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Original Article

Tumour necrosis factor-α −308G/A polymorphism is associated with insulin secretory defects in Bangladeshi prediabetic/diabetic subjects

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الملخص

أهداف البحث: يرتبط عامل نخر الورم، وهو سيتوكين شحمي، ارتباطا وثيقا باضطراب تحمل الجلوكوز ومقاومة الأنسولين لدى مرضى السكري من النوع الثاني. لم يتم تحديد العلاقة بين تعدد الأشكال في جين عامل نخر الورم ومقاومة الأنسولين لدى المرضى المصابين بالسكري أو مقدمات السكري في بنجلاديش بشكل كامل حتى الآن. تم تصميم الدراسة للكشف عن ارتباط تعدد الأشكال الجيني في جين نخر الورم بمقاومة الأنسولين عند مرضى فرط سكر الدم من أصل بنغلاديشي.

طرق البحث: في دراستنا، تم تجنيد 106 شخصا مصابا باضطراب تحمل الجلوكوز، و100مريض سكري من النوع الثاني، و109 من الأشخاص الأصحاء من أصل بنغلاديشي لتحديد تأثير تعدد الأشكال الجيني في جين عامل نخر الورم في الموضع 308 باستخدام التفاعل السلسلي للبوليميريز ثم طريقة تعدد أشكال أطوال الشدف المقتطعة.

النتائج: أظهر توزيع تكرار النمط الجيني لعامل نخر الورم -308 ضمن مجموعة التحكم ومجموعة اضطراب تحمل الجلوكوز ومجموعة مرضى السكري النوع الثاني ارتباطًا كبيرا، على الرغم من أن توزيع تكرار الأليل داخل المجموعات أظهر فرقا غير مهم إحصائيا. كما لوحظ أن نقص وظائف خلايا بيتا كان أقل بشكل ملحوظ لدى المرضى ذوي ضرب النمط الجيني. إلى جانب ذلك،

تشير نتائجنا إلى أن مؤشر كتلة الجسم وحالة الإقامة للمشاركين في الدراسة كانت مرتبطة بشكل إيجابي مع تعدد الأشكال الجيني.

الاستنتاجات: لذلك، يمكن استنتاج أن تعدد الأشكال الجيني في جين عامل نخر الورم في الموضع 308 قد يكون له علاقة سببية بقدرة إفراز الأنسولين المنخفضة وارتفاع مؤشر كتلة الجسم في مجموعات اضطراب تحمل الجلوكوز ومرضى السكري النوع الثاني في بنغلاديش، في حين أن نمط حياة سكان الحضر قد يكون له ارتباط مع تعدد الأشكال الجيني.

الكلمات المفتاحية: السكري النوع الثاني؛ اضطراب تحمل الجلوكوز؛ عامل نخر الورم ألفا؛ تعدد الأشكال الجيني؛ مقاومة الأنسولين

Abstract

Objectives: Tumour necrosis factor (TNF)-α, an adipocytokine, is closely linked to impaired glucose tolerance (IGT) and insulin resistance (IR) in type 2 diabetes (T2D) subjects. The relationship between the polymorphisms in the TNF-α gene and IR in Bangladeshi prediabetes and T2D subjects has not yet been fully identified. This study aims to reveal the association between TNF-α gene polymorphism and IR in hyperglycaemic patients of Bangladeshi origin.

Methods: In our study, 106 IGT, 100 T2D, and 109 healthy subjects of Bangladeshi origin were recruited to identify the impact of TNF-α gene polymorphism at position −308 with a G>A transition using PCR and subsequent restriction fragment length polymorphism (RFLP).

Results: The −308G>A TNF-α genotype frequency distribution within the control, IGT, and T2D groups showed a significant association ($\chi^2 = 21.077$; $P = 0.001$), although allele frequency distribution within the groups showed a

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statistically non-significant difference ($\chi^2 = 1.696$; $P = 0.091$). β-cell functional deficiency (HOMA-B%) was observed to be significantly ($P = 0.034$) lower in subjects with a variant genotype. In addition, our results indicate that the study subjects' body mass index (BMI) and residence status were positively correlated ($P \leq 0.05$) with −308G>A TNF-α gene polymorphism.

Conclusions: Therefore, it can be concluded that −308G>A TNF-α gene polymorphism may have a causative relationship with lower insulin secretory capacity and higher BMI in Bangladeshi IGT and T2D populations, while the urban population's lifestyle might be associated with this polymorphism.

