



Screening and Association between Diabetes Mellitus and Abo Blood Grouping in Amaoba Ikwuano and Amaba Ugwueke Abia State Nigeria

A. N. C. Amadi^{1*}, P. A. C. Ndukwe¹ and P. E. Agbai¹

¹*Department of Zoology and Environment Biology, College of Natural Sciences, Michael Okpara Univeristy of Agriculture, Umudike, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Author ANCA designed the study, performed the statistical analysis, and wrote the protocol ad the first draft of the manuscript. Author PACN managed the analysis while author PEA managed the literature review. All authors read and approved the final manuscript.

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ABSTRACT

Diabetes Mellitus is a genetic disorder with a high degree of inheritance and the importance of human blood group in human blood transmission is well established. Studies to establish the relationship between blood group and diabetes are varied. This study was to screen and determine the association between diabetes mellitus and ABO blood grouping in the study areas. Accu-check glucometer strip and anti-sera were used for the study. Out of the 209 samples from urban and rural areas, males were 120 (48.8%) and females 107 (51.20%). Majority of the subjects male and female (39.70% and 58.6%) from the urban area and male and female (35.29% and 24.44%) from the rural area are O⁺ respectively, followed by A+ male and female (29.41% and 29.03%) from the urban area and male and female (5.88% and 35.55%) from rural area respectively. Then B+, male and female (10.29% and 9.67%) from urban area also male and female (11.76% and 28.88%) from rural area. More males and females (52.94%, 35.56%) in the rural area were Diabetic than those

*Corresponding author: E-mail: amadi.anthonia@mouau.edu.ng;

in the urban area (38.24%, 24.19%) respectively this is statistically significant ($p < .05$). majority of the males and females with blood group O+ (28.57%, 77.78%) from the urban area and (40.00%, 43.75%) from the rural area were Diabetic, followed by those with blood group A+ (42.86%, 22.22%) from the urban area and (10.00%, 37.59%) for the rural area. There is statistical difference between the occurrence of Diabetes Mellitus among the various blood groups ($p < .05$). More subjects that were overweight male and female (67.85%, 61.11%) from the urban area and (60.00%, 50.00%) from rural area have Diabetes Mellitus. Questionnaire analysis shows that the causes of diabetes mellitus in the urban area were identified by most (31.6%) respondents to be high consumption of starchy food followed by high consumption of sugar (6.5%) while the rural area majority (56.6%) of the respondents indicated high consumption of sugar followed by witchcraft and the least (16.5%) had no idea about the cause of the ailment. Management of the disease in urban area was identified to be adhering to diet (28.3%) followed by adhering to medication (28.3%) then routine screening (25%) than the least was no idea (3.30%) while most (56.60%) of the respondents from the rural area have no idea on what are the management practices, than adhering to diet (20.60%) and exercise (2.60%) had the least response. In the treatment of the disease in both urban and rural area, majority (74.62%) of the respondents agreed that diabetes mellitus is best treated in the hospital while most (41.77) in the rural area believe in visiting the chemist respectively, However some in both urban and urban area still patronize herbal houses for treatment. There is therefore the need for proper public health enlightenment and education of the populace for regular screening test and management practices to immunize and avert the risk of complications that many arise.

Keywords: Screening diabetes mellitus; ABO blood grouping; relationship management; treatment.

1. INTRODUCTION

The major human blood grouping system is ABO. ABO blood grouping system is the only system in which antibodies are consistently and predictably present in the serum of normal individuals whose red cells lack the antigen [1]. The second type of blood group is the rhesus system, there are two phenotypes rhesus (+) and rhesus (-) depending on whether rhesus antigen is present on the red cell or not. Determination of ABO blood groups is done by detecting A and B antigen. In addition, known red cells are used to detect anti- A and B in the serum by the process called reverse grouping. Since their discovery by Landsteiner in 1900 [2], many researchers have attempt to determine the significance of particular ABO phenotype for susceptibility to diseases. Many reports have appeared in recent years suggesting an association between blood groups and diabetes mellitus its susceptibility to a number of diseases has been linked with ABO phenotype [3], but such correlation remains controversial [4;5]. Individuals with blood group O have about 25% less factor in their plasma. [6]. It is well established that low levels of these factors, which are proteins involved in the blood clotting, are the cause of excess bleeding and therefore may also increase the risk of both arterial (Ischaemic heart disease) and venous (Thromboembolic disease) problem [7,8] both

