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Association of fat patterning, type 2 diabetes mellitus and MTHFR gene polymorphism: a study among the two ethnic groups of Tripura, North-East India

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ABSTRACT

Introduction. Type 2 Diabetes Mellitus (T2DM) is a group of metabolic disorders resulting from insufficient action of insulin. The etiology of T2DM is multi-factorial that includes genetic factors, obesity and lifestyles. Recent reviews of overall and stratified meta-analyses demonstrated the association between MTHFR polymorphism (C677T) including fat distribution and risk of T2DM. Publications of Indian context regarding fat patterning and MTHFR genetic polymorphism of the North East Indian population are insufficient and scant among the ethnic population of Tripura.

Aim. In this backdrop, the present study is the first attempt to understand the relationship of fat patterning, MTHFR gene polymorphism and T2DM among two Tibeto-Burman speaker endogamous ethnic populations (Chakmas-the migrant group and Tripuris – the aboriginal group) of Tripura, North East India.

Material and methods. The present study consists of age matched 280 males (Chakmas 147 and the Tripuris 133) from Tripura. Anthropometric and metabolic (Fasting Blood Glucose) variables and to discern obesity, blood glucose level and genotyping of MTHFR was performed following standard techniques.

Results. The result revealed significant (p<0.05) association of obesity, TT genotypes and fasting blood glucose among the Chakmas with in comparison to the Tripuris.

Conclusion. In this first attempt from North East India on the aspects of association of fat Patterning, Type 2 Diabetes Mellitus and MTHFR gene polymorphism suggests that the Chakmas are more diabetic, and this might be due to the concomitant effects of T alleles and higher central obesity and Percent Body Fat (PBF). More population screening from other under-represented indigenous populations of North East India is needed for prevention of metabolic disorders.

Keywords. fat patterning, MTHFR, obesity, T2DM

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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Introduction

Type 2 Diabetes Mellitus (T2DM) is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from insufficient action of insulin. In contemporary time, the prevalence of this disease is increasing steadily all over the world. T2DM is known to be a complex and heterogeneous disease resulting from a set of interacting factors that can be genetic or environmental and also behavioral. Patients living with T2DM are often at risk of facing both short-term as well as long-term complications and can often lead to premature death because nowadays this disorder is the ninth major cause of death. Factors like high blood glucose level and obesity affects T2DM patients more than a non-diabetic person.

The etiology of diabetes in India is multi-factorial and includes genetic factors coupled with environmental influences such as obesity associated with rising living standards, steady urban migration, and lifestyle changes. The majority of genetic variations associated with Type 2 Diabetes Mellitus are thought to act by subtly changing the amount, timing, and location of gene activity. Genetic variations likely act together with health and lifestyle factors to influence an individual's overall risk of type 2 diabetes. Recent research such as candidate gene approach and Genome Wide Association Studies (GWAS) in the field of type 2 diabetes genetics had until recently succeeded in identifying few genuine disease-susceptibility loci. Ongoing studies focusing on the role of copy number variation and targeting low frequency polymorphisms should identify additional T2DM-susceptibility loci. Increased genetic activity in genes like MethyleneTetraHydroFolateReductase (MTHFR) can result in problems like high glucose level or insulin secreting pathway which leads to the physical condition known as T2DM.

Furthermore, epidemiological studies found that general adiposity assessed by Body Mass Index (BMI) is a powerful predictor of type 2 diabetes, which has a strong relationship to diabetes and insulin resistance. Subsequently, regional obesity (abdominal obesity) as measured by high waist circumference has been proposed as a better predictor of risk of T2DM development. However, fat patterning associated with genetic polymorphism and T2DM seems to be a contemporary approach for this disease condition were reported in some works. In obese or overweight people, the level of pro-inflammatory markers, and other substances that are involved in the development of insulin resistance, is increased. Fat distribution in terms of abdominal obesity may cause fat cells to release pro-inflammatory chemicals and these chemicals can make the body less sensitive to the insulin it produces by disrupting the function of insulin responsive cells and their ability to respond to insulin.