Keywords: -308G>A; Diabetes mellitus; Glucose intolerance; Insulin resistance; Polymorphism; Tumour necrosis factor-alpha

Introduction

Type 2 diabetes (T2D) is an accredited metabolic and multifactorial disorder that is defined by a high blood glucose level due to defects in insulin functionality or a deficiency in insulin secretion.[1,2] People with prediabetes have a greater chance of progressing to T2D.[3] Prediabetes can be defined as a blood glucose level that is intermediate between normoglycaemia and the diabetes threshold. This can be ascertained either through impaired fasting glucose (IFG) or impaired glucose tolerance (IGT).[4] Obesity, inactivity, and family history are the key factors that increase the possibility of developing T2D and have been found to be highly associated with complications related to this disease.[5] Apart from the role of environmental determinants, numerous genetic factors influence disease susceptibility.[6] Several studies have reported the existence of a close relationship between T2D and the different genes that encode molecules with diverse functions.[7]

Tumour necrosis factor (TNF)-α, a cytokine that originated predominantly from macrophages and adipocytes, can promote insulin resistance (IR) and ultimately assist the progression of T2D.[8] The TNF-α gene in humans is mapped at the 6p21.3 position of the chromosome encoding a polypeptide consisting of 157 amino acids.[9] The interrelation between the TNF-α gene's single nucleotide polymorphisms (SNPs) and T2D has been extensively examined in different populations. Kubaszek et al. reported that polymorphism in the promoter region of the TNF- α gene predicts the progression of T2DM from IGT.[10−12] Among other SNPs, TNF-α promoter SNPs such as −308G/A and −238G/A are well-characterised and have been found to be correlated with IR and T2D in different studied populations.[13,14] Indeed, −308 polymorphism has been shown to enhance transcriptional activity, affecting its gene expression with a rare A-allele over the G-allele, signifying the functionality of this

SNP.[15] The TNF-α gene with this unusual A-allele is supposed to be linked to body fat and hence obesity, as well as IR, contributing to the evolvement of T2D.[10,16] Although TNF-α SNP and its association with T2D have been widely studied, the results are inconsistent. This is probably due to the variation from population to population, which is not yet fully understood.[13,17,9] Previously, we reported that serum TNF-α is positively associated with Bangladeshi prediabetic populations and is also associated with insulin secretory defects.[18] Therefore, this study aims to explore the impacts of TNF-α −308G>A promoter SNP on IR and identify its association with prediabetes and T2D in the Bangladeshi population.

In this study, we show that −308G>A TNF-α gene polymorphism may have a causative relationship with lower insulin secretory capacity and higher BMI in Bangladeshi IGT and T2D populations, while the urban population's lifestyle might be associated with this polymorphism.

Materials and Methods

Study subjects

This research was carried out in the laboratory of the Department of Physiology and Molecular Biology, Bangladesh University of Health Science (BUHS), Dhaka. A total of 106 IGT and 100 T2D subjects of Bangladeshi origin were recruited, along with 109 age-matched healthy controls. World Health Organization (WHO) guidelines (1999) were followed to identify IGT and T2D subjects.[19] The study participants submitted signed informed consent letters. Data were collected using a pretested standard questionnaire. Patients who were newly diagnosed as either IGT or T2D were included, and patients suffering from any chronic illness, gestational diabetes, or mental disorder were excluded from our study. The power of the study was not calculated, but we calculated the sample size to derive the appropriate proportion of the population needed for this research, using the following statistical formula:

$$\text{Sample Size (SS)} = Z^2 \text{ x (p) x (1-p)/ } C^2$$

where Z = Z value (e.g. 1.96 for a 95% confidence level), p = percentage picking a choice, expressed as a decimal (0.5 used for the sample size needed), and C = confidence interval, expressed as a decimal (e.g. 0.04 = ±4).

Assessment of demographic and anthropometric parameters

Demographic information was collected by interviewing the subjects. Anthropometric parameters such as weight, height, and waist and hip circumference were measured for each of the subjects using standard methods, according to the WHO guidelines.[6]

Blood sample collection

Blood was drawn by venipuncture under the overnight fasting condition (10−12 hours) and after 2 hours of glucose administration (75 g dissolved in 250 ml water). After 30 minutes, samples were then centrifuged at 3000 rpm for 10 minutes to produce serum. The serum was preserved in the freezer

(−25 °C) for biochemical analysis. Five millilitre blood samples were stored in another freezer (−30 °C) for PCR analysis.

