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Study Protocol

Association of genetic variant of OXTR (rs53576) and MTNR-1B (rs10830963 and rs1387153) with symptoms of stress in Pakistani women with Gestational Diabetes.

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Abstract

Background: Pregnancy continues to be an event full of risks, complications and adverse outcomes for women despite advanced clinical and diagnostic methods and treatment options available in medicine. Diabetes, as a multifactorial and complex disease, is a possible outcome of a blend of numerous genetic variants of differing scarcities. The exact etiology of gestational diabetes mellitus (GDM) is unclear; however, the interaction of genetic and environmental factors, especially the genetic variants identified as the genetic loci for type 2 diabetes as candidate genes for GDM.

Methodology: It will be a case-control study with a genetic analysis of single nucleotide polymorphisms (SNPs) of candidate genes expressed using real-time PCR with sequencing. The study will also evaluate the primer sequence of SNPs.

Discussion: We will be able to share the findings after the completion of our research. The results of this study will able helpful in addressing the psycho-physiological needs of women with GDM, which may also be used to devise strategies to benefit the sustainable long-term behavioural change following the affected pregnancy.

Keywords

Gestational Diabetes Mellitus, Stress, OXTR, MNTR1B, Single Nucleotide Polymorphism.

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Introduction

Pregnancy continues to be an event full of risks, complications and adverse outcomes for women despite advanced clinical and diagnostic methods and treatment options available in medicine. One such complication is an alteration of glucose metabolism due to insulin resistance under the influence of placental hormones. In > 25% of pregnancies, it may progress into gestational diabetes mellitus (GDM) during the 2nd or 3rd trimester[1]. Diabetes, as a multifactorial and complex disease, is a possible outcome of a blend of numerous genetic variants of differing scarcities.

The exact etiology of GDM is unclear; however, several recent studies have highlighted the interaction of environmental and genetic factors, especially the genetic variants identified as the genetic loci for type 2 diabetes as candidate genes for GDM. Chromosome 3 contains the OXTR gene responsible for expressing Oxytocin- a nine-amino acid neuropeptide hormone produced in the hypothalamus. Oxytocin is involved in various biological effects, including social behaviours like the stress response, feeding behaviour and others. Oxytocin excess has been found associated with diabetes mellitus as it is involved in glucose homeostasis (gluconeogenesis, glycogenesis and glycogenolysis) by affecting insulin secretion and metabolism[2,3]. Studies have been evaluating the role of genetics in pathogenesis of diabetes especially GDM where many polymorphisms have been linked with DM[4]. However; a triad of the role of OXTR gene in influencing the stress among women presenting with GDM is yet to be thoroughly understood and cleared[5].

The melatonin receptor type 1B (MTNR1B) gene is positioned on an 11q14.3 locus and encodes receptors for melatonin[6]. MNTR1B plays a role in glucose metabolism regulation, and its overexpression polymorphisms (s10830963 and rs1387153) variants lead to impaired insulin secretion, impaired glucose homeostasis, and raised fasting plasma glucose levels. Thus; responsible for GDM in women ($p \leq 0.01$)[2,3,7] MTNR1B is second most frequently associated gene with GDM (15%). Interaction between MTNR1B polymorphism and lifestyle changes like stress among pregnant women with GDM have been noted[8,9]. GDM is associated with a variety of adverse outcomes for mother and child, especially the potentially modifiable psychological problems ranging from mental stress, anxiety and depression[10]. Limited studies have explored that continued exposure to psychological and environmental stressors is associated with maternal hyperglycemia during gravidity, which may enhance the threat of GDM[11].

There is rapidly growing evidence of genetic involvement in the development of chronic non-communicable diseases like GDM. GDM can also have an adverse influence on the mental wellbeing, physiological functioning and quality of life affected. The scarce studies on GDM and mental wellbeing advocate that the diagnosis of GDM is a threat for further health hazards like stress and anxiety during pregnancy, antenatal and postpartum depression, with probable adverse effects on treatment adherence[12,13]. The apparent stress of effectually managed GDM through lifestyle change coupled with the distress of catastrophe to accomplish glycemic control may cause depressive symptoms. The management of GDM is centered on glycemic control to prevent adversative obstetric consequences, while the possible influence of the diagnosis on women's psychological and emotional wellbeing is rarely considered in managing the condition[14,15].

There is no research work conducted in a local setting, highlighting the relationship of stress with GDM in the background of genetic mutations. Thus, exploring the genetic profile of pregnant women is of paramount importance. Identifying the epigenetic mechanism of stress and GDM can benefit sustainable long-term behavioural change measures to address the mental health of pregnant women. Further, the study will help the health providers treat the pregnant woman in a best-suited way and determine whether the newborn to a woman will be predisposed to psychological disorders in the future.

Methodology

Objective

The current study aims to describe and quantify the association of candidate genes (OXTR & MTNR1B) SNPs with psychological symptomatology in women with GDM. This information will simplify the provision of directed psychological mediations pre and early pregnancy to manage stress. These interventions will play a pivotal role in controlling the psychological stress symptoms during early pregnancy among women with GDM. Additionally, exploration of the genetic causes of GDM will ultimately give an improved understanding of the pathophysiological mechanisms that contribute to the disease.

