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Assessments of Urine Parameters in Diabetes Mellitus Patients in Jimma University Specialized Hospital, South West, Ethiopia, 2018

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Abstract

Background: Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes mellitus is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Long-term complications of diabetes cases are prominently associated with kidney abnormality which is so for change in urine parameters.

Objective: To assess alteration of urine parameters in DM patients attending in Jimma University specialized hospital, Southwest, Ethiopia, from Feb. 10 to mar 10,2018.

Method: Prospective cross-sectional study was conducted at Jimma University Specialized Hospital from February10 to March 10, 2018.The study subject was diabetic mellitus patients, who were selected by simple random sampling technique. The socio-demographic and some clinical characteristics of the study subjects were collected by using structured questionnaire and checklist respectively. Urine analysis was done to assess the physical, chemical and microscopic alteration of urine parameters. Data was analyzed using SPSS version 20. Frequencies and cross tabulations was used to summarize descriptive statistics. Bivariate and multivariate logistic regression analysis was used to see the association between variables. A p-value<0.05 was considered as statistically significant.

Result: A total of 275 Diabetic Mellitus patients were enrolled in this study of these, 150(54.5%) were females. Urine chemical test among the participant revealed positive for Protein 40(14.54%), ketone 100(36.35%), bilirubin 64 (23.14%), leukocyte esterase 155(56.38%), and glucose 188 (68.4%). It was found that DBP and SBP were significantly associated with a positive urine protein. Sex and type of DM were significantly associated with a positive urine ketone. Moreover; age, hypertensive and blood glucose were significantly associated with urine glucose. WBC, epithelial cell, RBC, and crystals were major microscopic findings for DM patients.

Conclusion and Recommendation: The finding showed relatively a higher proportion of DM subjects had altered urine parameters, which indicated a periodic assessment of urine parameters in the routine follow-up of DM patients is mandatory.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1].

The type of diabetes is based on the presumed etiology. There are two most common types of diabetes: type 1 and type 2 diabetes. In type 1 diabetes, the body does not produce insulin, and daily insulin injections are required. Type 2 diabetes is the result of failure to produce sufficient insulin and insulin resistance [2].

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues.

Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia [1].

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-kenotic hyperosmolar syndrome [1].

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with

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diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes [1].

Diabetes mellitus (DM) is the most frequent cause of chronic kidney failure in both developed and developing countries [3].

Diabetic nephropathy is a clinical syndrome characterized by albuminuria (>300 mg/day) confirmed on at least two occasions 3-6 months apart, permanent and irreversible decrease in glomerular filtration rate (GFR), and arterial hypertension [4].

The amount of glucose found in the urine is dependent not only on the glucose concentration in the blood, but also on the renal function. Urine testing for glycosuria is an important screening tool for DM. Glucose is usually detected when the renal threshold for blood glucose is exceeded (180 mg/dL; 10mmol/L) [5].

The presence of increased amounts of protein in the urine can be an important indicator of renal disease. It may be the first sign of a serious problem and may appear long before other clinical symptoms. There are, however, physiologic conditions such as exercise and fever that can lead to increased protein excretion in the urine in the absence of renal disease. There are also some renal disorders in which proteinuria are absent. In the normal kidney, only a small amount of low Molecular weight protein is filtered at the glomerulus [6].

Chronic non-communicable diseases have emerged as the next twenty-first century global epidemic and have already become the leading causes of death and disability worldwide [7].Among these, the global burden of diabetes mellitus (DM) is immense.

The world is facing an epidemic of diabetes mellitus [8]. Currently, more than 250 million people in the world have diabetes and it is predicted that this number will double in a little over 20 years [9, 10]. The epidemic is not evenly distributed around the world. While the world-wide prevalence of diabetes is 3-4%, several countries and regions experience a prevalence rate of diabetes of well over 10%. This includes some countries in the Middle East, where in some cases, the prevalence of diabetes among middle aged adults exceed 16% [11].