have a relationship with Hypocholesterolemia, which in turn has a relationship with diabetes mellitus and hypertension and explains why blood group O individuals are affected by this conditions than other blood groups [9,10], with adcreasing trend from group A, B to AB. An individual ABO blood group can also be a risk factor in coagulation effect. It has been reported that O individuals are proportionately more prone to bleeding than individuals of other blood groups, and A individuals with the highest incidence of thrombosis than in any other group [11]. Okon et al. [12] reported that it is likely that O- and A+ individuals are more susceptible to Diabetes mellitus. Detection of a positive relationship with ABO and rhesus blood groups may reflect increased susceptibility to and a negative relationship defense against diabetes mellitus. ABO and rhesus gene phenotype vary widely across races and geographical boundaries [13]. Previous studies have been comprehensively investigated the association between ABO and rhesus Blood group and diabetes mellitus in various countries. The frequency of blood group A among diabetic patients in Nepal [14] and increased frequency of blood group B among diabetic patients in Iraq [15], Qatar [16], Italy [17], India [18]. Rhesus negative blood group is more frequent in diabetic patients in Pakistan [19].

2. MATERIALS AND METHODS

2.1 Study Area and Research Design

The study was conducted in Amaoba in Ikwuano Amaba Ugwueke in Bende Local Government Area Abia State.

The design adopted for this research was a cross sectional, population based study comprised of 209 subjects. It tries to determine the incidence of diabetes mellitus using an accu-chek glucometre, and ABO blood grouping using slide agglutination method in Amaoba Ikwuano and Amaba Ugwueke both in Abia State.

2.2 Instrument and Procedures for Data Collection

The instrument used for data collection in this study was a data collection schedule sheet which attempted to elicit information on diabetes mellitus, and ABO blood groups, weight and height of the subjects present, a questionnaire was designed to know people's level of knowledge on diabetes mellitus in the study areas. Questionnaire was used to elicit information on respondent personal data; that is their age, sex, level of education, occupation, signs and symptoms, if they have been screened before and how they think the disease could be managed.

2.3 Ethical Approval

Ethical permission was obtained from the ethical committee of the department of zoology and environmental Biology, Michael Okpara University of Agriculture, Umudike, Abia State Nigeria.

Permission was also obtained from the village of ugweke and Amoeba on the agreement that the subject anonymity must be maintained and that every finding would be treated with utmost confidentiality Advocacy call was made to enlighten the people on the importance of the study after which consent of the subjects were sought and received before commencement of the study.

2.4 Collection of Specimen and Blood Glucose Measurement

Specimen was collected using the following: Accu-chek glucometre and test strip, soft clix lancet, anti-sera (A, B and D), cotton wool,

methylated spirit, syringe, sample bottles containing ethylene diamine tetra acetic acid (EDTA), tourniquet, glove, flat grouping tile and drop pipette.

The screening tests were carried out as and recorded.

The blood glucose level was measured using accu-chek glucometer by fasting blood sugar (FBS) method. An accu-chek glucometre with a measuring range 100-600 mg/dl (0.6-33.3 mmol/L). accu-chek active test strip was inserted into the glucometer with the appropriate code chip and the glucometer automatically turned on. A cotton wool was used to apply methylated spirit on the individual's fingertip (usually the third finger) to sterilize the area. Soft clix lancing device was prepared for pricking the fingertip and a small drop of blood was applied on the middle of the green colored square on the test strip after a dropping sign was displayed on the glucometer. The glucometer measured and displayed the level of glucose in the individual's blood which was recorded. The values obtained were classified according to WHO standards. A result of 60-100mg/dl shows normal sugar level, 100-126 shows pre-diabetes while 126 and above is diabetic according to WHO [20,21].

2.5 Determination of Blood Group

2ml of blood samples were taken intravenously using a syringe and a needle into a sample bottles containing ethylene diamine tetra acetic acid (EDTA). A drop pipette was used to collect the blood samples and dropped on the grouping tile in three places. The anti-sera (A, B and D) were dropped respectively on the three blood samples, another drop pipette was used to stir and slide at the same time. After some seconds, the samples were examined for agglutination. The blood samples on the tile were observed for agglutination or clumping and results recorded.

