

Factors associated with the occurrence of hypertension and dyslipidemia among diabetic patients attending the diabetes clinic of Ayder Comprehensive Specialized Hospital, Mekelle, Ethiopia

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Abstract

Background: The burden of diabetes mellitus is becoming a serious health issue in Ethiopia. Co-occurrence of hypertension and dyslipidemia with diabetes mellitus is responsible for increased morbidity and mortality among diabetes mellitus patients. However, there is paucity of data on the factors associated with the burden of hypertension and dyslipidemia among diabetes patients in Ethiopia.

Objective: Investigate the factors associated with the occurrence of hypertension and dyslipidemia among diabetic patients attending the diabetes clinic of Ayder Comprehensive Specialized Hospital, Mekelle, Ethiopia.

Methods: The study was conducted on patients with diabetes mellitus attending the diabetes clinic of Ayder Comprehensive Specialized Hospital, Mekelle, Ethiopia. A retrospective hospital based record review was conducted to collect data from patient cards who had follow up in the diabetic clinic of Ayder Comprehensive Specialized Hospital. Among the relevant data reviewed were anthropometric measurements such as height and weight, body mass index, laboratory estimation of triglycerides, total cholesterol and fasting blood sugar and blood pressure measurements. Bivariate and multivariable logistic regression was used for data analysis using SPSS version 16 software. Statistical significance was set at $P < 0.05$.

Results: Among 421 patients, 136(32.3%) had hypertension and eighty-nine (65.4%) of them were on antihypertensive medications. Prevalence of dyslipidemia was 50(11.9%). Thirty-seven (8.8%) of the diabetic patients were on both anti-hypertensive and lipid lowering agents. Regardless of the frequent follow up, the fasting blood glucose level of the diabetic patients remained uncontrolled. Hypertriglyceridemia was more common in diabetic patients than increased total cholesterol. Increased age ((AOR 41 – 50 years = 2.0, 95%CI: 1.07, 3.98; (AOR 51 – 60 years = 2.3, 95%CI: 1.2, 4.5)), BMI ((AOR overweight = 2.9, 95%CI: 1.2, 6.99; (AOR obese = 6.6, 95%CI: 1.8, 24.7)) and high triglyceride level (AOR 4.2, 95%CI: 2.6, 6.8) were found to have an impact on the coexistence of hypertension and diabetes mellitus. While only duration of diabetes (AOR 0.3, 95%CI: 0.14, 0.88) was associated with the coexistence of dyslipidemia and diabetes mellitus.

Conclusion and recommendation: Raised blood pressure and dyslipidemia were common occurrences among diabetic patients from Ayder Comprehensive Specialized Hospital, Mekelle. Hypertension and dyslipidemia should be given due attention in diabetes mellitus patients, especially in old patients, obese patients and those who have high triglyceride levels. Thus, diabetic patients should be treated based on the blood glucose, blood pressure and lipid control goals. Having regular follow up alone has no practical significance and hence diabetes programs should be integrated with hypertension and dyslipidemia programs.

Key words: Diabetes mellitus, hypertension, dyslipidemia, diabetes clinic, Ethiopia.

Background

According to the WHO projection, diabetes mellitus is ranked as the seventh leading cause of death by 2030 [1]. Recent estimates indicated that 387 million people in the world have diabetes mellitus. Among these, 46.3% are undiagnosed and 77% live in low and middle income countries. In 2014, an estimated 4.9 million deaths were due to diabetes and 80% were in low and middle income countries [1, 2]. In Africa, 22 million people were estimated to be living with diabetes in 2014 with a prevalence rate of 5.1% and 62.5% of them were undiagnosed [1]. According to the estimates of the World Health Organization (WHO) and International Diabetes Federation (IDF), the prevalence of diabetes in Ethiopia was 2.0% in 2011 and 2.9% in 2015, respectively [1, 3].

Non communicable diseases (NCD) account for 30% of deaths in Ethiopia and diabetes accounts for about 2.6% of these deaths in Ethiopia [1]. Raised blood pressure and obesity are among the major risk factors that lead to the development of NCDs. These risk factors are on the rise in Ethiopia. A national prevalence of raised blood pressure, overweight and obesity was estimated to be 35.2%, 7.4%, and 1.1%, respectively [4, 5]. The United Kingdom Prospective Study (UKPDS) group showed that lowering blood pressure decreases the incidence of cardiovascular complications and decrease the severity of micro vascular complications of diabetes mellitus with risk reductions ranging from 24% to 56% [6]. Despite the differences in disease prevalence by sex and by racial/ethnic group, the positive associations between BMI and hypertension,

diabetes mellitus and dyslipidemia are consistent [7].