In 2012 there were 1.5 million deaths worldwide directly caused by diabetes? It was the eighth leading cause of death among both sexes and the fifth leading cause of death in women in 2012 [12]. In 2013, DM-related complications were a major cause of disability and reduced quality of life, and an estimated 5 million people aged 20–79 years worldwide died prematurely from the disease [13].

Epidemiological studies have shown that about 1/3rd of type 2 diabetics and every other type 1 diabetic patient is likely to develop sight threatening retinopathy within their life time [14].

The burden of diabetes and diabetic related mortality and disability are rising in Africa. Increasing sedentary lifestyle, coupled with rapidly growing urban culture and modified diets, are predicted to triple the prevalence of diabetes mellitus in the next 25 years [15].

Ethiopia is the second most populous country in sub Saharan Africa where more than 80% of the population lives in the countryside. The country experiences a heavy burden of disease mainly attributed to communicable infectious diseases and nutritional deficiencies. Currently, it is also challenged by the growing magnitude of chronic non communicable diseases. The estimated prevalence of DM in adult population of Ethiopia is 1.9%

Prevalence of diabetic nephropathy in Ethiopia, from the studies of diabetic Complications, is 15.7% to 29.5%. Fifteen percent of DM patients also develop diabetic nephropathy in Black lion referral hospital. From the estimations above, if the prevalence of diabetic nephropathy continues like this, there were 270,000 cases of diabetic nephropathy in Ethiopia.

Ethiopia is one of the top five countries with the highest number of people affected by DM in Sub-Saharan African courtiers. The prevalence of DM in Ethiopia was 2.5% in the year 2000 and was estimated to rise to 3.5% by 2030. So the impact of checking urine parameter in the diagnosis management of DM will be immense.

Methods and Materials

Study area and seating

This study was conducted at Jimma University medical center (JUMC) that is one of the oldest public hospitals in Ethiopia. It was established in 1937 by Italian invaders for the service of their soldiers. It is located in Jimma city 352 km southwest of Addis Ababa. After the withdrawal of the colonial occupants, it has been governed under the Ethiopian government by the name of Ras Desta Damtew Hospital and later Jimma Hospital during Dergue regime and currently renamed as Jimma University Medical Center. It is teaching and referral hospital in the southwestern part of the country, providing the services for approximately 18,000 inpatients, 160,000 outpatients including 11,000 emergency cases and 4500 deliveries in a year coming to the hospital from the catchment population of about 15 million peoples. The hospital has many chronic follow-up clinics for both pediatric and adult patients. The diabetes clinic runs twice weekly (Monday and Tuesday) and provides integrated diabetic care for more than 2500 both type-1 and type-2 diabetic patients. Cognizant of the fast growing service and teaching role of the hospital, the federal government considered construction of a new and level best 860 bedded hospital which is currently functional as of teaching referral hospital.

Data collection method and instrumentation

Questionnaire based interview: For this study we used structured questionnaire to collect data related to socio demographic characteristics, genetic predisposition, Racial/ethnic background and type of DM. The data related to these variables was collected by well trained Nurses at chronic illness clinic in Jimma University Medical center by face to face interview.

Checklist based review of clinical profile: For this study we used checklist to collect information about the type of diabetes mellitus and other related clinical profile.

Laboratory analysis

After a brief explanation, the study participants were asked for their consent to be interviewed by clinical nurses to give fasting blood sample. Then five ml of blood was withdrawn by laboratory professionals from the study participants, who had fasted overnight (10-12 hours), for laboratory analysis. In addition, we collected 12 ml urine with clean, dry and leak proof urine cap .The sample is kept at room temperature. First we take the physical examination results by using optically clear test tubes then using the same test tube we check for the chemical parameters by using Healthy Wiser Urinalysis Reagent Strips, by immersing the reagent strip for brief time in the urine specimen and compering the color on the strip to the color chart on the reagent strip container. Then the positivity or negativity of the specimen for specific urinary chemical parameter was determined depending up on

the color chart on the reagent strip container. The instructions that came with the strips indicating how to wait to correctly for the color change was diagnostic, and was strictly followed. For the microscopic examination we first open the urine cap and well-mixed the sample of urine (12ml) in test tube was centrifuged at 1000 rpm for 3minutes and the supernatant was poured off then a well A well-mixed sediment of urine was used for microscopic examination under High and low power field .