2.6 Statistical Analysis

Data was computed statistically using chi-square and simple percentage. chi-square was applied to know the association of blood groups to Diabetes Mellitus. 'p' value <0.05 was considered statistical significant.

3. RESULTS

Adults in the rural area are more (45.57%) diabetes than adults in the urban area (35.58%) (Table 2).

Majority of the subjects male (39.70%) and female (58.06%) from the urban area male (35.29%), female (24.44%) from the rural area are O⁺ followed by A⁺, male (29.41%), female (29.03%) in the urban area and male (5.88%), female (35.55%) in rural area (Table 3).

Most (34.62%) of the respondents in the urban area are Civil servants followed by students (31.52%) then traders (17.39%) self employed (10.86%) and the least are farmers (5.43%). While in the rural majority are farmer (83.15%) followed by traders (8.42%) the least are civil servants (7.5%) Table 4.

Incidence of fasting blood sugar according to sex in the study area shows that male (38.24%) are more diabetic in rural area than in the urban area also female in the rural area are more (52.94%) diabetic that those in the urban area (Tables 5 and 6).

But incidence of the disease revealed that in the Urban area, under blood group O⁺, males with diabetes mellitus were significantly higher than non-diabetic males (46.86% vs. 28.57%; $p < 0.05$). For patients with blood group A⁺ and B⁻, diabetic males were significantly lower (15.62% vs. 42.86%; $p < 0.05$ and 0.00% vs. 10.71%; $p < 0.05$) as compared to non-diabetic males respectively. While blood group B⁺ has significantly more males with *Diabetes mellitus* than non-diabetic male. Blood groups O⁻, A⁻, AB⁺ and AB⁻ were not significantly different ($p > 0.05$).

For females in urban areas, only blood groups O⁺ (48.27% vs. 77.77%) and B⁺ (17.24% vs. 0.00%) showed significant differences ($p < 0.05$) when compared with diabetic and non-diabetic females.

Results obtained in rural areas, showed that blood groups O⁺, O⁻, A⁺ and A⁻ had less diabetic males which was significantly higher than in non-diabetic males. While blood groups B⁺, B⁻, AB⁺ and AB⁻ had more diabetic males when compared to non-diabetic males (Table 7 and 8).

Incidence of Body Mass Index in male and female with diabetes mellitus in the study area show that majority of those with diabetes mellitus are overweight and obese, Male (67.85%) and female (61.11%) in the urban area and male (60.00%) and female (50.00%) in the rural area are overweight. Others male (14.29%) and female (16.61%) in the urban area and male

(10.00%) and female (25.00%) in the rural area are obese (Table 9).

On causes of diabetes mellitus, most (31.62%) respondents from the urban area believe that consumption of starchy food, followed by high consumption of sugar (27.90%), hereditary (13.95%) and witchcraft (33.19%) followed by high consumption of sugar (29.46%) hereditary (20.74%) no idea (16.59%). Most (61.53%) in the urban area and (42.01%) in the rural area indicated that frequent urination, sugar ants around urine (37.27%) weakness (30.76%) are the prevalent symptoms others (3.84%) from the urban area and (11.83%) rural area have no idea of the symptoms of the disease.

In management of the disease while majority of the people in the urban area believe that adhering to diet would help in the disease management. Most (56.66%) in the rural area have no idea about management practices of diabetes mellitus.

In both urban and rural areas majority have not been diagnosed of the disease 64.62% and 54.43% respectively.

Method of treatment varies, while majority (74.62%) of the people in the urban area prefer visiting the hospital, most respondents in the rural prefer the chemist for their medication, followed by herbal (19.23%) in the urban area, and rural area (22.77%). Table 10.

4. DISCUSSION

Many diabetics are unaware of their diabetic status until they have reached complication stage [22]. This may account for the many incidental cases in the study area as most (64.62% and 54.43%) in urban and rural areas have not been previously diagnosed of the disease although majority (92.3% and 62.03%) subjects in the urban and rural areas aware of the disease.

It was observed in the study that more males (41.30% and 58.83%) in the urban and rural are diabetic than the females (29.03% and 35.20%). This findings disagreed with the work of [23] the result could be attributed to the fact that men tend to distribute fat more readily around the liver and other body organs unlike the women who deposit fat under the skin and the hip. And also alcohol intake and dietary habit can influence the way they gain weight and can equally make them insensitive to insulin [24].

