to 250 words

7. Editorial

- By invitation of the Editorial Board, but an editorial topic can be proposed and submitted
- Word limit of 1,000 words: excluding references and title; no Abstract
- References up to 15.

8. Perspectives

- By invitation of the Editorial board, but a topic can be proposed and submitted
- Word limit of 1,500
- References up to six

9. Obituaries

- By invitation of the Editorial board, but readers are welcome to suggest individuals (members of the EMA) to be featured.

Preparation of manuscripts

- Manuscripts must be prepared in English, the official language of the Journal.
- On a single separate sheet, there must be the title of the paper, with key words for indexing if required, and each author's full name and professional degrees, department where work was done, present address of any author if different from that where work was done, the name and full mailing address of the corresponding author, including email, and word count of the manuscript (excluding title page, abstract, references, figures and tables). Each table/figures/boxes or other illustrations, complete with title and footnotes, should be on a separate page.
- All pages should be numbered consecutively in the following order: Title page; Abstract and keywords page; main manuscript text pages; References pages; acknowledgment page; Figure-legends and Tables
- The Metric system of weights and measures must be used; temperature is indicated in degrees Centigrade.
- Generic names should be used for drugs, followed by propriety brand name; the manufacturer name in parenthesis, e.g. diazepam (Valium, Roche UK)
- Statistical estimates e.g. mean, median proportions and percentages should be given to one decimal place; standard deviations, odds ratios or relative risks and confidence intervals to two decimal places.
- Acronyms/Abbreviations should be used sparingly and must be given in full, at first mention in the text and at the head of Tables/foot of Figure, if used in tables/figures.eg. Blood Urea Nitrogen (BUN). Interstitial lung disease (ILD).
- Use the binomial nomenclature, reference to a bacterium must be given in full and underlined - underlining in typescript becomes italics in print (e.g. *Hemophilus influenzae)*, and later reference may show a capitalised initial for the genus (e.g. *H. influenzae)*
- In the text of an article, the first reference to any medical phrase must be given in full, with the initials following in parentheses, e.g., blood urea nitrogen (BUN); in later references, the initials may be used.
- Manuscripts for submission should be prepared in Microsoft Word document file format

Submission of manuscripts

- As part of the submission process, authors are required to check off their submission's compliance with journals requirements
- All manuscripts must be submitted to the Editor-in-Chief of the Journal with a statement signed by each author that the paper has not been published elsewhere in whole or in part and is not submitted elsewhere while offered to the *Ethiopian Medical Journal*. This does not refer to abstracts of oral communications at conferences/symposia or other proceedings.
- It is the author's responsibility to proof-read the typescript or off-print before submitting or resubmitting it to the Journal, and to ensure that the spelling and numerals in the text and tables are accurate.
- Authors should submit their work through the Ethiopian Medical Journal website; ema.emj@telecom.net.et.

Conflict of interest

Authors should disclose at the time of submission of manuscripts any conflict of interest, which refers to situations in which financial or other personal considerations may compromise, or have the appearance of compromising their professional judgment in conducting or reporting the research results They should declare that there is no conflict of interest to declare if there is none,

Manuscripts review procedures

The procedures for manuscripts review include:

- Within one week of receipt of a manuscript, the Editorial Board will review it in reference to (i) conformity with the Journal's "guidelines to authors (revised version available in all issues starting January 2020)", (ii) relevance of the article to the objectives of the *EMJ*, (iii) clarity of presentation, and (iv) plagiarism by using appropriate software
- The Editorial Board has three options: accept manuscripts for external review, return it to author for revision, or reject it. A manuscript not accepted by a board member is blindly reviewed by another board member. If not accepted by both, the manuscript is rejected by the Editorial Board. Decision will be made by the suggestion of a third Editorial Board member if the decisions of first two do not concur.
- Once accepted for external review, the Editorial Board identifies one (for brief communication, case reports, and teaching articles) or two (for original articles) reviewers with appropriate expertise. The reviewers will be asked to review and return manuscripts with their comments online within two weeks of their receipt. Reviewers have four options; accept, accept with major revision, accept with minor revision, or reject.
- A Manuscript accepted subject revision as suggested by reviewers will be returned to the corresponding author. Author(s) will be given four weeks to respond to reviewers' comments, make necessary changes, and return the manuscript to the Editorial Board. A Manuscript not returned within the specified time will be considered withdrawn by the author(s).
- Manuscripts with minor revisions will be cleared by the Editorial Board and accepted for publication. Those with major revisions will be returned to external reviewers and follow the procedures as outlined for the initial review.

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