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A STUDY ON THE ASSOCIATION OF ACE I/D GENE POLYMORPHISM, OBESITY, BLOOD PRESSURE AND SUSCEPTIBILITY OF TYPE 2 DIABETES MELLITUS AMONG THE KURMIS OF WEST BENGAL, INDIA.

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Abstract:

Earlier studies reported significant association of obesity, hypertension and Type2 Diabetes Mellitus (T2DM). Genetic and many disease-associated alleles have been identified through GWAS and applied to T2DM and indicated roles of renin-angiotensin system (RAS) in insulin signaling pathway and insulin resistance has been well documented. Angiotensin converting enzyme (ACE) gene catalyzes the conversion of angiotensin I to angiotensin II and also inactive the vasodilatation and hence renin-angiotensin system (RAS) in insulin signaling pathway and insulin resistance has been reported. To best of the knowledge we are reporting for the first time regarding association of ACE gene polymorphism with body composition, physiological and metabolic variables among any endogamous ethnic group (Kurmis) from of West Bengal, Eastern India. To achieve the purpose, total 197 (male 99 and female 98) randomly selected apparently healthy unrelated adult individuals of Kurmi population of Purulia District, West Bengal, India were incorporated in the present study. Anthropometric variables, physiological variables (blood pressure) and metabolic variables (PP blood sugar) have been collected using standard techniques. Extracted genomic DNA was PCR amplified and genotyped to understand ACE gene I/D polymorphism. The result demonstrated significant ($p<0.05$) sexual dimorphism in PBF. MAP and PP blood sugar found to be in normal range among the Kurmis. ACE gene polymorphism showed no deletion of the Kurmis and hence, only the prevalence of ACE II (insertion-Insertion) genotype has been noticed. The present study vindicated on the basis of body composition in terms of fat patterning, physiological and metabolic variables and ACE gene polymorphism that there is very low or no risk of T2DM among the Kurmis of West Bengal, India.

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Introduction:

Obesity is well recognized as a major risk factor for coronary heart disease [1], type 2 diabetes [2] and hypertension [3]. Diabetes Mellitus type 2 (DM2) is a metabolic disease that develops by a decrease in sensitivity of insulin receptors as an effect of the disruption certain metabolic functions in the processing of glucose. T2DM patients have, uncontrolled glucose levels, and commonly have problems with obesity and cardiovascular disease [4] has been debated [2] Epidemiological studies suggest that an excess deposition of fat in the abdominal region may be more predictive for the risk of myocardial infarction, stroke, and diabetes than in general obesity [5] Richard et al. 1995. Many technologies are available to measure adipose tissue and its distribution, such as the underwater weighing method, air-displacement plethysmography, dual-energy X-ray absorptiometry (DXA), computerized axial tomography (CAT) and magnetic resonance imaging (MRI). However, the use of these methods is limited, because of inaccessibility and the high cost of equipments. Thus, simple methods such as bioelectrical impedance analysis (BIA) and anthropometric methods are still the norm in field studies and for public use [6].

The number of individuals with diabetes is on the rise and the figure is expected to reach 552 million by 2030 owing to many factors such as population growth, aging, urbanisation, obesity, and physical inactivity [7]. Although environmental factors are reasons often considered, it is important to keep in mind that environmental factors, by themselves, represent only a fraction of cases. Individuals with family history of T2DM are at higher risk of developing the disease as shown in familial studies [8] suggest strong genetic contribution.

The genome-wide association studies (GWAS) allowing genetic and many disease-associated alleles have been identified through GWAS and applied to T2DM [9] However, the utilization of the candidate gene approach has been a pioneer and at a forefront of genetic association studies. Although, genome-wide significance level has limited the common and rare variants capturing, therefore, contribute to the missing heritability. Thus, candidate gene approach is still valuable inspite of the GWAS era. The roles of renin-angiotensin system (RAS) in insulin signaling pathway and insulin resistance has been well documented [10]. The blockade of the system has been found to have beneficial effects in the prevention of T2DM [11]. Angiotensin converting enzyme (ACE) gene is a potent vasoconstrictor and directly involved in the process of cell proliferation, differentiation, apoptosis and angiogenesis [12]. Contemporary study [13] reported ACE gene catalyzes the conversion of angiotensin I to angiotensin II and also inactive bradykinin. Bradykinin is vasodialotor which bringing about its proteolysis [14]. ACE gene is located on 17(17q13) and contains 26 exons. ACE gene has insertion-deletion (I/D) polymorphism at 287 bp region in intron 16 [15], which leads to three genotype DD, DI and II,. Variable findings in different populations on the association between (I/D) polymorphism in the ACE gene and Diabetic nephropathy has encourage to be studied on this matter [16]. Ethnic variation of ACE (I/D) polymorphism has been reported in earlier studies [17]. The polymorphism of ACE gene has been raising the susceptibility of T2DM in addition with environmental factors. [18]

In this circumstance, the present study is an attempt to understand the body composition in terms of fat patterning and or obesity and distribution of some physiological variables, and its association with ACE gene polymorphism. To best of the knowledge we are reporting for the first time regarding association of ACE gene polymorphism with body composition physiological and metabolic variables among any endogamous ethnic group from Eastern India.