Biochemical analysis

The glucose−oxidase method was used to measure the serum glucose level. Serum total cholesterol (TC), triglyceride (TG), and HDL-C were measured using cholesterol oxidase assay, glycerophosphate oxidase assay, and cholesterol oxidase assay, respectively. To calculate serum LDL-C, Friedewald's equation was used.[20] Serum creatinine and serum SGPT were measured with the alkaline picrate method and the UV-spectrophotometric method using commercial kits (Randox Laboratories, Ltd., UK), respectively. Fasting serum insulin levels were determined using the ELISA method (Linco Research Inc., USA). We employed homeostatic model assessment (HOMA) to measure β-cell functional deficiency (HOMA B%), insulin sensitivity (HOMA S%), and insulin resistance (HOMA IR) based on fasting serum glucose and fasting serum insulin level. HOMA IR and HOMA B% were obtained using the following formulas.[21]

(a) HOMA-IR = Glucose x Insulin/22.5
(b) HOMA-B % = 20 x Insulin/Glucose-3.5, if the glucose in molar units (mmol/L)

Genetic analysis of TNF-α genotypes

Extraction of DNA and PCR analysis

We used the Commercial GenElute DNA extraction kit (QIAGEN, USA), and then performed PCR using specific primers for TNF-α gene amplification [(5'- AGGCAATAGGTTTTGAGGGCCAT -3' (sense); 5'-TCCTCCCTGCTCCGATTCCG -3' (antisense)] [31]. The master mix included 4 µL of genomic DNA (10−50 ng/ml), 10 pmol of each of the primers, 200 µmol/L dNTPs, 0.35 units of HotStar Taq polymerase (QIAGEN, USA), 1 µL of PCR buffer, and 3.23 µL of nuclease-free water in a final volume of 10 µL. PCR was carried out in a thermal cycler (Biometra, Germany) according to the following steps: i) initial denaturation (95 °C for 15 minutes), ii) denaturation (95 °C for 30 seconds and 34 cycles), iii) annealing (58 °C for 45 seconds), iv) elongation (72 °C for 45 seconds), and v) final elongation (72 °C for 10 minutes). Amplicons were identified via agarose gel electrophoresis (2% agarose gel) following ethidium bromide staining.

Restriction fragment length polymorphism

Genotyping of TNF-α −308G>A polymorphism was determined via the RFLP method using the *Nco I* restriction enzyme.[22] Five µL of PCR products were taken in a separate tube and digested with the *Nco I* restriction enzyme at 37 °C for 4 hours (New England Biolabs, USA) in a water bath. Next, we performed agarose gel electrophoresis using 3.5% gel to resolve the fragments and then verified using UV spectrometry. In this experiment, we used ethidium bromide for staining.

Statistical analysis

The Statistical Package for Social Science (SPSS), Windows version 17 (SPSS Inc., Chicago, Illinois, USA) was used to analyse the data. An unpaired Student's *t* test was performed to assess the comparison between the two groups. ANOVA-Bonferroni analysis was performed to explore the relationship with the anthropological and biochemical characteristics. In appropriate situations, we performed a Chi-square (χ^2) test. Statistically significant values were regarded as having a *P* value of <0.05.

Results

Anthropological and biochemical parameters

Among the anthropometric and biochemical parameters, IGT and T2D subjects' BMI, WHR, SBP, FSG, PPG, TG, cholesterol, LDL-C, fasting insulin, and HOMA IR were observed to have higher values than in the control group (*P* = 0.001), whereas HOMA %B and HOMA %S were found to be significantly reduced in the IGT and T2D groups (*P* = 0.001) (Table 1, Figure 1).

