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Epidemiology of Diabetes Mellitus and Molecular Characterization of Bacterial Isolates Recovered from Cases with Asymptomatic Bacteriauria in Trinidad and Tobago

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Authors' contributions

This work was carried out in collaboration between all authors. Author PEA designed the study, wrote the protocol, coordinated the study and wrote the first draft of the manuscript. Authors PEA, AJV and OAO coordinated data collection, performed laboratory and data analysis. Author PJ managed the molecular analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To highlight the observed features including socio-demographic, economic and biochemical characteristics seen among uncontrolled diabetic adults that should be areas of concern or focus by healthcare providers during the management of diabetes in the country. Also to perform molecular characterization of bacterial organisms prevalent among a cross section of diabetic patients with asymptomatic bacteriauria.
Study Design: This was a cross sectional prospective and descriptive study.

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Place and Duration of Study: Study was carried out among patients attending two non-communicable chronic diseases health centers in Trinidad & Tobago over a 6 months period in 2012.

Methodology: Following informed consent, diabetic volunteers were recruited to participate in the study. Participants fulfilled study criteria that included absence of urinary symptoms, not catheterized, no history of UTI or any form of uropathy. Blood samples were screened for Hb1Ac, serum electrolytes and urea values; urine for microscopy, culture and sensitivity. Enterobacteriaceae isolates from urine culture were subjected to screening for CTX-M, TEM, and SHV by amplification of gene fragments by conventional PCR and for KPC, and NDM and OXA48 targets by real-time PCR using Sybergreen melting curve analysis.

Results: Four hundred and fourteen diabetics were surveyed. Significant (15.7%; 65/414) bacteriauria was noted in sixty five subjects. Majority, 81.5% (53/65) with positive urine cultures had high HBA1c values. *Escherichia coli* 48.57% (34/70) and *Klebsiella pneumonia* 25.7% (18/70) were the most recovered organisms, with 87.1% (61/70) from urine samples and 75.4% (49/65) from female subjects. Urine samples from males 24.6% (16/65) yielded mostly *Staphylococcus epidermidis* 14.3% (10/7) and *Staphylococcus aureus* 5.7% (4/70) respectively. All Enterobacteriaceae isolates were negative for KPC, NDM and OXA-48. Although the blaTEM and bla SHV were detected in both the *E. coli* and *K. pneumoniae* isolates as expected.

Conclusion: *Escherichia coli* was the prevalent Enterobacteriaceae among the patients with asymptomatic bacteriauria. Poor diabetic control is significantly and strongly associated with bacteriauria that was more prevalent among female diabetics. Although none of the antimicrobial resistant targets were encountered among the Enterobacteriaceae, there is still the need to keep an eye on these targets and diabetic subjects in the country.

Keywords: Asymptomatic bacteriauria; enterobacteriaceae; diabetes mellitus; Trinidad & Tobago; PCR; TEM; SHV; NDM.

1. INTRODUCTION

Diabetes mellitus is now an epidemic disease in the Caribbean and in Trinidad & Tobago like in several other places, it is a priority and very important public health issue because it affects several individuals resulting in huge economic losses [1]. Its complications, mainly vascular, make it a leading cause of heart attacks, kidney failure, strokes or cerebrovascular accidents, blindness and amputation [2-4]. Trinidad & Tobago is an heterogeneous society in terms of her culture, ethnicity, religion and socioeconomic status, with just a population of about 1.3 million people of which more than 150,000 suffer from diabetes [5].

According to a report in Trinidad & Tobago, over 60% of all deaths are due to chronic non communicable diseases (CNCDs) such as cardiovascular diseases, cancer, stroke and diabetes [6]. And within the Caribbean region, Trinidad and Tobago has the highest overall percentage of deaths due to CNCDs. This report by the Ministry of Health shows diabetes prevalence rate to be approximately 15%; is more common in females than in males and more prevalent in persons of East Indian origin [6].

World Health Organization (WHO) and International Diabetes Federation (IDF) Diabetes Atlas report reveals that the prevalence of diabetes in Trinidad & Tobago is between 100,000 to 150,000. In Trinidad & Tobago, reports or data on prevalence of diabetes is disseminated by the Ministry of Health based on information received from Central Statistical Office (CSO). Such information may not be accurate because such rates do not include data from the private health institutions and for those from the government or public health institutions, the data may be duplicated or omitted. The IDF reports that as of 2012, over 371 million people have diabetes. The number of people with diabetes is increasing in every country. Half of people with diabetes are undiagnosed; 4.8 million people died due to diabetes and more than 471 billion USD were spent on healthcare for diabetes [7].