Studies have showed that hypertension and dyslipidemia are diseases that coexist with diabetes mellitus [8 – 11]. The prevalence rates of hypertension and obesity in patients with T2DM are high throughout the world. Controlling hypertension and reducing obesity in these patients is important to limit the morbidity and costs for the health care systems derived from diabetic complications [12]. However, there is a paucity of data on the co-occurrence of hypertension and dyslipidemia among diabetic patients in Ethiopia. Thus, this study was conducted to investigate the factors associated with the occurrence of hypertension and dyslipidemia in diabetes mellitus patients attending the diabetes clinic of Ayder Comprehensive Specialized Hospital in Mekelle City, Ethiopia.

Methods

Study setting: The study was conducted in Ayder Comprehensive Specialized Hospital, Mekelle, Ethiopia, which is the biggest tertiary hospital in Tigray region, with catchment area of 8 million population. It is the second largest hospital in the nation and has 500 inpatient beds in the four major departments and other specialty units. Internal medicine is one of the major departments with various specialties such as cardiac, gastrointestinal, pulmonary, renal, and diabetes clinics. The diabetes clinic which was established in 2010 GC is giving service for more than 2000 patients under follow up. For better service provision, two nurses are trained on 1) screening acute and chronic complications of diabetes mellitus (DM), 2) the techniques of retinal screening

and grading, and 3) diabetic education on how to inject insulin, diet recommendation and exercise duration. Patients on follow up are seen by a senior, resident or general practitioner twice per week. The diabetic clinic is equipped with materials such as retinal camera, fund scope and monofilaments that are used to screen for chronic complications.

Study design: A retrospective cross sectional record review was conducted to collect data from patient cards who had follow up in the diabetic clinic.

Source population: All follow up diabetic patients attending the diabetes clinic of Ayder Comprehensive Specialized Hospital in Mekelle City, Ethiopia.

Study population: Patients visiting the diabetes clinic of Ayder Comprehensive Specialized Hospital between February 2015 and April 2015.

Exclusion criteria: Patients below 30 years of age, with follow up for less than a year, blood pressure measurement in less than 50% of their visit and with no lipid profile measurements were excluded from the study.

Sample size and sampling methods: The sample size was estimated using a formula for estimation of single population proportion with the assumption of 95% confidence level, margin of error of 5% and a prevalence of 50% for maximum sample size as empirical evidences for the occurrence of raised blood pressure and dyslipidemia among diabetic patients was not known in the study communities. To compensate the non-response rate, 10% of

the estimated sample was added up to make the total sample of 422 diabetes mellitus patients. All follow up diabetic patients who visited the diabetes clinic of Ayder Comprehensive Specialized Hospital for three consecutive months (February through April 2015) were selected and their cards reviewed for history of diabetes mellitus, blood pressure measurements and lipid profile results.

Data collection: The cards of the patients above the age of 30 were collected using the Medical Record Numbers from a log book found in the diabetes mellitus clinic of the Hospital and were searched from the card room using the SMART CARE software. Data were collected by nurses in the diabetic clinic. The nurses were trained on how to fill the data extraction formats by the principal investigator prior to the start of data collection. All the cards with complete information were reviewed for history of diabetes mellitus, blood pressure measurements and lipid profile results by the principal investigator and nurses in the diabetic clinic. This was written in a pre-tested checklist (data extraction format) prepared for data collection.

Operational definitions

- BMI- $<18.5\text{kg/m}^2$ (underweight), $18.5\text{-}24.9\text{kg/m}^2$ (normal weight), $25\text{-}29.9\text{kg/m}^2$ (over weight) and $\geq 30\text{kg/m}^2$ (obese).
- Hypertension- when the highest blood pressure measured is $\geq 140/90\text{mmHg}$ or if patient is already on antihypertensive medications.