Allele and genotype frequency of the TNF-α gene −308 G>A

The genotype frequency distribution within the groups showed a statistically significant association in the χ^2-test (χ^2 = 21.077; *P* = 0.001). The allele frequency distribution within the groups showed a non-significant association (χ^2 = 1.696; *P* = 0.091) (Table 2).
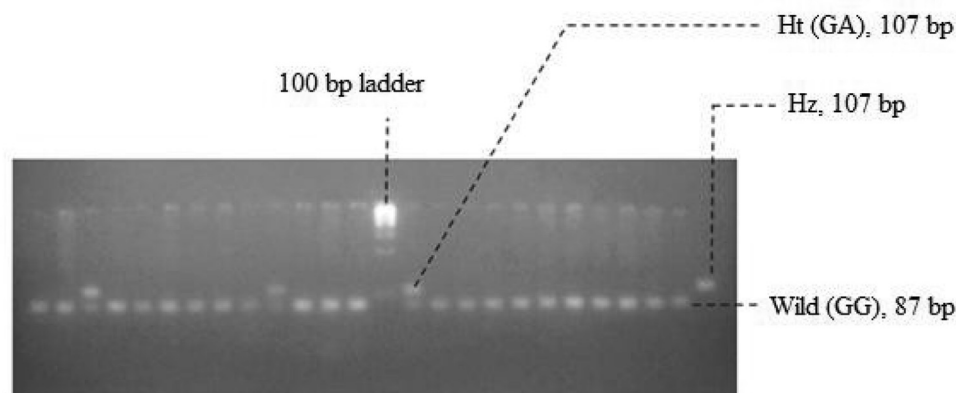

Figure 1: Representative electrophoretic patterns of TNF-α gene −308G>A polymorphism analysis by *Nco I* restriction enzyme digestion. Fragments produced were as follows: Homozygous wild genotype 87 bp and 20 bp; heterozygous (Ht) variant genotype 20 bp, 87bp, and 107 bp; and homozygous (Hz) variant genotype 107 bp. 20 bp is too small and cannot be resolved in 3.5% Agarose gel.

Table 1: Anthropological and biochemical parameters.

Variables	Control (n = 109)	IGT (n = 106)	T2DM (n = 100)	p-value
Age (years)	42 ± 12^a	44 ± 8^a	44 ± 9^a	0.493
BMI (kg/m^2)	22.1 ± 3.9^a	25.5 ± 3.99^b	25.1 ± 3.6^b	**0.001**
WHR	0.90 ± 0.08^a	$0.93 \pm .06^b$	$0.95 \pm .08^b$	**0.001**
SBP (mmHg)	111 ± 13^a	117 ± 16^b	115 ± 16^b	**0.016**
DBP (mmHg)	74 ± 9^a	77 ± 11^a	77 ± 11^a	0.072
FSG (mmol/L)	4.6 ± 0.7^a	5.7 ± 0.8^a	10.6 ± 4.2^b	**0.001**
PPG (mmol/L)	5.7 ± 1.2^a	8.5 ± 1.4^b	17.8 ± 6.1^c	**0.001**
Triglyceride (mg/dL)	134 ± 65^a	181 ± 102^b	228 ± 141^c	**0.001**
Cholesterol (mg/dL)	167 ± 31^a	195 ± 36^b	211 ± 46^c	**0.001**
HDL-C (mg/dL)	37 ± 7^a	34 ± 7^a	35 ± 17^a	0.354
LDL-C (mg/dL)	103 ± 28^a	124 ± 34^b	129 ± 46^b	**0.001**
F Insulin (µIU/L)	10.9 ± 9.4^a	16.1 ± 9.6^b	20.8 ± 13^c	**0.001**
HOMA %B	141 ± 56^a	127 ± 53^b	91 ± 73^c	**0.001**
HOMA %S	100 ± 52^a	61 ± 38^b	45 ± 29^c	**0.001**
HOMA IR	1.3 ± 0.7^a	2.1 ± 1.0^b	2.9 ± 1.5^c	**0.001**

Data are expressed as mean $\pm$ SD. Values in the full row which do not contain the same superscript have significance at P-value <0.05. BMI: Body Mass Index, WHR: Waist-hip ratio, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, F Insulin: Fasting Insulin, HOMA%B: β-cell functional deficiency, HOMA%S: Insulin sensitivity, HOMA IR: insulin resistance.

Table 2: -308G >A TNF-α genotype and the allele frequency.

Genotype	Control [Frequency (n)]	IGT [Frequency (n)]	T2DM [Frequency (n)]	p-value
Wild type (GG)	0.954 (104)	0.774 (82)	0.913 (95)	**0.001**
Ht type (GA)	0.046 (5)	0.189 (20)	0.087 (9)	
Hz type (AA)	0	0.038 (4)	0	
Allele frequency (G/A)	0.977/0.023	0.869/0.133	0.957/0.044	0.091

Data are presented as frequency (number); Ht: Heterozygous variant, Hz: Homozygous variant.