Data analysis

Data was cleaned, edited, checked for completeness and entered to EPI info version 3.5.3 and then transferred to SPSS version 20 statistical package for analysis. Bivariate and multivariate logistic regression was used to see the association between dependent variables and independent variables. A p-value< 0.05 was considered as statistically significant.

Data quality management

To ensure the reliability and validity of the study, the following activity was done just before and during performing the procedures.

For the interview we translated the questionnaire the local language and we also used data collectors who are able to speak the local language.

Prior to the use of the questionnaire, it was checked for its completeness and we also under take for checking the completeness and consistency.

We also gave two days training for data collectors on how to collect using both the questionnaire and checklist.

To assure the quality of the data from laboratory analysis standard operating procedures (SOPs) was strictly followed during specimen collection and other laboratory procedures.

All reagents strip kits was checked for their expiry date and used according to the manufacturer's instructions.

Training/orientation was given for specimen collector to apply standard operational diagnostic procedures to ensure the quality of each test.

The data collection, application of standard procedure, accuracy of test results was supervised by principal investigator.

Result

Socio-demographic characteristics of the study participants

From the total of 275 DM patients who attend JUS Hospital during the study period, from those 150(54.5%) were female and 125(45.45%) were male participants. The highest number of participants, 160 (58.1%), belong to the age group >49 years old while the lowest number. From the total of study participant 238 (86.5%) were urban dwellers and the rest 37(13.45%) were lives in rural area. One hundred eighty one (66%) were married and 38(13.9%) participant were widowed. Concerning educational status 138(50.2%) of them were higher education. Most of the participants 110(40%) were governmental. and 113(41.1%) had <1000 monthly income (Table 1).

Urine physical parameter of study participants

Among the physical finding it was common to find, 124(45.1%) Dark yellow in color, 146(53.1%) sweetie fruit in its Odor, 113(41.2%) Turbid in transparency (Table 2).

Table 1: Socio-demographic characteristics of diabetes mellitus patients attending at Jimma University specialized hospital, Southwest Ethiopia, 2018.

Characteristics		Number	Percentage (%)
Age	18-49	115	49%
	>49	160	58.10%
Sex	Male	125	45.45%
	Female	150	54.50%
Marital status	Single	20	7.30%
	Married	181	66%
	Divorced	36	13%
	Widowed	38	13.90%
Religion	Orthodox	70	25.45%
	Muslim	165	60%
	Protestant	25	9.10%
	Catholic	15	5.45%
Residence	Urbane	238	86.50%
	Rural	37	13.45%
Occupation	Governmental	110	40%
	Non-governmental	34	12%
	Hose wife	67	24.40%
	Private business	44	16.40%
	Other	20	7.30%
Ethnicity	Oromo	198	72%
	Amhara	14	5.10%
	Tigray	7	2.54%
	Wolayita	30	11%
	Other	26	9.45%
Educational status	No education	78	28.30%
	primary education	41	14.90%
	Secondary education	18	6.54%
	Higher education	138	50.20%
Monthly income	<1000	113	41.10%
	1001-2000	65	23.60%
	2001-3000	37	13.50%
	3001-4000	31	11.30%
	>4000	29	10.50%

Table 2: The prevalence of urine physical parameters of diabetes mellitus patients attending at Jimma University specialized hospital, Southwest Ethiopia, 2018.

No.	Physical characteristics'		Number	Percentage (%)
1	Color	Colorless	43	15.63%
		Dark yellow	124	45.10%
		Clear red	77	28%
		Cloudy red	31	11.30%
2	Transparency	Clear	20	7.30%
		Hazy	44	16%
		Cloudy	98	36%
		Turbid	113	41.20%
3	Foam	Dark	136	49.45%
		Beer	139	50.54%
4	Odor	Sweetie fruity	146	53.10%
		Aromatic	20	7.30%
		Pungent smile	109	40%

Urine chemical parameter of study participants

Out of 275,188(68.4%) were positive for urine glucose, 40(14.54%) for protein, 155(56.38%) for leukocyte esterase, 100(36.35%) for ketone and 64(23.94%) for bilirubin (Table 3).