Studies by previous researchers in the country have shown that there are differences or variations seen in the prevalence of diabetes among several age groups and ethnicities in the country [8-10]. Also in the country other workers have reported that poor glycaemic control is particularly worse at the primary care setting [11, 12]. And increased prevalence of cardiovascular

disease risk factors which are associated with poor glycaemic control among diabetic patients has equally been described in the country [13]. None of these studies have highlighted specific features, risk or relative factors associated with or seen prevalent among these diabetic patients whose glucose blood levels were out of control.

This study was therefore carried out to highlight the observed characteristic features including the socio-demographic, economic and biochemical characteristics seen among uncontrolled blood sugar diabetic adults that should be areas of concern or focus by healthcare providers during the management of diabetes in the country. It also sought to perform molecular characterization of bacterial organisms prevalent among a cross section of diabetic patients with asymptomatic bacteriauria.

2. MATERIALS AND METHODS

2.1 Study Design

This cross sectional observational study was carried out over a six month period at two non-communicable chronic diseases clinics for adult patients. The clinics are located at two different geographical (northern and central) areas in Trinidad. During the study period, weekly visits were made to these Health Centers, on a Tuesday and Friday respectively. Participants were recruited if they fulfilled the following criteria – regularly attending the clinic for the past twelve or more months with confirmed diagnosis of diabetes mellitus, non-pregnant and above the age of 18 years. They were excluded if they refused to participate or failed to give the appropriate specimens.

2.2 Data Collection

All participants gave written informed consent after being well informed about the study, potential benefits, risks and the procedures. Using a designed questionnaire to capture history of diabetics and drug treatment were recorded. Other data - age, ethnicity, educational level, occupation, monthly income, any history of recent hospitalization, surgery, dialysis, family history of diabetes, information on the types of antidiabetic medications, history of any other underlying medical conditions such as hypertension or any other cardiovascular, respiratory, urogenital systemic diseases or haematological and immunological disorders. In addition, vital signs (pulse, blood pressure), standing height (centimetres), weight (kilogram) and abdominal gait were taken for all participants.

2.3 Laboratory Investigation

A 5-mL blood sample was collected by venipuncture in EDTA tube for HbA1c and red top plain tubes for cholesterol levels and blood urea nitrogen (BUN) values. Each participant was immediately given universal specimen container to collect a midstream urine sample for microscopy and albumin assay. Urine samples were processed on the same day but the blood samples were processed in batches.

Urinalysis was performed to determine albumin content of the urine. The glycosylated haemoglobin test (HbA1c), cholesterol, serum electrolytes and blood urea nitrogen were analyzed using the Cobas system (CobasR Integra 400/400 plus, Roche Diagnostics, USA). The tests were performed following the manufacturer instructions as in product insert. The HbA1c determination was not only carried out to confirm diagnosis of diabetes but also to identify the subjects who have poor glycaemic control. The normal HbA1c value was taken to be ≤ 6.5 based on the International Expert Committee recommendation and adopted by the American Diabetes Association [14].

The urine samples were cultured for bacterial organisms using standard microbiological methods and speciation of organisms were made using the automated Microscan walkaway (Siemens, USA) machine. The isolates of *Enterobacteriaceae* from urine culture were subjected to screening for extended beta lactamases enzymes (ESBLs) genes of class A that include the ones designated CTX-M for cefotaxime, TEM and sulphydryl variable or group (SHV), by amplification of gene fragments by conventional polymerase chain reaction (PCR) and agarose gel electrophoresis for detection [15]. Cabapenamase-resistant genes: *Klebsiella pneumoniae* carbapenemase (KPC), New Delhhi metalo-beta-lactamase (NDM), and OXA48 targets were detected by real-time PCR using Quantitect SYBR Green PCR kit (Qiagen Inc. Mississauga ON.) by melting curve analysis. Cell suspension of each organism in 0.5N saline to 50% T in the Vitek colorimeter was prepared and heat at 95ºC for 10 min. Five microliter of clear supernatant obtained after spinning the tubes at 13000 x g for 2 min was used as the