Clinical parameters in regard to the −308G>A TNF-α gene

As clinical parameters, we considered age, BMI, WHR, blood pressure, FSG, PPG, HOMA%B, HOMA%S, and HOMA IR (Table 3). Among them, the study subjects' BMI and HOMA%B had a significant association with the variant genotype (P = 0.037 and P = 0.034, respectively).

Frequency of the −308 G>A TNF-α genotype in regard to residence

In regard to residential status, we divided our study population into a rural group and an urban group. The frequencies of the wild and variant genotype for both groups are shown in Table 4. A significant association was found among the groups in the χ^2-test (χ^2 = 14.78 and

Table 3: Clinical parameter analysis in regard to −308G>A genotype.

Variables	Wild (n = 277)	Variant (n = 38)	t/p-value
Age (years)	43 ± 10	44 ± 8	$-0.534/0.594$
BMI ((kg/m^2)	24.0 ± 4.1	25.5 ± 4.0	$-2.099/\mathbf{0.037}$
WHR	0.93 ± 0.08	0.91 ± 0.07	0.799/0.425
SBP (mm/Hg)	114 ± 15	117 ± 19	$-1.198/0.232$
DBP (mm/Hg)	76 ± 10	75 ± 13	0.405/0.686
FSG (mmol/L)	6.9 ± 3.7	6.3 ± 2.4	0.922/0.357
PPG (mmol/L)	9.3 ± 5.6	9.7 ± 4.1	$-0.403/0.687$
F Insulin (µIU/L)	14.7 ± 10.9	14.9 ± 11.4	$-0.094/0.925$
HOMA %B	130 ± 61	106 ± 54	$2.131/\mathbf{0.034}$
HOMA %S	77.1 ± 50.4	69.3 ± 39.2	0.855/0.394
HOMA IR	1.9 ± 1.2	1.8 ± 0.8	0.396/0.692

Data are expressed as mean $\pm$ SD. BMI: Body Mass Index, WHR: Waist-hip ratio, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, FSG: Fasting glucose, PPG: postprandial glucose, F Insulin: Fasting Insulin, HOMA%B: β-cell functional deficiency, HOMA%S: Insulin sensitivity, HOMA IR: insulin resistance.

Table 4: Frequency of the −308 G>A TNF-α genotype variant in regards to residence.

Residence	Wild (n)	Variant (Ht & Hz) (n)	χ^2/p-value
Rural	0.95 (152)	0.05 (8)	14.78/0.001
Urban	0.81 (123)	0.19 (29)	

Data are represented as frequency (number). Ht: Heterozygous variant, Hz: Homozygous variant.

$P = 0.001$). Since T2DM and IGR were found to be higher in the urban population, TNF-α-308G/A polymorphism was also found to be higher in this group. This suggests a direct correlation between hyperglycaemia and TNF-α-308G/A polymorphism in the urban population.

Discussion

TNF-α, an important adipocytokine, can inhibit insulin activity by impairing insulin signalling pathways and exert a potent effect in the early development of T2D through the destruction of pancreatic beta cells.[14,23] Polymorphism in the TNF-α gene has a startling impact on susceptibility to T2D.[14] The effect of the −308G>A TNF-α SNP on obesity, IR, and T2D is inconsistent among different populations.[23,17] Several studies have reported a probable correlation between the −308G>A variant of the TNF-α gene and susceptibility to T2D in subjects from diverse populations.[13,17] Some studies have also reported the opposite result through a negative association with IR and obesity.[9,23] However, ethnic differences may be responsible for the variation in these results, since TNF-α −308G>A polymorphism can vary among different ethnic populations. In addition, an inappropriate study design, an insignificant sample size, or related environmental factors may have played a role in obtaining such results.[12]

In this study, multiple biochemical tools related to T2D and IGT were considered. Among them, the study subjects' BMI, WHR, SBP, lipid profiles (TG, cholesterol, and LDL), and glucose and insulin levels showed a statistically significant difference within the control, IGT, and T2D groups ($P \leq 0.05$). These are common phenomena in prediabetic and diabetic subjects, as documented in other studies.[18,24] HOMA is a widely used tool for assessing IR, insulin sensitivity, and the insulin secretory capacity of pancreatic β-cells. Our study subjects' HOMA %S, HOMA%B, and HOMA IR were also found to exhibit a significant difference among the groups ($P \leq 0.05$). These findings are concordant with Song et al.'s study involving an exclusively female population; they showed that HOMA indices are associated with diabetes risk.[25]