Microscopic result of study participant

Among the microscopic finding it was common to find, 125(45.45%)

Table 3: The prevalence of urine chemical parameters of diabetes mellitus patients attending at Jimma University specialized hospital, Southwest Ethiopia, 2018.

Variable		Protein		Glucose		Ketone	
		P %	N %	P %	N %	N %	P%
Age	≤49	10(3.6)	95(34.5)	97(35.3)	23(8.4)	61(22.9)	52(19)
	>49	30(10.9)	140(51)	91(33.1)	64(23.3)	39(14.9)	123(44.7)
Sex	Male	15(5.5)	109(39.6)	99(36)	29(10.54)	84(30.5)	24(8.7)
	Female	25(9.1)	126(45.8)	89(32.36)	58(21.1)	16(58.9)	151(57.1)
TDM	Type 1	10(3.6%)	110(40)	60(21.8)	18(6.54)	82(29.8)	37(13.5)
	Type 2	30(10.9%)	125(45.5)	128(46.54)	69(25.1)	18(6.5)	138(50.9)
FHDM	Yes	17(6.2)	135(49.1)	24(8.72)	28(10.9)	42(15.3)	21(7.6)
	No	23(8.4)	100(36.4)	164(59.6)	59(21.45)	58(21.1)	154(56)
ALC	Yes	13(4.7)	59(21.5)	31(11.27)	34(12.4)	9(3.4)	19(6.9)
	No	27(9.8)	176(64)	157(57.1)	53(19.3)	91(33.1)	156(56.7)
HT	Present	15(5.5)	54(19.6)	93(33.8)	21(7.6)	22(8)	78(28.4)
	Absent	25(9.1)	181(65.8)	95(34.54)	66(24)	78(28.4)	97(35.3)
SBP	<140	31(11.3)	45(16.4)	147(53.5)	71(25.8)	87(31.6)	105(38.2
	≥140	9(3.27)	190(69.1)	41(15)	16(5.8)	13(4.7)	70(25.5)
DBP	<90	21(7.6)	172(62.5)	138(50.9)	65(23.6)	94(34.2)	111(40.4)
	≥90	19(6.9)	63(22.9)	50(18.9)	22(8)	6(2.2)	64(23.3)
BG	≤130	22(8)	100(36.4)	74(27)	19(69.1)	14(5.1)	59(21.5)
	>130	18(6.5)	135(49.1)	114(41.5)	68(24.7)	86(31.3)	116(42.2)
BMI	<25	5(1.8)	159(57.8)	117(42.5)	55(20)	93(33.8)	44(16)
	25-29.9	20(7.3)	47(17.1)	53(19.3)	18(6.54)	5(1.8)	70(25.5)
	≥30	15(5.5)	29(10.5)	18(6.54)	14(50.9)	2(0.3)	61922.2)

Table 4: Microscopic result of diabetes mellitus patients attending at Jimma University Medical Center, Southwest Ethiopia, 2018.