PCR template. The following isolate cultures were used as the positive controls: KPC American Tissue Culture Collection (ATCC) - (*Klebsiella pneumoniae* ATCC BAA® 1705™); NDM (*K. pneumoniae* ATCC BAA® 2146™) and for oxacillinase group (OXA-48; *E. coli*, Toronto Public Health Laboratory). The PCR primers used were: 5'-attggctaaagggaaacacgacc-3' and 5'gtagacggccaacacaat-3' (KPC); 5'-gaccgcccagatcctcaa-3' and 5'-ataccgcccatcttgtc-3'(NDM); 5'- ttcggccacggagcaaatcag-3', 5'-ggttcgattctagtcgagta-3' and 5'-gatgtgggcatatccatattcat-3' (OXA-48). Real-Time PCR and melting curve analysis was done using a Rotor-Gene Q (Qiagen) instrument.

The study was approved by the institutional review Ethics Committee of The University of the West Indies at St. Augustine

2.4 Statistical Analysis

Descriptive statistics were used to summarize the data collected for the mean, standard deviation, percentages, tables and figures. All statistical procedures were conducted using SPSS version 20.0 and Epi Info version 3.5.3 (CDC, Atlanta USA). The chi squared value was used to compare the significance of the frequency rates of several variables in the patients with normal and elevated HbA1c values. A *P*-value of < 0.05 was regarded as statistically significant.

3. RESULTS AND DISCUSSION

A total of 414 diabetic Trinidad & Tobago nationals comprising 302 (73%) females and 112 (27%) males fulfilled the study criteria and were used for analysis. A greater number of the participants (66.7%, 276/414) were from the health center located in the central part of the country. As self-reported by the participants, more than half (61.5%; 255/414) were of East Indian descent, 23.9% (99/414) were of African descent, 13% (54/414) were of mixed descent and the rest (1.6%, 7/414) were of Chinese/Spanish/Syrian/Lebanese descent.

HbA1c values of all subjects (Table 1) revealed that 68.4% (283/414) had normal values while 31.6% (131/414) had abnormal values of ≥ 6.5 confirming uncontrolled diabetes status. Just over 27.7% (35/131) of the subjects with abnormal HbA1c values are Type 1 (insulin dependent) diabetes mellitus while 73.3% (96/131) are Type 2 (noninsulin dependent) diabetes mellitus. Evaluation of the drug treatment of the Type 2 diabetic group revealed that 62.5% (60/96) were using Glicazide; 33.3% (32/96) were on Glibenclamide and 2.1% (2/96) were each on Glipizide and Glimepiride respectively. As noted in Table 2, the age group 60 – 69 of individuals with both normal and abnormal blood glucose levels had the most frequent rate accounting for 35.69% and 43.94% respectively.

A 12.3% (51/414) had abnormal micro albumin in their urine. Further analysis of these 51 subjects with abnormal micro albuminuria revealed that 70.6% (36/51) also had elevated Hb1Ac. More than half 52.9% (27/51) of the subjects who had abnormal micro albumin in their urine were Type 1 diabetes mellitus. Specific anthropometric and biochemical data are summarized in Table 3. As indicated the body mass index (BMI), blood urea nitrogen BUN) and blood pressure (both systolic and diastolic) measurements were statistically significantly varied or different between those diabetics whose blood sugar levels are under control in comparison to the uncontrolled subjects.

As depicted on Table 4, the HbA1c results was used to place all the subjects into group A (subjects with normal blood sugar level) 68.4% (283/414) or group B (comprising subjects with abnormal or uncontrolled blood sugar level) 31.6% (131/414). The parameters average monthly income, educational level, period subject had suffered from diabetes in years and occupation of the diabetic subject) compared among these two groups revealed some statistically significant results. Comparison of the duration of the diabetes among the uncontrolled diabetic group revealed that abnormal HbA1c values were significantly higher in those who have had diabetes for 6 – 9 years (29.8%), 10 – 14 years (15.3%), 15 – 19 (11.4%) or over 20 years (15.3%) than their comparators that had 7%, 4.2%, 4.6% and 5.0% respectively. And in terms of occupation, among those who had no job or unemployed, only 23.7% of them had abnormal HbA1c values in comparison to 11.3% noted among those whose sugar were under control. These results revealed that diabetic subjects who were unemployed (or had no job), custodians and others with job categorizations as masons, welder, taxi drivers etc. significantly had poor glucose control.