Very limited evidence is available to prove the effect of TNF-α gene promoter SNP on T2D progression in subjects with prediabetes. In our study, although allele frequency distribution between the groups showed a non-significant difference ($P = 0.091$), the genotype frequencies of the −308G>A variant of the TNF-α gene among the control, IGT, and T2D subjects showed significant differences ($P < 0.001$). This finding could be an indication of the link between −308G>A TNF-α polymorphism and prediabetes, which may influence the progression of T2D in the Bangladeshi population. Our findings are consistent with those of Liu et al., who observed a close link between −308G>A TNF-

α gene polymorphism and an increased chance of developing T2D in the Chinese population.[26] Our results also support a meta-analysis of the Asian population; however, they contradict Feng et al.'s meta-analysis, which found no significant association.[27,12] Although it appears that −308G>A TNF-α polymorphism is only limitedly associated with Bangladeshi IGT and T2D subjects, further studies with a large dataset are required to validate the current results.

In our study, the wild and variant TNF-α genotypes were compared with different variables. Our results indicate that the −308G>A genotype of TNF−α is significantly linked to BMI ($P = 0.037$). Therefore, it can be suggested that BMI may be related to −308G>A TNF−α polymorphism, probably acting as a predictor of obesity and ultimately a risk indicator in the development of T2D. Um et al. studied obese Korean women and reported that −308G>A TNF-α polymorphism is unrelated to BMI, but Dalziel et al.'s study of a white population supports our findings, as −308G>A polymorphism was observed to have an association with obesity.[28,17] Another study showed that subjects with an A allele over the G allele were at a greater risk (23%) of developing obesity.[13] However, Um et al. indicated a significant association between this polymorphism and WHR based on lower WHR in subjects with GA or AA genotypes. However, their result is not consistent with our observations. Our data showed that −308G>A TNF-α polymorphism has no significant effects on WHR.[28] Furthermore, our study indicated no significant role of −308G>A TNF-α polymorphism with respect to age, which contradicts the findings of Heijmans et al., who reported that −308G>A TNF-α polymorphism is significantly related to T2D in old age.[29] Ethnic variations within different countries could be the prime reason for such contradictions. For this reason, we were interested in performing this experiment on Bangladeshi subjects, as no studies have yet been done to confirm the effects of this polymorphism under the prediabetic condition and in the conversion of diabetes mellitus within this population.

Kubaszek et al. reported that lifestyle changes interact with TNF-α −308G>A polymorphism.[10] Consistent with this, urban subjects participating in the present study showed a significantly ($P = 0.001$) higher frequency of variants in TNF-α −308G>A polymorphism. This variation may be explained by lifestyle changes among urban people or by increased environmental pollution in urban areas. Thus, it can be suggested that the −308G>A TNF-α SNP might be associated with lifestyle change or environmental pollution.

Our study demonstrated that the circulating insulin level did not differ in the subjects with the TNF-α −308G>A wild versus variant genotypes and that there were also no differences for HOMA-IR and HOMA%S. Our findings are similar to those Liu et al. obtained within a Chinese

population. They did not observe any effects of the −308G>A TNF-α variant on fasting serum insulin level and HOMA-IR.[23] In our study, differences in HOMA%B between the genotypes (wild GG and variant GA, AA) showed statistical significance ($P = 0.034$). A significant decrease in HOMA%B was observed in the study subjects with the variant genotype (Table 4), which indicates that β-cell function may be decreased in the presence of a variant genotype, leading to decreased glucose uptake, and may ultimately play role in the development of T2D. However, no data were reported to show such an association between HOMA%B and TNF-α variant genotypes.

Overall, our present experiment lays the foundation for determining the obvious effects of TNF-α gene polymorphism in Bangladeshi prediabetic and diabetic subjects and suggests that −308G>A SNP could be a potent susceptible target. The experiment is limited by a relatively small data size, which may limit some of our assumptions. Therefore, further research that includes gene and protein expression analyses with a large sample size will be beneficial to draw a certain conclusion by verifying the effects of TNF-α SNPs on Bangladeshi people's disease (prediabetes or diabetes) susceptibility.