Variable		Bilirubin		Urobilinogen		Leucocytes esterase		Nitrite	
		P %	N %	P %	N %	P%	N%	P%	N%
Age	≤49	25(9.1)	169(61.5)	34(12.4)	98(35.3)	64(23.3)	53(19.3)	32(11.6)	124(45.1
	>49	39(14.2)	42(15.3)	45(16.4)	48(17.5)	91(33.1)	67(24.4)	43(15.6)	76(27.6)
Sex	Male	23(8.4)	153(55.6	29(10.5)	117(42.5	47(17.1)	81(29.5)	53(19.3)	67(24.4)
	Female	41(14.9)	58(21.1)	50(18.2)	29(10.5)	108(39.3	39(14.2)	22(8)	133(48.4
TDM	Type 1	19(6.9)	120(43.6	33(12)	88(32)	28(10.2)	56(20.4)	34(12.4)	122(44.4
	Type 2	45916.4)	91(33.1)	46(16.7)	68(24.7)	127(46.2	64(23.3)	41(14.9)	78(28.4)
FHDM	Yes	21(7.6)	134(48.7	21(7.6)	98(35.6)	18(6.5)	31(11.3)	23(8.4)	145(52.3
	No	43(15.6)	77(28)	58(21.1)	48(17.5)	137(49.8	99(36)	52(18.9)	55(20)
ALC	Yes	41(14.9)	89(32.4)	18(6.5)	56(20.4)	11(4)	101(36.7	36(13.1)	110(40)
	No	23(8.4)	122(44.4	61(22.2)	90(32.7)	144(52.4	19(6.9)	39(14.2)	90(32.7)
HT	Present	17(6.2)	130(47.3	34(12.4)	59(21.5)	59(21.5)	42(15.3)	27(9.8)	132(48)
	Absent	47(17.1)	81(29.5)	45(16.4)	87(31.6)	96(34.9)	78(28.4)	48(17.5)	68(24.7)
SBP	<140	43(15.6)	107(40)	52(19)	109(38.5	88(32)	47(17.1)	19(6.9)	145(52.3
	≥140	21(7.6)	104(37.8	27(9.8)	37(13.5)	67(24.4)	73(26.5)	56(20.4	55(20)
DBP	<90	18(6.5)	139(50.5	23(8.4)	99(36)	97(35.3)	91(33.1)	57(20.3)	80(29.1)
	≥90	46(16.7)	72(26.2)	56(20.4)	47(17.1)	58(21.1)	29(10.5)	18(6.5)	120(43.6
BG	≤130	39(14.2)	100(36.4	34(12.4)	63(22.9)	44(16)	56(20.4)	33(12)	79(28.7)
	>130	25(9.1)	111(40.4	45(16.4)	83(30.2)	111(40.4	64(23.3)	42(15.3)	121(44)
BMI	5	23(8.4)	32(11.4)	47(17.1)	30(11)	76(27.6	66(24	42(15.3)	27(9.8)
	-29.9	22(8)	80(29.1)	26(9.5)	45(16.4)	60(21.8	42(15.3	21(7.6)	64(23.3)
	≥30	19(6.9)	99(36)	6(2.2)	71(25.8)	19(6.9	12(4.4	12(4.4)	109(39.6)

for epithelial cells/ LPF followed by116 (42.2%) WBC/HPF, 98(35.7%) few RBC/HPF (Table 4).

Association of Urine parameters with factors

It was found that DBP and SBP were significantly associated with a positive urine protein. Moreover, sex, HT and type of DM were found to be statistically with high significance association with a positive urine ketone(p≤,0.001≤respectively). Sex, SBP and DBP were significantly associated with positive leukocyte esterase among the study participants (p≤0.001, P=0.004, and P=0.019) respectively. Furthermore; age, hypertensive, ALC and blood glucose were significantly associated with urine glucose.

Discussion

Diabetes is a chronic disease significantly affecting the quality of life of many people. With the rising number of DM patients worldwide, it has become necessary to institute measures to prevent diabetes mellitus, improve on existing treatment modalities and also to diagnose, control and treat complications of DM. Screening of DM patients for urine parameters is an important tool in the management of patients with

DM. Management of DM is dynamic. With better understanding of the pathogenesis of DM, there have been many changes in the treatment of the disease.

The expanding field of inquiry into DM is likely to be the source of future break-through in treatment. Urine physical and chemical parameter will also play an enormous part in this regard. In this study alteration of urine parameters in DM patients have been evaluated. In this study, 40(14.54%) participants had urine proteinuria which was somehow consistent with a study conducted in India (9.4%), (25) Denmark (13.8%)(23).

On the other hand, the current study was much lower than a study conducted in Bahrain, UAE, and Oman (42.5%, 34.5%, and 29%), respectively(22) and in Shakiso, Ethiopia (30.8%)(27) among type 2 DM patients. The difference might be the difference in study design, lifestyle, socioeconomic status or environmental/genetic factors.