The frequency of the subjects who had abnormal HbA1c and earned less than $1000.00 TTD per month were 61.1%. Over sixty one percent (61.4%) of the subjects who had abnormal or

uncontrolled diabetes only completed Primary level education and when compared with their counterparts who had controlled sugar level, the difference was statistically significant, 61.4% vs. 38.6%, P =0.001. There is also a significant difference when other occupations such as civil servants, security personnel, unskilled labourer and farmers were compared.

Significant bacteriauria was noted in sixty five subjects. Majority, 81.5% (53/65) of the positive urine cultures were in those with abnormal HbA1c values. Most 87.1% (61/70) of the bacteria were recovered in urine samples from female 75.4% (49/65) subjects. Urine samples from males 24.6% (16/65) yielded mostly *Staphylococcus epidermidis* 14.3% (10/7) and the *Staphylococcus aureus* 5.7% (4/70), respectively. *Enterobacteriaceae* recovered most frequently were *Escherichia coli* 48.57% (34/70) and *Klebsiella pneumoniae* 25.7% (18/70). All *Enterobacteriaceae* isolates were negative for KPC, NDM and OXA-48. Although the *bla*TEM and *bla* SHV were detected in both the *E. coli* and *K. pneumoniae* isolates as expected.

There were very many pertinent characteristic features observed among diabetic subjects surveyed during this study. Notably was the fact that the prevalence of abnormal level of blood glucose among type 2 diabetic patients in Trinidad & Tobago was 31.6%. This value is high in comparison to value noted in Sweden [16]; almost similar to what is reported in Arab speaking countries [17] but lower than what is reported in many Asian countries such as China, India and Japan [18,19]. This high value of poor diabetic control in the country has previously been reported elsewhere and in the country where it is driven by diet, obesity and lifestyle factors [20]. According to report by Ezenwaka CE et al. [21] carbohydrate-based foods prepared and consumed in the country is strongly related to poor glycaemic control among East Indian descents. This fuels the increase of prevalence of diabetes and its complications among this ethnic group of people in the country [21] as was also noted in this current study. The HbA1c assay was not only useful in confirming the diabetes diagnosis of these patients but it was also accurate in determining their blood sugar status whether adequately or poorly controlled. The target of health care providers for these diabetic clinics all over the country should therefore be to adequately and effectively establish glycaemic control among these patients since this has historically been reported or associated with less frequent serious complications such as proliferative retinopathy, nephropathy, and cardiovascular diseases [22,23].

Observing such a high frequency of poorly controlled blood sugar levels among patients attending these health care facilities in Trinidad and Tobago is very worrisome. It means that more efforts in terms of health education and finances should be expended in the management of diabetes in the country. However, commendations must be given to the Ministry of Health in the country for implementing the Chronic Disease Assistance Plan (CDAP) which covers all the citizens of Trinidad and Tobago afflicted by Chronic Non Communicable Diseases like: Diabetes, Hypertension, Cardiac Disease, Asthma, Arthritis, Glaucoma, Mental Depression, Enlarged Prostate, etc; with around 51 prescription drugs, free of charge at all public health facilities and most of the private pharmacies nationwide. Since January 2008, this plan has further been extended by providing free blood glucose testing strips to Diabetics.

Table 1. Gender and HbA1c values distributions of 414 diabetic patients attending primary health care facilities in Trinidad & Tobago (%)

HbA1c value*	Frequency	Gender		*p*-value
		Male	Female	
<6.5	283(68.4)	77(68.75)	206(66.40)	0.917
6.6 – 7.5	49(11.8)	5(4.46)	44(14.57)	0.003
7.6 – 8.5	25(6.0)	9(8.04)	16(5.30)	0.352
8.6 – 9.5	18(4.4)	6(5.36)	12(3.97)	0.589
9.6 – 10.5	15(3.6)	6(5.36)	9(2.98)	0.248
10.6 – 11.5	10(2.4)	2(1.79)	8(2.64)	0.579
11.6 – 12.5	8(1.9)	3(2.68)	5(1.66)	0.451
12.6+	6(1.5)	4(3.57)	2(0.66)	0.048