This study also showed that diabetes mellitus was higher in rural area than urban area. This may be as a result of high consumption of starch food and sugar in the rural area due to ignorance and low educational and economic background as most are present farmers and could not adhere to diet and provide balance diet.

Majority of male and female in both rural and urban are with blood group O and A have diabetes mellitus and this is in agreement with [12] who observed that diabetes mellitus occur in all blood group O and A as both rhesus positive and Negative are affected.

More subjects that are overweight and obese have diabetes than those with normal weight in this study, however, Zagar et al. [25,26] observed that relatively lean adults with a lower Body Mass Index (BMI) may be at equal risk to diabetes mellitus as those obese.

Respondents in the urban area attributed the cause of diabetes mellitus to be consumption of starchy food whereas in the rural area high consumption of sugar was most significant to the causes of diabetes mellitus which could be as a result of the meaning of the disease name in the dialect of the people in the rural area studied is called "Oria Suga" meaning sickness caused by high consumption of sugar, although [27] observed that diabetes mellitus has no clear cut causes.

In both urban and rural area, respondents attributed frequent urination as the most significant symptoms of diabetes mellitus.

This is in agreement with Okonkwo [28] and Amadi and Nwokolo [29] observed symptoms for diabetes mellitus which include; frequent urination, excessive thirst, blurred vision.

A good number of respondents in the urban area have knowledge of diabetes mellitus as majority of them have been previously diagnosed of the diseases as compared to those from rural area, this could be as a result of disproportionate

allocation of health recourse between urban and rural area which may lead to poor diabetes mellitus screening and preventive services [30].

The respondents in the urban area believed that adhering to prescribed diet was the best management procedure for diabetes mellitus. This is in agreement with Kazeem [31] dietary management of diabetes mellitus. Majority of the respondents in the rural area have no idea of the management of diabetes mellitus this could be as a result of their little or no knowledge of the disease and ignorance.

Majority (41.77%) of the respondents from the rural area receive their treatment from the chemist, those from the urban (74.62%) have theirs from the hospital. Still some from the urban and rural area (19.23% and 22.77%) respectively use herbs for their treatment.

This study also revealed that there is no association between a particular ABO blood group to the susceptibility of diabetes mellitus but the incidence of the disease showed that in both urban and rural areas, under blood group O+ and B+, males and females with diabetes mellitus were significantly higher than non-diabetic males and females. While some other study reveal that diabetes mellitus is more common individuals with blood group B compared with non-diabetic patients. Government and relevant agencies should emphasis on screening exercise at least once a year to dictate the ailment on time for better treatment and management. Drugs and screening kits should be made available in homes, health centres and hospitals and a subsidized rate for effective management practices.

Table 1. Overall prevalence according to sex in the study

Sex	Frequency (N)	Percent
Male	102	48.8
Female	107	51.20
Total	209	100

Table 2. Overall prevalence of diabetes mellitus in the study area according to sex and location

Urban			Rural		
Sex	Frequency	No of prevalence(%)	Sex	Frequency	No of prevalence(%)
Male	68	28(41.3)	Male	34	20(58.83)
Female	62	18(29.03)	Female	45	16(35.20)
Total	130	46(35.38)		79	36(45.57)

Table 3. Distribution of ABO blood grouping according to sex in the study area

BG	Urban area						Rural area					
	N/E	%	M	%	F	%	T	%	M	%	F	%
O+	63	48.46	27	39.70	36	58.06	23	29.11	12	35.29	11	24.44
O-	4	3.07	4	5.88	0	0.00	8	10.12	8	23.52	0	0.00
A+	38	29.23	20	29.41	18	29.03	18	22.78	2	5.88	16	35.55
A-	4	3.07	3	4.41	1	1.61	3	3.79	2	5.88	1	2.22
B+	13	10.00	7	10.29	6	9.67	17	21.51	4	11.76	13	28.88
B-	4	3.07	4	5.88	0	0.00	2	2.53	2	5.88	0	0.00
AB+	3	2.30	2	2.94	1	1.61	5	6.39	1	2.94	4	8.88
AB-	1	0.76	1	1.47	0	0.00	3	3.79	3	8.82	0	0.00
TOTAL	130	100	68	100	62	100	79	100	34	100	45	100

Key: BG= Blood Group, N/E = Number Examined, M = Male, F= Female, %= Percentage.