MATERIALS AND METHODS

A total of 197 randomly selected apparently healthy unrelated (free from any major disease) adult individuals (male 99 and female 98) of Kurmi population of Purulia District, West Bengal, India were incorporated in the present study. Purulia is one of the severely under developed district of India in West Bengal. Anthropometric data were obtained using techniques [19]. Blood pressure (Systolic blood pressures SBP and Diastolic blood pressure- DBP) were also taken following the standard technique [19] and classified in accordance with the Seventh Report of the Joint National Committee (JNC-7) recommendation [20]. The mean arterial pressure (MAP) was derived (Equation: MAP = [(2 x diastolic) +systolic] / 3. Diastole counts twice as much as systole because 2/3 of the cardiac cycle is spent in diastole) to understand the average blood pressure in an individual as the average arterial pressure during a single cardiac cycle. Postprandial glucose (PP) Glucose level were measured, by "Advance Micro-draw" Glucometer (Hypoguard, Minneapolis, MN 55439, USA), strictly following instruction manual and classified following the American Diabetes Association criteria [21]. Reliability of all machine measurement was checked on a regular basis.

Majority of the participants were found to be involved different activities like agricultural labourer and or day labourer. Verbal informed consents were taken from the participants before the work. For obtaining socio-demographic information a specially prepared and pre-tested heart disease risk factor schedule containing information regarding socio demographic variables, physical activities, sleep duration etc. have been used. Derived measures regarding fat patterning and obesity (BMI, WHR etc.) were calculated using standard equations. Assessment of body composition in terms of fat patterning form different aspects of the body viz. PBF (Percent of body fat) using bioelectrical impedance analysis and other body composition variables for componential fat determination have also been done using body scanner (OMRON HBF- 300, OMRON Corporation, Tokyo, Japan). For ACE gene polymorphism genomic DNA was extracted from the buccal swab using standard technique [22] with slight modifications. PCR products were genotyped for I (Insertion) and D (Deletion) alleles of ACE gene using locus specific primer *viz.* Forward Primer-5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ Reverse Primer-5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′. PCR product was taken for 2.5% agar gel electrophoresis [15]. Obtained data were doubly checked and analyzed using the SPSS (Version- 16.0). Pearson's correlation has been done and cut off was set as $p=0.05$.

The Studied Population

The population for the present study is Kurmi, also known as the Kurmi- Mahato and Mahato, they are sometimes referred to as Koli-Kurmi, as they have been living with tribal of Chotonagpur plateau for centuries and have been instrumental in disseminating agricultural technology and craftsmanship among them. The Kurmis live in the North and South Divisions of Chotonagpur, Santhal parganas of Bihar, Mayurbhanj, Keonjhore, Sundargarh, Balasore and cuttack district of Orissa, Purulia, Bankura, midnapore, Burdwan, Birbhum, Nadia, 24 Parganas, north Bengal and tea garden of Assam [23] and observed a few endogamous subgroups such as Kurum, Adh-kurmi and Madhyam-kurmi and etc. The Kurmis of West Bengal also have surnames such as Mahato, Deb, Debsigha, Sinha, Pramanik and Majhi. They speak Kurmali at home and Bengali with others. They claim an equal social status with the Santhal, Bhumij, Kora, Mudi and others and maintain ceremonial friendship ties with them. [24].

Results

Distribution of Anthropometric and physiological variables (Table 1) revealed significant ($p<0.05$) sexual dimorphism in percent body fat (PBF) in terms of

significantly ($p<0.05$) higher PBF among the females. However, BMI being one of the general adiposity traits demonstrated no significant sexual dimorphism and eventually, showed the general adiposity as calculated from BMI being below normal for both the males and females.

Table 1. Distribution of anthropometric, physiological and metabolic variables of the studied population

Variables	Male (n=99) Mean ± SD	Female (n=98) Mean ± SD
Age(years)	45.13±13.86	56.00±11.00
BMI	21.79±3.640	22.30±4.79
PBF	27.07±7.035	36.81±6.91*
SBP(mm/hg)	140.32±23.34	146.45±32.51
DBP(mm/hg)	83.81±8.94	83.35±13.15
Blood Glucose (mg/dl)	105.74±25.88	103.77±24.27
Waist hip Ratio (WHR)	0.92±0.05	0.90±0.06

*p<0.05

No significant correlation has been revealed among the anthropometric, metabolic and physiological variables in the males (Table 2). However, significant correlation ($p<0.01$) was found (Table 3) between BMI and WHR and as well as BMI and PBF among the females (Table 3). Regression analysis (not shown) expressed 39% of variation of PBF to BMI, while WHR expressed about 21% variation among females However, no correlation for both the sexes have been noticed with physiological variable (MAP) and metabolic variable (PP blood sugar) and also found to be in normal range.