Recent review of overall and stratified meta-analyses of the association between MTHFR polymorphism C677T and risk of type 2 diabetes mellitus, delineates a significant effect of C allele (CC genotype) compared to CT and TT genotypes. More than 15 different genes were investigated for their possible influence on plasma homocysteine levels of which MethyleneTetraHydroFolateReductase (MTHFR) was one of the most studied. MTHFR (677TT mutation) is associated with type 2 diabetes in and rs1801133 Single Nucleotide Polymorphism(s) (SNPs) has been found among the Asian population. A study also reported that the TT genotype of MTHFR C677T contributes to susceptibility to T2DM and supports the hypothesis that elevated Homocysteine is causally related to increased risk of T2DM. Indian studies reported variable result as no association with MTHFR C677T gene polymorphism and T2DM in South Indian Population and North East India has found the MTHFR gene polymorphic in both case and control for hyperhomocysteinemia.

India, occupying the center-stage of Palaeolithic and Neolithic migrations, found to be somewhat under-represented in genome-wide studies of variation. Being at the cross-roads of migration, Indian populations have undergone complex and ancient admixture events over a long period of time and have been the melting-pot of disparate ancestries originating from different parts of Eurasia and South-East Asia. Although the date of entry of modern humans into India remains uncertain but it is reasonable to consider that by the middle Palaeolithic period (50,000–20,000 years before present [ybp]), humans appear to have spread onto many parts of India. Contemporary ethnic India is a land of enormous genetic, cultural, and linguistic diversity. A more recent study exploring Indian genomic diversity demonstrated four major ancestral genetic components in mainland India that included four dominant ancestries in populations from mainland India: Ancestral North-Indian (ANI), Ancestral South-Indian (ASI), Ancestral Tibeto–Burman (ATB) and Ancestral Austro-Asiatic (AAA).

Aim

The literature reviews on fat distribution and genetic polymorphisms from North East India reveals scanty studies from Tripura. In this context, the present study to best of the knowledge, is the first attempt to discern the relationship of fat patterning, Type 2 Diabetes Mellitus and MTHFR gene polymorphism among two Tibeto-Burman speaker endogamous ethnic populations (Chakmas – the migrant group and Tripuris – the aboriginal group) of Tripura, North East India.

Material and methods

The present study consisted of one hundred forty seven (147) male participants from the migrant Chakma
population from Manu, Longthorai Valley of Tripura and one hundred thirty three (133) male participants from the aboriginal Tripuri population from Agartala, Tripura. Prior to the study verbal and/or written consent from each participant was obtained. Mouthwash was collected from all the participants in 15 mL centrifuge tubes. Genomic DNA was isolated from the mouthwash following a standard technique. The quantity and quality of DNA was checked by Spectrophotometry and gel electrophoresis. DNA was stored at −20 °C. Genotyping of MTHFR (C677T) (rs1801133) was performed using PCR-RFLP with the locus specific primers (Forward Primer was 5'-TGA AGG AGG TGT CTG CGG GA-3' and reverse primer was 5'-AGG ACG TGTCGGT AAG TG-3'). PCR product was digested with HinfI enzyme (Biolab) following the manufacturer's protocol. Restriction fragment size analysis was performed by visualization of digested PCR product after separation by 3% Agarose gel electrophoresis. Height, weight, waist circumference, and hip circumference were measured using standard techniques. BMI (Body Mass Index), WHR (Waist-Hip Ratio) and WSR (Waist to Stature Ratio) were calculated using standard formulas. Fasting blood glucose was measured using Accu-Chek blood glucose monitoring following the manufacturer’s protocol. The classification was done by the standard classification. Data checked and analyzed by SPSS (windows version 18.0). Allele frequencies for MTHFR allele frequency were estimated by MLH (Maximum Likelihood) estimation. Descriptive and Inferential statistics in terms of paired ‘t’ test was done to understand the mean difference between the two ethnic groups. On the other hand, genotype modeling has been done to find out the effects of the MTHFR genotypes along with odds ratio. Cut off was set as p = 0.05.