Conclusion

Based on our findings, we can assume that TNF-α −308G>A polymorphism may have a causative relationship with pancreatic β-cells' lower insulin secretory capacity. Moreover, −308G>A variant genotypes of the TNF-α gene are significantly associated with a higher BMI, which might act as a predictor of obesity in prediabetic and T2D subjects. Finally, urban people seem to be largely affected by variant genotypes of the TNF-α −308G>A gene.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The work was carried out with ethical approval from the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC), Institute of Biological Sciences, University of Rajshahi, Bangladesh (Resolution no 05 of the 2nd meeting held on 4th January, 2012). The certificate reference number is 21/320/IAMEBBC/IBSc (issued on 7th January, 2012).

Authors contributions

The authors have declared the following contributions: concepts and design by MMH, MOF, and ZH; experiments performed by SP, MD, and TS; data analysed by MMH, SP, MD, TS, MOF, and ZH; tables and figures prepared by MMH, SP, MD, and TS; manuscript written by MMH, SP, MD, MOF, and ZH. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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References

1. Bruce KD, Byrne CD. The metabolic syndrome: common origins of a multifactorial disorder. **Postgrad Med J 2009**; 85(1009): 614−621.

2. Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, et al. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. **Nat Genet 2000**; 26(2): 163−175.

3. Dutta D, Choudhuri S, Mondal SA, Maisnam I, Reza AHH, Ghosh S, et al. Tumor necrosis factor alpha −238G/A (rs 361525) gene polymorphism predicts progression to type-2 diabetes in an Eastern Indian population with prediabetes. **Diabetes Res Clin Pract 2013**; 99(3): e37−e41.

4. Lucas R, Parikh SJ, Sridhar S, Guo D-H, Bhagatwala J, Dong Y, et al. Cytokine profiling of young overweight and obese female African American adults with prediabetes. **Cytokine 2013**; 64(1): 310−315.

5. Linmans JJ, Spigt MG, Deneer L, Lucas AEM, de Bakker M, Gidding LG, et al. Effect of lifestyle intervention for people with diabetes or prediabetes in real-world primary care: propensity score analysis. **BMC Fam Pract 2011**; 12: 95.

6. Ningombam SS, Rajkumari S, Chhungi V, Newmei MK, Devi NK, Mondal PR, et al. Type 2 diabetes and FTO rs9939609 gene polymorphism: a study among the two tribal population groups of Manipur, North East India. **Int J Diabetes Dev Ctries 2019**; 39(1): 60−65.

7. Willer CJ, Bonnycastle LL, Conneely KN, Duren WL, Jackson AU, Scott LJ, et al. Screening of 134 single nucleotide polymorphisms (SNPs) previously associated with type 2 diabetes replicates association with 12 SNPs in nine genes. **Diabetes 2007**; 56: 256−264.

8. Ruan H, Hacohen N, Golub TR, Van Parijs L, Lodish HF. Tumor necrosis factor-α suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3- L1 adipocytes. **Diabetes 2002**; 51(5): 1319−1336.

9. Vikram NK, Bhatt SP, Bhushan B, Luthra K, Misra A, Poddar PK, et al. Associations of −308G/A polymorphism of tumor necrosis factor(TNF)−α gene and serum TNF-α levels with measures of obesity, intra-abdominal and subcutaneous abdominal fat, subclinical inflammation and insulin resistance in asian Indians in north India. **Dis Markers 2011**; 31(1): 39−46.

10. Kubaszek A, Pihlajamäki J, Komarovski V, Lindi V, Lindström J, Eriksson J, et al. Promoter polymorphisms of the TNF-α (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes. **Diabetes 2003**; 52(7): 1872−1876.

11. Susa S, Daimon M, Sakabe J-I, Sato H, Oizumi T, Karasawa S, et al. A functional polymorphism of the TNF-α gene that is

associated with type 2 DM. **Biochem Biophys Res Commun 2008**; 369(3): 943–947.

12. Feng R-N, Zhao C, Sun C-H, Li Y. Meta-analysis of TNF 308 G/A polymorphism and type 2 diabetes mellitus. **PLoS One 2011**; 6(4):e18480.

13. Sookoian SC, González C, Pirola CJ. Meta-analysis on the G-308a tumor necrosis factor- α gene variant and phenotypes associated with the metabolic syndrome. **Obes Res 2005**; 13(12): 2122–2131.