- Isolated systolic hypertension- when the systolic hypertension is above 140mmHg while the diastolic blood pressure is below 90mmHg.
- LDL- <100mg/dl (optimal), 100-129mg/dl (near optimal), 130-159mg/dl (borderline high), 160-189mg/dl (high) and \geq 190mg/dl (very high).
- HDL- <40mg/dl (low), 40-59mg/dl (border line high) and \geq 60mg/dl (high).
- Triglyceride- <150mg/dl (normal), 150-199mg/dl (border line high) and \geq 200mg/dl (high).
- Total cholesterol- <200mg/dl (desirable), 200-239mg/dl (border line high), \geq 240mg/dl (high).
- Dyslipidemia- when triglyceride becomes \geq 200mg/dl and total cholesterol \geq 240mg/dl.

Data management and analysis: Data collected were cleaned, edited, coded, categorized and entered into a computer using Epi-DATA version 3.1 and analyzed using SPSS version 16 software. An outlier analysis was carried out. Descriptive statistics like frequencies and percentages was used to describe categorical variables. Mean with standard deviation was used to describe parametric continuous variables. Bivariate and multivariable logistic regression was used to analyze the data and determine the independent predictors of hypertension and dyslipidemia among diabetic patients. Variables that showed

significant association with hypertension and dyslipidemia in the bivariate analyses were entered into the multivariable logistic regression model. Variables with p-value less than 0.05 were declared as statistically significant predictors. Adjusted Odds Ratio with its 95% confidence interval was used to interpret independent effect of predictors on raised blood pressure and dyslipidemia among diabetic patients.

Ethical considerations: Ethical clearance was obtained from the Institutional Review Board of the College of Health Sciences of Mekelle University. Confidentiality regarding patient and institutional information was maintained. Each patient record was coded. Similarly, patient name and other personnel identifiers were not recorded on the checklist.

Results

Socio-demographic characteristics of the study subjects

A total of 421 cards were selected for analysis after data cleaning. Above half, 247 (58.7%) were males and among 416 who had written addresses, more than three fourth, 331 (79.6%) were urban dwellers. Above one fourth of the patients, 120 (28.5%) were in the age range of 41 to 50 years. The mean age was 49.9 with a standard deviation of 11.4. The minimum age was 30 and the maximum 85. Almost six of ten patients, (249/59.1%) have their BMI in the normal range (18.5-24.9). The mean BMI of the diabetic patients was 22.8 with a standard deviation of 3.7. Only 19 (4.5%) patients have their BMI in the obesity range, $>30 \text{ Kg/m}^2$ (Table 1).

Table 1: Socio demographic characteristics of DM patients in Ayder Comprehensive Specialized Hospital, Mekelle, Ethiopia, 2015 (n = 422).

Variable		Frequency	Percentile
Sex	Male	247	58.7
	Female	174	41.3
Address (n = 416)	Urban	331	79.6
	Rural	85	20.4
Age, years	30-40	115	27.3
	41-50	120	28.5
	51-60	103	24.5
	>60	83	19.7
BMI, Kg/m ²	<18.5	59	14
	18.5-24.9	249	59.1
	25-29.9	94	22.3
	>=30	19	4.5

Clinical characteristics of patients

In 209(49.6%) of the patients, the duration of diabetes ranged from 1 to 3 years with a mean duration of illness of 2.85years with a standard deviation of 1year. Majority of the patients 297(70.5%) had their follow up visit every month. Hundred ninety-nine (47.3%) patients were on insulin therapy while 172(40.9%) were on combined Glibenclamide and metformin therapy. The mean duration of follow up in Ayder Comprehensive Specialized Hospital for more than one third of the patients 149(35.4%) was 2.62 years and standard deviation of 1.03 years. Two hundred twenty-one (53.5%) patients had uncontrolled diabetes (FBS above 130), which was assessed by the recent fasting blood sugar that they had during the last visit to the diabetic clinic. Eighty-nine

(65.4%) of the hypertensive patients are on antihypertensive medications. Among them majority 65(73.03%) are on enalapril. Eighty-eight (20.9%) patients are on lipid lowering agents and majority of them are 80(90.91%) on simvastatin. 37(8.8%) of patients are on both anti-hypertensive and lipid lowering agents (Table 2).