Table 4. Distribution of respondents based on occupational status

Occupational status	Urban area		Rural area	
	Frequency (n)	Percent	Frequency (n)	Percent
Civil servant	45	34.62	6	7.59
Students	41	31.52	0	0.00
Farmers	7	5.43	66	83.15
Self-employed	14	10.86	0	0.00
Trader	23	17.39	7	8.42
Total	130	100	79	100

Table 5. Levels of fasting blood sugar (FBS) in male and female with diabetes mellitus in the study area

FBS interval	Urban area				Rural area			
	Male freq.	Percent	Female freq.	Percent	Male freq	Percent	Female freq	Percent
< 60	8	11.76	15	24.19	2	5.88	2	4.44
60-89	25	36.76	29	46.77	7	20.58	9	20.00
90-100	2	2.94	0	0.00	0	0.00	6	13.33
101-120	5	7.35	0	0.00	5	14.70	9	20.00
121-126	2	2.94	3	4.83	2	5.88	2	4.44
127-150	5	7.35	2	3.22	8	23.52	10	22.22
151-180	3	4.41	4	6.45	3	8.82	1	2.22
181-250	14	20.58	4	6.45	4	11.76	6	13.33
251-300	4	5.88	4	6.45	2	5.88	0	0.00
301-400	0	0.00	1	1.61	1	2.94	0	0.00
TOTAL	68	100	62	100	34	100	45	100

Hypoglycaemia < 60mg/dl, Normal 60-100mg/dl, Pre-diabetes =100-120mg/dl, Diabetes = >126mg/dl

Table 6. Incidence fasting blood sugar and sex in the study area

Variables	Urban Area		Rural Area	
	Male Frequency (%)	Female Frequency (%)	Male Frequency (%)	Female Frequency (%)
Hypoglycemia < 60 mg/dl	8 (11.76)	15 (24.19)	2 (5.88)	2 (4.44)
Normal 60<100mg/dl	27 (39.71)	29 (46.77)	7 (20.59)	15 (33.33)
Pre diabetes 100<120mg/dl	67 (8.82)	3 (4.84)	7 (20.59)	11 (24.44)
Diabetes >120mg/dl	26 (38.24)	15 (24.19)	18 (52.94)	16 (35.56)
Total	68 (100)	62 (100)	34 (100)	45 (100)

Table 7. Level of fasting blood sugar (FBS) in male and female in relation to ABO blood grouping

Blood group	Male						Female							
	Non-DM	Percent	No. of DM	Percent	p-value	No. of HPG	Percent	Non-DM	Percent	No. of DM	Percent	P-value	No. of HPG	Percent
Urban area														
O+		46.86	8	28.57	0.04	2	25.00	14	48.27	14	77.77	0.01	9	60.00
O-	2	6.25	2	7.14	0.81	1	12.50	0	0.00	0	0.00	-	0	0.00
A+	5	15.62	12	42.86	0.00	3	37.50	9	31.03	4	22.22	0.23	4	26.66
A-	2	6.25	2	7.14	0.81	0	0.00	1	3.44	0	0.00	0.06	0	0.00
B+	6	18.75	1	3.57	0.00	0	0.00	5	17.24	0	0.00	0.00	1	6.66
B-	0	0.00	3	10.71	0.00	1	12.50	0	0.00	0	0.00	-	0	0.00
AB+	1	3.13	0	0.00	0.08	1	12.50	0	0.00	0	0.00	-	1	6.66
AB-	1	3.13	0	0.00	0.08	0	0.00	0	0.00	0	0.00	-	0	0.00
Total	32	100	28	100		8	100	29	100	18	100		15	100
Rural area														
O+	2	16.67	8	40.00	0.00	0	0.00	6	26.09	7	43.75	0.03	2	33.33
O-	0	0.00	6	30.00	0.00	0	0.00	0	0.00	0	0.00	-	0	0.00
A+	0	0.00	2	10.00	0.00	0	0.00	7	30.43	6	37.50	0.39	0	0.00
A-	0	0.00	2	10.00	0.00	0	0.00	0	0.00	0	0.00	-	2	33.33
B+	3	25.00	1	5.00	0.00	0	0.00	8	34.78	3	18.75	0.03	0	0.00
B-	3	25.00	1	5.00	0.00	0	0.00	0	0.00	0	0.00	-	0	0.00
AB+	3	25.00	0	0.00	0.00	0	0.00	2	8.70	0	0.00	0.00	2	33.33
AB-	1	12.50	0	0.00	0.00	2	100	0	0.00	0	0.00	-	0	0.00
Total	12	100	20	100		2	100	23	100	16	100		6	100