ACE gene polymorphism (Table 4) has been taken into consideration as potent vasoconstrictor and directly involved in the process of cell proliferation, differentiation, apoptosis and angiogenesis and the result demonstrated no deletion in both sexes. Therefore all the studied participants belong to II (Insertion-Insertion) genotype II.

Discussion

Current trend in Public Health research have created demand for relevant information from Biological Anthropology, in particular, and Social Sciences. The existing contributions reflect that Biological Anthropology can provide significant new theories and data that dramatically expand the understanding of different processes that known to affect public health. Furthermore, such work demonstrated the capacity to expand the understanding of "*normal*" human biology across the life course. Analysis of specific local contexts and patterns of temporal variation, using a socio-ecological and lifespan perspective, posed challenges to notion of panhuman biological uniformity and the search for a single descriptive frame work in human biology. Biological Anthropology integrates multiple level of analysis, from ultimate (evolutionary) to proximate (molecular) causes. T2DM once considered as a disease limited to developed counties, the occurrence of

Table 2. Distribution of correlations of different anthropometric and physiological variables among the males

	BMI	WHR	PBF	BLOOD SUGAR (PP)	MAP
BMI	1	-0.006	0.295	0.102	0.136
WHR	-0.006	1	0.114	0.056	0.047
PBF	0.295	0.114	1	0.042	0.000
BLOOD SUGAR (PP)	0.102	0.056	0.042	1	0.072
MAP	0.136	0.047	0.000	0.072	1

Table 3. Distribution of correlations of different anthropometric and physiological variables among the females.

	BMI	WHR	PBF	BLOOD SUGAR (PP)	MAP
BMI	1	0.458**	0.626**	0.189	0.195
WHR	0.458**	1	0.240	0.109	0.064
PBF	0.626**	0.240	1	0.205	0.252
BLOOD SUGAR (PP)	0.189	0.109	0.205	1	0.081
MAP	0.195	0.064	0.252	0.081	1

**p< 0.01 level

Table 4. Distribution of ACE gene genotype of the studied participants.

ACE gene polymorphism	Male	Female
DD genotype	0	0
DI genotype	0	0
II genotype	99	98

diabetes mellitus is increasing throughout the world, both in industrialized and in developing countries with the developing countries experiencing an accompanying epidemiological transition [25] Since the publication of Thompson (1929) [26] on the theory of demographic transition, the notion of epidemiological transition was introduced by Omran (1971) [27] to describe the changes in health, occurring during the demographic transition, where the largest burden of disease gradually shifts from infectious diseases to chronic, non-communicable diseases (NCDs) like T2DM. Subsequently it was revealed that T2DM as one of the major categories of NCDs is associated with a two- to four-fold excess risk of death from coronary heart disease (CHD) [28]. Thus, considering epidemiological transition as an effect of demographic transition contemporary studies [29] recognized T2DM as much a disease of poor and disadvantaged people as it is of fat and unfit people. Association of T2DM and obesity [30] has also become an important health problem in developing countries particularly in India [31], which is currently experiencing a rapid epidemiological transition. Positive association of obesity with T2DM has been established repeatedly in many cross-sectional and prospective studies [32-34]. Thus, in order to develop ethnic specific T2DM prevention strategies, the relationship of anthropometric measures to T2DM risk factors requires in depth elucidation. The genome-wide association studies (GWAS) allowing genetic and many disease-associated alleles have been identified through GWAS and applied to T2DM [9]. Hence, eventual contemplation for the potential link between obesity, hypertension and insulin resistance genes could stand as prospective area of research on biomedical aspect concerning to public health. In this context, the present study reports no significant excess adiposity among the Kurmis. However, PBF was found to be significantly ($p<0.05$) higher among the females might be due postmenopausal age (56.00±11.00 years) and eventually corroborative with earlier studies [35, 36].

With regard to blood pressure and PP blood sugar all the participants of Kurmis of the present study has been found to be in normal range. ACE I/D gene polymorphism revealed only II (insertion – insertion)

genotypes among the Kurmis. Since the present study on Kurmis, is the maiden attempt, comparison of the results regarding anthropometric, physiological, metabolic and ACE gene polymorphism remained unresolved. The present study also vindicated on the basis of body composition in terms of fat patterning, physiological and metabolic variables and ACE gene polymorphism that the Kurmis might not be susceptible for T2DM. However, limitation of this study is the sample size.

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