The lowest systolic blood pressure (BP) of 414(98.3%) was below 139mmHg with mean Bp of 106.9 ± 12.7 mmHg while the highest in 264(62.7%) was below 139mmHg with mean Bp of 129.97 ± 15.23 mmHg. More than 50% of patients are found to have their blood pressures in the normal range. Majority of the patients 416(98.8%) had their lowest diastolic blood pressure below 89mmHg with mean Bp of 69.57 ± 8 mmHg while 331(78.6%) had their highest BP

below 89mmHg with mean of 80.77 ± 7.73 mmHg.

Table 2: Clinical history and medication of patients with diabetes mellitus in Ayder Comprehensive Specialized Hospital, Mekelle, Ethiopia, 2015 (n = 421).

Variable		Frequency	Percentile (%)
Duration of illness	1-3 years	209	49.6
	3-5 years	110	26.1
	5-10 years	60	14.3
	>10 years	42	10
Frequency of follow up	Every month	297	70.5
	Every 2 months	103	24.5
	Every 3 months	3	0.7
	Irregular	18	4.3
Medication	Glibenclamide	7	1.7
	Metformine	36	8.6
	Insulin	199	47.3
	Glibenclamide and metformine	172	40.9
	Metformine and insulin	7	1.7
Duration of follow up	1 year	62	14.7
	2 years	149	35.4
	3 years	97	23
	More than 3 years	113	26.8
Hemoglobin A1C	<7	39	31.2
	>7.1	86	68.8
Recent range of FBS (mg/dl)	<79	16	3.9
	80-130	176	42.6
	>130	221	53.5
Antihypertensive medications used	Enalapril	65	73.03
	Hydrochlorothiazide(HCT)	7	7.87
	HCT and enalapril	15	16.85
	Enalapril and amlodipine	2	2.25
Lipid lowering agents used	Simvastatin	80	90.91
	Atorvastatin	7	7.95
	Lovastatin	1	1.14

Nearly a third, 136(32.3%), of the patients had blood pressure $\geq 140/90$. In this study,

only 25 of the 421 patients had measured their LDL and HDL values, but 416 patients

had triglyceride and total cholesterol values. Close to three quarter (71.4%) of the patients had lowest triglyceride below 150mg/dl with mean of 136.09 ± 84 while around half (51%) of the patients had highest triglyceride below 150mg/dl with mean of 193.69 ± 142.6 mg/dl. Three hundred

forty-four (86.6%) patients had their lowest total cholesterol below 200mg/dl with a mean of 150.7 ± 52.23 mg/dl. While around two third (63%) of patients had their highest total cholesterol below 200mg/dl with mean of 189.2 ± 54.99 mg/dl (Table 3).

Table 3: FBS, lipid profile and systolic and diastolic blood pressure readings of patients with diabetes mellitus in Ayder Comprehensive Specialized Hospital, Mekelle, Ethiopia, 2015 (n = 421).

Variable		Lowest		Highest	
		Frequency	%	Frequency	%
Range of FBS in the first year, mg/dl	<79	36	8.6	2	0.5
	80-130	245	58.2	23	5.5
	>130	140	33.3	396	94.1
Range of FBS in thesecond year. mg/dl	<79	34	9.1	2	0.5
	80-130	239	64.1	36	9.7
	>130	100	26.8	335	89.8
Range of FBS in thethird year, mg/dl	<79	21	9.9	0	0
	80-130	138	65.1	22	10.4
	>130	53	25	190	89.6
Range of FBS in thefourth year, mg/dl	<79	9	6.7	1	0.7
	80-130	94	69.6	11	8.1
	>130	32	23.7	123	91.1
Systolic, mmHg	<139	414	98.3	264	62.7
	>140	7	1.7	157	37.3
Diastolic, mmHg	<89	416	98.8	331	78.6
	>90	5	1.2	90	21.4
Triglyceride, mg/dl	<150	212	51	282	71.4
	150-199	80	19.2	52	13.2
	>200	124	29.8	61	15.4
Total cholesterol, mg/dl	<200	262	63	344	86.6
	200-239	88	21.2	36	9.1
	>240	66	15.9	17	4.3

Factors associated with coexistence of hypertension and dyslipidemia in diabetic patients