**Based on the colorimeter assay or method used, the normal value for the HbA1c was <6.5. Any value ≥ 6.5 was regarded as abnormal*

Table 2. Age and gender distributions of 414 diabetic patients with normal and abnormal blood sugar levels attending primary health centers in Trinidad & Tobago (%)

Age group	Normal group; N=283			Abnormal group; N=131			*p*-value
	Frequency	M	F	Frequency	M	F	
<20	0	0	0	0	0	0	0
20 – 29	4(1.41)	1	3	2(1.52)	0	2	0.93
30 – 39	15(5.30)	4	11	5(3.79)	2	3	0.63
40 – 49	51(18.02)	10	41	16(12.12)	5	11	0.15
50 – 59	80(28.27)	17	63	37(28.03)	8	29	0.99
60 – 69	101(35.69)	20	81	58(43.94)	14	44	0.10
70+	32(11.31)	18	14	13(9.85)	6	7	0.74
Total	283(100)	70(24.7)	213(75.3)	131(100)	35	96	

Table 3. Anthropometric and biochemical data of diabetics whose blood sugar levels were normal and abnormal in Trinidad & Tobago

Characteristics	Normal	Abnormal	*p*-value
Height (m)	1.50±0.26	1.54±0.28	0.73
Weight (kg)	84±30	84±34	1.00
BMI (kg/m^2)	29.1±7.2	32.5±9.4	0.001
Heart rate (bpm)	66±9	70±10	≤0.001
Systolic BP (mmHg)	120±15	135±20	≤0.001
Diastolic BP (mmHg)	70±10	78±12	≤0.001
BUN	17±10	19±12	≤≤0.001
Creatinine	1.15±0.37	1.10±0.42	0.22
Total cholesterol (mg/dl)	180±65	176±78	0.59
Triglycerides (mg/dl)	134±48	133±51	0.85
HDL cholesterol (mg/dl)	46±15	45±18	0.56
LDL cholesterol (mg/dl)	110±53	109±55	0.86

BMI = body mass index, bpm = beats per minute, BUN = blood urea nitrogen, HDL = high density lipoprotein, LDL = low density lipoprotein

Table 4. Comparison of characteristics observed among 414 diabetic patients with abnormal and normal HbA1c values attending primary health centers in Trinidad & Tobago

Observed characteristics	N	Normal	Abnormal	*p*-value
Average monthly income				
<$1000 TTD	194	114(40.3)	80(61.1)	≤ 0.001
1001 – 3000	171	130(45.9)	41(31.3)	0.004
3001 – 5000	26	21 (7.4)	5 (3.8)	0.115
5001 – 7000	10	9 (3.1)	1 (0.7)	0.061
7001 – 10,000	1	1 (0.30)	0 (0)	0.320
>10000	0	0 (0)	0 (0)	1.00
No response	12	8 (2.8)	4 (2.8)	0.90
Total	414	283 (68.4)	131 (31.6)	
Educational level				
None	14	9 (3.1)	5 (3.8)	0.77
Primary school	241	148 (52.3)	93 (71.0)	≤ 0.001
Secondary school	130	105 (37.1)	25 (19.1)	≤0.001
Vocational/Tech Institution	26	18 (6.4)	8 (6.1)	0.921
University	2	2 (0.7)	0 (0.0)	0.156
Special Need School	1	1(0.3)	0 (0.0)	0.320
Post Graduate	0	0 (0)	0(0)	1.000
Total	414	283(68.4)	131 (31.6)	
Duration (years)				
<2	133	125 (44.1)	8 (6.1)	≤ 0.001

Observed characteristics	N	Normal	Abnormal	*p*-value
2 – 5	120	95(33.6)	25 (19.1)	0.001
6 – 9	62	25 (8.8)	37 (28.2)	≤ 0.001
10 – 14	32	12 (4.2)	20 (15.3)	0.001
15 – 19	26	10 (3.5)	16 (12.2)	0.005
20+	38	16 (5.7)	22 (16.8)	0.002
Do not know	5	2 (0.71)	3 (2.2)	0.330
Total	414	283 (68.4)	131 (31.6)	
Occupation				
None	63	32 (11.3)	31 (23.7)	0.003
Housewife	140	90 (31.8)	50 (38.2)	0.220
Civil Servant	37	27 (9.5)	10 (7.6)	0.580
Custodian	32	23 (8.1)	9 (6.9)	0.065
Self employed	29	22 (7.7)	7 (5.3)	0.410
Security personnel	17	14 (4.9)	3 (2.3)	0.290
URP worker	26	19 (6.7)	7 (5.3)	0.670
Unskilled labourer	20	15 (5.3)	5 (3.8)	0.630
Farmer	8	6 (2.1)	2 (1.5)	1.000
Others	42	35 (12.4)	7 (5.3)	0.035
Total	414	283 (68.4)	131 (31.6)	