Results
Distribution of anthropometric and metabolic variables (Table 1) of the age matched participants revealed statistically significance (P<0.05) between the Chakmas and Tripuris. Except for the general obesity measured through BMI, Chakmas demonstrated significantly (P<0.05) higher stature, PBF and as well central obesity measured by WC and WHR in comparison to the Tripuris. Furthermore, significantly (P<0.05) higher mean fasting blood glucose level was found among the Chakmas compared to the Tripuris.

Distribution of MTHFR gene polymorphism (Table 2) demonstrated significant (P<0.05) difference in the genotypes between the Chakmas and Tripuris due to differential distribution (polymorphism) C and T homozygote and thus reflected on significantly (P<0.05) higher T alleles among the Chakmas in comparison to the Tripuris. The result of fasting blood glucose level (mg/dL) evinced higher prevalence of T2DM among the Chakmas in accordance to ADA (American Diabetic Association, 2016) and showed significantly (p<0.05) higher mean value (131.42±53.65 mg/dL) of fasting blood glucose level compared to that of the Tripuris (108.96±2.13 mg/dL).

Table 1. Anthropometric and metabolic characteristics of the studied population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Chakma (Mean±SD)</th>
<th>Tripuri (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.12±8.29</td>
<td>45.09±7.28</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>154.05±9.01*</td>
<td>151.32±7.47</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>55.40±11.28</td>
<td>54.31±10.11</td>
</tr>
<tr>
<td>BMI</td>
<td>23.02±4.55</td>
<td>23.70±4.34</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>86.85±10.32*</td>
<td>78.73±7.98</td>
</tr>
<tr>
<td>WHR</td>
<td>0.92±.06*</td>
<td>0.87±.03</td>
</tr>
<tr>
<td>PBF</td>
<td>28.82±7.52*</td>
<td>20.71±6.84</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>131.42±53.65*</td>
<td>108.96±21.83</td>
</tr>
</tbody>
</table>

*p<0.05, BMI - Body Mass Index; WC - Waist Circumference; HC - Hip Circumference; WHR - Waist to Hip Ratio; PBF - Percent Body Fat

Table 2. Distribution of MTHFR gene polymorphism among the Chakmas and Tripuris

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>N</td>
</tr>
<tr>
<td>Chakma</td>
<td>147 (87.07)</td>
</tr>
<tr>
<td></td>
<td>119</td>
</tr>
<tr>
<td>Tripuri</td>
<td>133 (90.98)</td>
</tr>
</tbody>
</table>

Figures in (parenthesis) denotes the percentage

Discussion
The overall cardinal result of the maiden study on these ethnic groups (Chakmas and Tripuris), one being migrant (The Chakmas) and another being aboriginal (The Tripuris) revealed that the Chakmas were significantly (p<0.05) heavier, obese and had significantly (p<0.05) higher fasting glucose level in comparison to the Tripuris. In accordance to WHO cut off Chakmas males revealed much higher WC (86.85±10.32 cm) and on the Tripuris demonstrated significantly (p<0.05) lesser WC (78.73±7.98 cm), which is within the normal range and
the present study found in corroborations with earlier studies from abroad and India.\(^7,8,9\) The present study also found the association of significantly (p<0.05) higher central obesity marked by WC and PBF along with significantly (p<0.05) higher TT genotypes and higher fasting blood glucose level (131.42±53.65 mg/dl), which is significantly higher than (IEC 2009, ADA 2010, WHO 2011) among the Chakmas. In contrast the Tripuris had lower fasting blood glucose level (108.96±21.83 mg/dL) associated with lower abdominal obesity, PBF and lower TT genotypes.\(^31\)

**Conclusion**

The first attempt from North East India with regard to the association of fat distribution, genetic polymorphism of MTHFR gene and T2DM gene polymorphism envisaged that the Chakmas are more diabetic than the Tripuris might be due to the concomitant effects of T alleles and higher central obesity and PBF. More population screening from much under-represented indigenous populations of North East India is needed for understanding and preventing of metabolic disorders.

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**References**