The objectives of this study are:

Primary Objectives

- To measure the OXTR (rs53576) gene polymorphism with symptoms of stress in gestational diabetic women.
- To evaluate the association of OXTR (rs53576) gene polymorphism with symptoms of stress in gestational diabetic women.
- To evaluate the association of MNTR 1B (rs10830963 and rs1387153) gene polymorphism with symptoms of stress in gestational diabetic women.

Secondary Objectives

- To assess the effects of single nucleotide polymorphism on the presentation of women with gestational diabetes mellitus.
- To associate the biochemical derangements in GDM and stress with SNP in various forms of stress.

Study design

It will be a case control study with genetic analysis of SNPs of candidate genes will be expressed using real time PCR with sequencing. The study will also evaluate the primer sequence of SNPs.

Ethics

Ethical permission for the present study has already been opted by the Institutional Review Committee University of Karachi (Approval No. IBC KU-191/2021).

Participants

Pregnant women presenting to the outpatient department for antenatal care and those diagnosed with gestational diabetes mellitus will be approached to consent to participate in the study. GDM patients who fulfill the selection criterion will be registered after they provide verbal and written consent. Informed consent will be obtained from each study subject after the nature of the study is fully explained.

Eligibility criteria

Inclusion criteria:

- 18 years of age
- Diagnosed case of gestational diabetes mellitus (as per operational definitions)
- Consent to participate in study

Exclusion criteria:

- Pre-existing diabetes mellitus
- Polycystic Ovary Syndrome
- Multiple gestation
- Autoimmune disease
- Chronic systemic disease like (Cardiovascular, Urogenital, Immunological)
- Preeclampsia, pregnancy induced hypertension, and hypertension

Assessment Procedures

General physical examination

A general physical examination will be performed on each study subject. The vital signs, anthropometric measurements, assessment of potential causes of stress, along with the identification of medical problems will also be noted in the proforma.

Sample collection and assay analysis procedure

Fasting whole blood samples will be collected in the second trimester (24-48 weeks) to analyze basic biochemical data, including fasting glucose, lipid profiles, and HbA1c during the pregnancy course. For Genetic evaluation, DNA will be extracted by a commercially available Promega Genomic DNA extraction kit, and its quantification

will be performed by gauging the UV absorbance of all the samples using a Nanodrop-ND1000 by Thermo Fisher Scientific, Waltham, MA.

Analysis of single nucleotide polymorphism
Polymerase Chain Reaction (PCR) will be performed.