Age, BMI and triglyceride levels had significant association with the coexistence of hypertension and diabetes mellitus. Participants at the age range of 41-50 years were at 2-fold increased risk of developing hypertension (AOR 2.0, 95%CI: 1.07, 3.98) than those at the age range of 30-40 years. While those at the age range of 51-60 years were at 2.3-fold increased risk of developing hypertension (AOR 2.3, 95%CI: 1.2, 4.5) compared to those at the age range of 30-40 years. Over weight and obese participants had a 2.9 (AOR 2.9, 95%CI: 1.2, 6.99) and 6.6 (AOR 6.6, 95%CI: 1.8, 24.7) -fold increased risk of developing hypertension compared with individuals who have their BMIs in the normal range. High triglyceride, that is, above 201 mg/dl was associated with a 4.2-fold increased risk of developing hypertension (AOR 4.2, 95%CI: 2.6, 6.8). Duration of diabetes was found to have significant association with coexistence of dyslipidemia and diabetes mellitus. Participants with duration of diabetes 3-5 years were found to have a 70% decreased risk of developing dyslipidemia (AOR 0.3, 95%CI: 0.14, 0.88) than those with duration of diabetes 1-3 years (Table 4).

Table 4: Bivariate and multivariable analysis results of the factors associated with the coexistence of hypertension and dyslipidemia among diabetic patients from Ayder Comprehensive Specialized Hospital, Mekelle, Ethiopia.

Variable		Hypertension		COR (95% CI)	AOR (95% CI)	Dyslipidemia		COR (95% CI)	AOR(95% CI)
		Yes	No			Yes	No		
Age	30-40	22	93	1	1	5	110	1	1
	41-50	44	76	2.4(1.3,4.4)	2.0(1.07,3.98)	17	103	0.3(0.10, 1.01)	0.4(0.15,1.53)
	51-60	45	58	3.2(1.8,6.0)	2.3(1.2,4.5)	18	85	1.2(0.52,2.78)	1.2(0.54,3.06)
	>60	25	58	1.8(0.9,3.5)	1.2(0.6,2.6)	10	73	1.5(0.67,3.55)	1.5(0.66,3.68)
Address	Urban	119	212	1	1	44	287	1	1
	Rural	17	68	2.2(1.2,3.9)	0.7(0.39,1.48)	6	79	2(0.83,4.90)	1.3(0.5,3.5)
BMI, Kg/m ²	<18.5	10	49	1	1	2	57	1	1
	18.5-24.9	66	183	0.07(0.02,0.24)	1.2(0.56,2.8)	27	222	0.18(0.02,1.21)	0.3(0.04,2.40)
	25-29.9	46	48	0.12(0.04,0.37)	2.9(1.2,6.99)	18	76	0.6(0.17,2.37)	0.8(0.21,3.04)
	>=30	14	5	0.34(0.11,1.02)	6.6(1.8,24.7)	3	16	1.26(0.33,4.80)	1.3(0.34,5.26)
Triglyceride, mg/dl	<= 200	65	234	1	1	--	--	--	--
	>= 201	71	51	0.2(0.12,0.31)	4.2(2.6,6.8)	--	--	--	--
Duration of diabetes, years	1-3	--	--	--	--	17	192	1	1
	3-5	--	--	--	--	12	98	0.28(0.11,0.67)	0.3(0.14,0.88)
	5-10	--	--	--	--	11	49	0.39(0.15,0.99)	0.4(0.16,1.14)
	>10	--	--	--	--	10	32	0.71(0.27,1.88)	0.7(0.28,2.13)

Discussion

Age, BMI and triglyceride levels had significant association with the coexistence of hypertension and diabetes mellitus. Similarly, duration of diabetes was found to have significant association with coexistence of dyslipidemia and diabetes mellitus. One third of the diabetic patients had hypertension while only 4.5 % patients were obese. Only 32.3% of diabetic patients had hypertension in our study while it was 60% in a study done in an outpatient unit of the university of Nigeria teaching hospital, 66.7% in a study done in a semi urban Cameroonian population, 26.1% in a study done in Sidamo zone Ethiopia and 19.6% in the general population of Ethiopia [13 – 15]. The lowest prevalence of hypertension in diabetic patients in our study could be due to decreased prevalence of risk factors (obesity and dyslipidemia) that are common for both hypertension and DM. About 34.5% of patients who had hypertension in our study were not initiated in anti-hypertensive medications which were higher than the study done in Nigeria (12.3%). Nearly 85% of the patients who were on antihypertensive agents had controlled hypertension (systolic blood pressure of <130) which was quite higher than the Nigeria's study which was only 12%. Enalapril, an ACE inhibitor, was the most frequently prescribed drug similar to the study done in Nigeria. Despite the well controlled hypertension, the patients had poor glycemic control.