14. Feng R, Li Y, Zhao D, Wang C, Niu Y, Sun C. Lack of association between TNF 238 G/A polymorphism and type 2 diabetes: a meta-analysis. **Acta Diabetol 2009**; 46(4): 339–343.

15. Kroeger KM, Carville KS, Abraham LJ. The −308 tumor necrosis factor-α promoter polymorphism effects transcription. **Mol Immunol 1997**; 34(5): 391–399.

16. Fernández-Real JM, Vendrell J, Ricart W, Broch M, Gutiérrez C, Casamitjana R, et al. Polymorphism of the tumor necrosis factor-alpha receptor 2 gene is associated with obesity, leptin levels, and insulin resistance in young subjects and diet-treated type 2 diabetic patients. **Diabetes Care 2000**; 23(6): 831–837.

17. Dalziel B, Gosby AK, Richman RM, Bryson JM, Caterson ID. Association of the TNF- α−308 G/A promoter polymorphism with insulin resistance in obesity. **Obes Res 2002**; 10(5): 401–407.

18. Hossain M, Faruque MO, Kabir G, Hassan N, Sikdar D, Nahar Q, et al. Association of serum TNF-α and IL-6 with insulin secretion and insulin resistance in IFG and IGT subjects in a Bangladeshi population. **Int J Diabetes Mellitus 2010**; 2(3): 165–168.

19. Paul S, Ahmed S, Khan I, Hassan Z, Ali L. Type 2 diabetes mellitus (T2D) subjects of Bangladeshi origin with fast N-acetyltransferase 2 (NAT2) acetylator phenotype show lower insulin sensitivity than slow acetylator phenotype. **Int J Diabetes Dev Ctries 2013**; 33(4): 213–218.

20. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. **Clin Chem 1972**; 18(6): 499–502.

21. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. **Diabetologia 1985**; 28: 412–419.

22. Guimaraes AL, Correia-Silva Jde F, Sa AR, Victoria JM, Diniz MG, Costa Fde O, et al. Investigation of functional gene polymorphisms IL-1beta, IL-6, IL-10 and TNF-alpha in individuals with recurrent aphthous stomatitis. **Arch Oral Biol 2007**; 52(3): 268–272.

23. Liu H-L, Lin Y-G, Wu J, Sun H, Gong Z-C, Hu P-C, et al. Impact of genetic polymorphisms of leptin and TNF-α on rosiglitazone response in Chinese patients with type 2 diabetes. **Eur J Clin Pharmacol 2008**; 64(7): 663–671.

24. Knowler WC, Pettitt DJ, Saad MF, Charles MA, Nelson RG, Howard BV, et al. Obesity in the Pima Indians: its magnitude and relationship with diabetes. **Am J Clin Nutr 1991**; 53(6): 1543S–1551S.

25. Song Y, Manson JE, Tinker L, Howard BV, Kuller LH, Nathan L, et al. Insulin sensitivity and insulin secretion determined by the homeostasis model assessment (HOMA) and risk of diabetes mellitus in a multi-ethnic cohort of women: the women's Health initiative observational study. **Diabetes Care 2007**; 30(7): 1747–1752.

26. Liu Z-h, Ding Y-l, Xiu L-c, Pan H-y, Liang Y, Zhong S-q, et al. A meta-analysis of the association between TNF-α −308G>A polymorphism and type 2 diabetes mellitus in han Chinese population. **PLoS One 2013**; 8(3):e59421.

27. Wu N, Zhou L, Guo D, Wang Y, Han P. Association of the promoter polymorphism of tumor necrosis factor alpha 308 G/A gene with the type 2 diabetes mellitus: a meta-analysis. **Chinese J Practical Internal Med 2010**; 30(10): 927–929.

28. Um JY, Lee JH, Joo JC, Kim KY, Lee EH, Shin T, et al. Association between tumor necrosis factor-alpha gene polymorphism and Sasang constitution in cerebral infarction. **Am J Chin Med 2005**; 33(4): 547–557.

29. Heijmans BT, Westendorp RGJ, Droog S, Kluft C, Knook DL, Slagboom PE. Association of the tumour necrosis factor α −308G/A polymorphism with the risk of diabetes in an elderly population-based cohort. **Gene Immun 2002**; 3: 225–228.