URP = Government Unemployment Program; Others = Tradesman, Mason, Cashier, Chef, mechanic, welder, caterer, process plant operator, Taxi driver, Tailor/Seam-mistress

In this study, majority of the individuals with abnormal blood sugar level in the Trinidad & Tobago were of East Indian ethnic group or descent, in the age group of 60 – 69 (Table 2) and are females too. These are similar to the findings reported by The DECODA study group [24] and also by Ramachandra A et al [20]. Diabetes prevalence increased with age and reached the peak at 70–89 years of age in Chinese and Japanese subjects, peaked at 60–69 years followed by a decline at the 70 years of age in Indian subjects [24,25]. The increased frequency of diabetes mellitus among subjects of East Indian descent than those of the African descent still confirms what has already been reported by other researchers in the country [8-13]. The observation of having similar age and ethnic groups (East Indian descent in Trinidad & Tobago) having similar distribution of diabetes could be that this is a genetically related factor as good number of the nationals of Trinidad & Tobago are of East Indian descent. This may be attributed to or driven by population growth, improved living standards, obesity, accelerated urbanization, drastic changes in nutrition and reduced physical activity.

Elevated micro-albuminuria and blood urea nitrogen were seen more among the uncontrolled diabetic group of subjects in the country. As reported in literature, diabetes is the leading cause of chronic kidney failure, or end-stage renal disease and in diabetics (both types 1 and 2), microalbuminuria predicts renal disease and relates to premature mortality as well as being a marker for pronounced diabetic vascular disease - endothelial dysfunction and chronic low-grade inflammation [26]. The reason for this may still be associated with dietary habits of patients in the country as previously reported [21]. A cross-sectional study of over 2,600 people with diabetes found that a protein intake greater than 20% of calories was associated with an increased urinary albumin excretion rate [27,28]. The frequency of the subjects who had abnormal blood sugar control (elevated HbA1c) and earned less monthly income were noted to be very high in this study. These patients also did not attain a high educational level beyond the secondary school certificate or diploma. These subjects depended on the supply of their medication for controlling their diabetes on the government prescription program in the country called chronic disease assistance plan (C-DAP).

Duration or time the subjects had suffered with diabetes also appeared to significantly contribute to the subject's poor control of their sugar level. The more the years the participants had suffered with diabetes the more they were prone to have poor glycaemic control. The reason for this could be that they are lacking in understanding and experience in terms of their diet or medication usage, in other words they had not mastered the art. A good number of the patients had very high body mass index (BMI) and the authors would strongly recommend for these patients to participate in weight reduction program and

increase their physical activity level in order to reduce the risk of developing complications associated with diabetes.

3.1 Limitations

This study was not designed to follow up on subjects with poor blood sugar control. However, the observations in this study of the several factors associated with these diabetics will serve as a template for further studies on adequate control of blood sugar levels in diabetic patients in the country.

4. CONCLUSION

In Trinidad and Tobago, diabetics in the country who have abnormal blood sugar levels were more of the type 2; of East Indian descent; were in the age group of 60 – 69; were more of females; had elevated micro-albuminuria and blood urea nitrogen. Poor blood sugar control among the diabetics were also observed to be more among subjects who had very low monthly income and only attained primary and secondary school level education in the country. The longer the duration patient has had diabetes the poor blood sugar level control was also observed among the diabetics in the country. All these observations point to areas where concerted efforts should be focused or directed at among the diabetics care to stem the scourge and complications in Trinidad & Tobago. During follow up of these patients attention should be focused on education and modification of life style activities to help the diabetics in the country.

CONSENT

Written informed consent was obtained from all patients who participated in this study.

ETHICAL APPROVAL

The study was approved by the institutional review Ethics Committee of The University of the West Indies at St. Augustine.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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