Age, one of the non-modifiable risk factor for non communicable diseases, was a

significant risk factor for hypertension among diabetes patients in line with various studies reported elsewhere [16 – 18]. The working age people (41 – 60 years) were more likely to develop hypertension and hence are more likely to become disabled and die prematurely as a result of their disease. The premature death and disability of the emerging middle class from the coexistence of these non communicable diseases saps the country's labor supply and diminishes workforce productivity [19]. This makes it harder for Ethiopia to capitalize on the demographic dividend that would otherwise occur from having a larger proportion of young working-age people relative to developed countries.

Increased BMI was associated with hypertension in the diabetes patients. Overweight/obesity, which increases plasma volume and cardiac output, not only causes high blood pressure, but increases the risk of cardiovascular disease in adults [20]. The significant association of BMI with hypertension among diabetic patients could be due to adaptation of sedentary life style, altered food habits and obesity [21]. Hence, apart from the medications, maintaining healthy body weight through physical activity and dietary changes to lower excess energy intake can help reduce blood pressure in people with diabetes [22, 23].

Elevated triglycerides were significantly associated with the coexistence of hypertension and diabetes mellitus in this study and other studies elsewhere [24 – 26].

Thus, dyslipidemia should be aggressively treated in patients with diabetes as dyslipidemia associated with hypertension and diabetes further increases the risk of cardiovascular diseases [27]. A study from Japan indicated that Japanese patients with diabetes and dyslipidemia were placed at 3.7-fold risk of ischemic heart disease (IHD) compared to those with normal lipid profiles [28]. An increase in physical activity and decrease in body fat are known to improve lipid profiles [22, 23]. Thus, reduced physical activity and poor dietary practices could be the reasons for the elevated triglycerides in the diabetes patients.

In this study, those with 3-5 years duration of diabetes mellitus were at a 70% decreased risk of developing dyslipidemia than those with duration of diabetes 1-3 years. But, in a study done in India, the prevalence of dyslipidemia was found to be highest in patients with 5-10 years duration of diabetes mellitus [29]. This could be because some of the patients (21%) with increased duration of diabetes are put on statins as primary prophylaxis.

Our study has some limitations. It is a retrospective and hospital based study and has included patients who had follow up in a single diabetes mellitus clinic. So a community based study should be done to confirm the results of this study. The nature of the study design which is a retrospective chart review restricted our study not to explore the association of acute and chronic complications with the triad. Failure to record sufficient information of patient

diagnosis on the charts was the major hindrance for not capturing complete picture of the cases.

Conclusion

Nearly a third of the diabetic patients had hypertension (32.3%) and 12% had dyslipidemia suggesting that raised blood pressure and dyslipidemia were common occurrences among diabetic patients from Ayder Comprehensive Specialized Hospital, Mekelle. Regardless of the frequent follow up, the fasting blood glucose level of the diabetic patients remained uncontrolled. Hypertriglyceridemia was more common in diabetic patients than increased total cholesterol. Increased age, BMI and high triglyceride level were associated with the coexistence of hypertension and diabetes mellitus and duration of diabetes was associated with the coexistence of dyslipidemia and diabetes mellitus among diabetic patients from Ayder Comprehensive Specialized Hospital, Mekelle, Ethiopia. Thus, health care professionals need to be concerned about the increase in weight of their patients because of the well-established relationships between excess body weight and the co-occurrence of the serious medical conditions such as diabetes, hypertension and dyslipidemia which are the major risk factors for cardiovascular diseases.

Recommendations

Hypertension and dyslipidemia should be given due attention in diabetes mellitus patients, especially in old patients, obese patients and those who have high triglyceride levels. Thus, diabetic patients

should be treated based on the blood glucose, blood pressure and lipid control goals. Having regular follow up alone has no practical significance and hence diabetes programs should be integrated with hypertension and dyslipidemia programs.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contributions

MAA, TG, HT and MA were involved in designing the manuscript. MAA was involved in data collection, analysis and interpretation of the data. HT and MA were involved in reviewing the analysis and interpretation and gave advices on how to develop the manuscript. MAA and TG have involved in developing the manuscript. Finally, all authors have approved the manuscript.

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