The prevalence of proteinuria was higher in type 2 DM 30 (10.9%) than in type1 DM 10(3.6%)patients in the current study. This might be due to type 2 DM patients are more likely do have hypertensive, obesity, and advanced age which commonly associated with proteinuria.

Moreover, our result demonstrated proteinuria has a significant association with hypertensive DBP (12%) (P-value=0.002). The presence of proteinuria suggests kidney disease which is a common complication of DM and requires further evaluation.

Another finding of the current study showed, 188(68.3%) had urine positive for glucose among the participants. the current study was higher than a study conducted in Japan for Urine Glucose Screening Program at Schools in Japan to detect Children with Diabetes and Its Outcome-Incidence and Clinical Characteristics of Childhood Type 2 Diabetes was Approximately 30– 60% of children who show positive test for urine glucose.

The result demonstrated that urine glucose was significantly associated with hypertension, alcoholic consumption, age and sex .for further cooperation I can't get research done to compare with prevalence of glycosuria.

And also Another finding of the current study showed, 100(36.35%) had urine positive for ketone among the participants which was somehow consistent with a study conducted in selected hospitals in Addis Ababa for Clinical Characteristics of Diabetic Ketoacidosis in Children with Newly Diagnosed Type 1 Diabetes was (35.8%) presented with DKA at first diagnosis of diabetes.

The prevalence of ketone was higher in type 1 DM 69(25.1%) than in type2 DM 18(6.54%) patients in the current study. this is due to type 1 diabetes have lost the ability to produce any insulin, so ketone can occur when insulin doses are missed, or when the body's insulin requirements rise due to stress or illness, Moreover, sex, HT and type of DM were significantly associated with a positive urine ketone. Another point in this study was that when I compare the urine physical parameter with the chemical parameters of DM patients, Turbidity or cloudiness of DM patient's urine be caused by excessive cellular material or protein in the urine of DM Patients. the presence of bacteria, red or white blood cells in DM patients urine is caused by Cloudy urine of the DM patients.

Conclusion and Recommendation

In this study, a higher proportion of DM subjects had altered urine parameters those Altered urine chemical test among the participant was protein 14(14.54%), ketone 100 (36.35%), leukocyte esterase 155(56.36%), and glucose 188(68.4%). It was found that DBP and SBP were significantly associated with a positive urine protein. Sex, HT and type of DM were significantly associated with a positive urine ketone. Moreover; age, hypertensive and blood glucose were significantly associated with urine glucose. WBC, epithelial cell, casts, and crystals were major microscopic findings. By recognizing the above the following recommendation was given:-

There should be periodic assessment of urine parameters in the routine follow-up of DM patients

Kidney function tests should be performed to assess DM related complication

Further longitudinal studies should be done to identify determinant factors

Early diagnosis, the improvement of care and constant monitoring of patients should be the focus of primary prevention.

Availability of Data and Materials

All the data and the materials we used in this research will be available up on request any legal concerned body at any time.

Declaration

Ethical consideration

Ethical clearance was obtained from the school of CBE/graduate and undergraduate research coordinating office of Jimma University health science faculty and laboratory result attached or written on patient's card and results of samples seen in laboratory was recorded in code given with ID number. For patients, I was make clear explanation as I keep secretly and on the benefits of the study and therefore, informed consent was sought from the study subject. But we have also made sure that patient name has no relation with research laboratory results.

Consent for Publication

All of the study participants in this study have gave their consent after we have clearly explain the aim of the study, about the procedure, the benefit , incentive if any and about the confidentiality and their right to refuse or withdraw from the study any time they like .

Conflict of Interest

The author(s) have no competing interests for financial support, publication of this research, patents and royalties through this collaborative research. All authors were equally involved in discussed research work. There is no financial conflict with the subject matter discussed in the manuscript

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Author's Contribution

Akililu Getachew, Ermias Yonas were involved in conception and design, and acquisition of data. Akililu Getachew took the lead in data generation, analysis and drafting the manuscript. Ermias Yonas revised the draft manuscript critically for important intellectual content. All authors were involved in analysis and interpretation of the data, as well

as final approval of the version to be published.

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