KEY: DM= Diabetes mellitus, HPG= Hypoglycaemia, BG= Blood group

Table 8. Incidence of diabetes mellitus in male and female in relation to ABO blood group

Variables	Urban Area		Rural Area	
	Male	Female	Male	Female
	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)
0 +	8 (28.57)	14 (77.78)	8 (40.00)	7 (43.75)
0 -	2 (7.43)	0 (0.00)	6 (30.00)	0 (0.00)
A +	12 (42.86)	4 (22.22)	2 (10.00)	6 (37.50)
A -	2 (7.14)	0 (0.00)	2 (10.00)	0 (0.00)
B +	1 (3.57)	0 (0.00)	1 (5.00)	3 (18.75)
B -	3 (10.71)	0 (0.00)	0 (0.00)	0 (0.00)
AB +	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
AB -	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Total	28(100)	18 (100)	20 (100)	16 (100)

Table 9. Incidence of body mass index (BMI) in male and female with diabetes mellitus (DM)

BMI Internal	Urban Area		Rural Area	
	Male	Female	Male	Female
	No. of DM (%)	No. of DM (%)	No. of DM (%)	No. of DM (%)
18.5 – 25.0 kg/m ² Normal	5 (17.86)	4 (22.22)	6 (30.00)	4 (25.00)
25.0 – 29.0 kg/m ² Overweight	19 (67.85)	11 (61.11)	12 (60.00)	8 (50.00)
Above 30 kg/m ² Obsess	4 (14.29)	3 (16.61)	2 (10.00)	4 (25.00)
Total	28 (100)	18 (100)	20 (100)	16 (100)

Table 10. Knowledge, attitude and management practices of diabetes mellitus

Diabetes	Urban area frequency (n)	Percent (%)	Rural area frequency (n)	Percent (%)
Causes				
Witchcraft	0	0.00	23	29.46
High consumption of sugar	36	27.90	26	33.19
Consumption of starchy food	41	31.62	0	0.00
Lack of exercise	11	8.37	0	0.00
Hereditary	18	13.95	17	20.74
High alcohol intake	9	6.51	0	0.00
Overweight	9	6.97	0	0.00
No idea	6	4.65	13	16.59
Total	130	100	79	100
Signs and symptoms				
Frequent urination	80	61.53	33	42.01
Sugar ant around the urine	5	3.84	30	37.27
Weakness	40	30.76	7	8.87
No idea	5	3.84	9	11.83
Total	130	100	79	100
Management				
Exercise	22	17.93	2	2.66
Medication	34	26.89	5	6.66
Routine screening	32	25.86	11	13.33
Adhering to diet	38	29.31	16	20.66
No idea	4	3.44	45	56.66
Total	130	100	79	100
Aware				
YES	120	92.31	30	37.97
NO	10	7.69	49	62.03
TOTAL	130	100	79	100
Previously diagnosed				
Yes	46	35.38	36	45.57
No	84	64.62	43	54.43
Total	130	100	79	100
Method of treatment				
Chemist	5	3.85	33	41.77
Hospital	97	74.62	10	12.66
Herbal	25	19.23	18	22.77
Self-medication	3	2.31	10	12.66
No idea	0	0.00	8	10.13
Total	130	100	79	100

5. CONCLUSION

Diabetes Mellitus is one of the major burning issues worldwide. It varies from location, sex, nutritional habits and genetical issues. Several assessed causes are known but majority of the reasons behind the disease are unknown. Proper awareness and empowerment programs are needed for precaution and prevention of the disease. Health awareness campaigns are to be organized especially in the rural areas where the disease is more prevalent due to lack of

knowledge and proper health education and facilities.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee

has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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