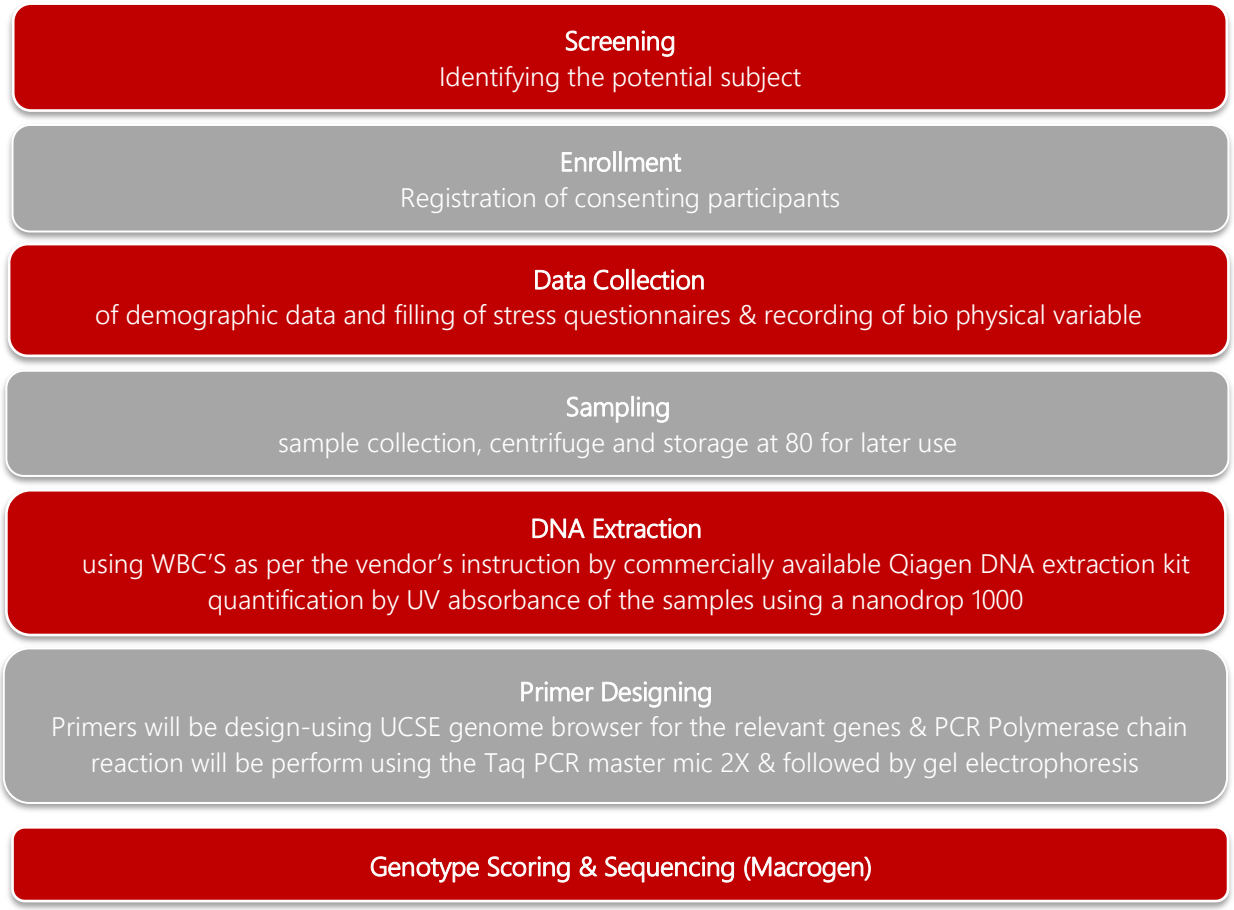

Figure 1: Flowchart of the study procedure

Genotyping and sequencing of PCR products
An independent technical staff (blinded about the case control status of the study subjects) will score genotypes. With genotype quality controls to be performed in 10% of the samples by duplicate checking and further reconfirmation of genotyping accuracy to be achieved by sequencing several samples with detected polymorphic variants.

Perceived stress scale (PSS10)
The Perceived Stress Scale (PSS10) will be used to assess stress levels of the participants according to proven protocol.

Diabetes-related stress scale
An eight items scale to assess patients for psychological adaptation and acceptance towards diabetes of assess.

Sample Size
Sample size estimation is based on available data of OXTR (rs53576) and MNTR 1B (rs10830963 and rs1387153) gene polymorphism frequency. Allele risk data will be applied using STATA 14 (Stata Crop, College Station, United States), considering an 80% power & a two-tailed alpha of 0.05. Sample size of 50 in each group will be enough to detect an association between the alleles and GDM.

Duration

The study will be conducted over 2 years, starting from July 2021 and ending in July 2023, in Karachi, Pakistan. DNA extraction will be performed on-site AT Aga khan university Hospital, and genotyping will be performed at the Macrogen Japan Genome analysis and clinical diagnostics service

Statistical Analysis

Data will be analyzed using IBM SPSS version 25 with descriptive analysis of categorical data to be shown as frequencies and percentages, while continuous variables will be articulated as mean and standard deviation. To compare continuous and categorical variables, the Mann-Whitney U test, Kruskal–Wallis H test and Pearson's $\chi 2$ test of independence will be used wherever valid. For both SNPs, Hardy-Weinberg equilibrium (HWE) will be calculated, and data will be studied for allele frequency and genotype determination by applying $\chi 2$ statistics. Connotation and effect size of the minor allele with study parameters will be resolute with regression analyses. A p-value of <0.05 will be considered significant in all calculations. Receiver-operating characteristic (ROC) analysis will be performed to establish the predictive ability of OXTR (rs53576) and MNTR 1B (rs10830963 and rs1387153) gene polymorphism concentration for stress in women with GDM and find out the cut-off value by Youden index.

Discussion

The presence of stress in pregnancy and GDM adversely influence maternal health, increases the likelihood of obstetric complications, preterm birth, and neonatal complications[16,17] on the one hand. In contrast, it may usually increase the risk of stress and anxiety on the other hand[18,19]. Although; GDM is a transient disorder in the majority of pregnancies but, it is inclined to be superimposed by stress, which has long-lasting adverse effects on maternal and child health[20]. If left untreated, it can cause or exaggerate after-delivery problems, including postpartum depression.

It has also been suggested that pre-existing depression and stress during early and mid-pregnancy can also be a risk factor for glucose intolerance in later pregnancy; this may be a bidirectional relationship between psychological stress disorders and GDM in pregnancy[21]. Many environmental, nutritional and genetic factors are related to the development of GDM, which potentiates the stress condition/ symptomatology of pregnancy. Among the genetic factors, OXTR & MNTR1B single nucleotide polymorphisms (SNPs) have been of particular focus[22]. Although, there are studies that found an association between OXTR & MNTR1B SNPs gestational diabetes while other studies documented involvement of OXTR & MNTR1B SNPs in psychological disorders like depression, anxiety and/or stress we could not find out studies in our population which have evaluated the association of the above given genetic risk factors with stress development in GDM.

We will be able to share the findings after the completion of our research. We expect to see a positive association between single nucleotide polymorphism of Oxytocin receptor genes (OXTR rs53576) and Melatonin receptor type 1B (MTNR1B rs10830963 and rs1387153) with stress symptoms among GDM women in the Pakistani population. The results of this study will able helpful in addressing the psycho-physiological needs of women with GDM, which may also be used to devise strategies to benefit the sustainable long-term behavioural change following the affected pregnancy.

Conflicts of Interest

The authors have declared that no competing interests exist.

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