IDENTIFICATION OF V59L AND A953G GENOTYPES DISTRIBUTION IN AQP7 GENE AND THEIR ASSOCIATION WITH GLYCEROL IN OVERWEIGHT/OBESE MALAY PATIENTS

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Identification of V59L and A953G Genotypes Distribution in AQP7 Gene and Their Association with Glycerol in Overweight/Obese Malay Patients

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Abstract:

Introduction: One of the major public health issues that contributes to cardiovascular risk factors is obesity. Previous evidence suggested that abnormal glycerol metabolism and aquaglyceroporin 7 (AQP7) dysfunction in promoting glycerol influx and efflux from the adipose tissue are among the mechanisms involved in obesity. This pilot study aims to identify the genotype distribution in *AQP7* polymorphisms and to find their association with plasma glycerol.

Methodology: A cross-sectional study was undertaken at Hospital Universiti Teknologi MARA (HUiTM) Sungai Buloh, Selangor, Malaysia, to enroll 100 study participants (56 normal and 44 overweight/obese participants). Anthropometry data was collected from all participants. Blood samples were taken by venipuncture to measure plasma glycerol and the genotypes of two SNPs in AQP7 (V59L rs4008659 and A953G rs2989924) were determined for both groups. The genotype distribution and allele frequencies of both SNPs in the *AQP7* were determined and their association with plasma glycerol was estimated by logistic regression.

Results: Participants in the overweight/obese group had higher plasma glycerol (median = 0.78 mg/dL, Q1-Q3=0.47-1.42) compared to the normal group. V59L and A953G genotypes distribution between normal and overweight/obese groups showed no significant difference. Logistic regression analysis showed that participants with A953G (rs2989924) TC genotype had 71% decreased risk of developing abnormal plasma glycerol when factors such as age, gender, and waist: hip ratio (WHR) were controlled. Genotypes of V59L (rs4008659) did not show any association with plasma glycerol.

Conclusions: This study highlights the potential role of A953G (rs2989924) TC genotype in reducing the risk of having impaired glycerol metabolism reflecting its potential protective nature against obesity.

Keywords— **Adipose tissue, aquaglyceroporin, glycerol, obesity, polymorphisms**

INTRODUCTION

Obesity is a major global healthcare problem attributed to many factors including genetic, lifestyle, environmental and modernization. As part of the metabolic syndrome (MetS), obesity is linked to other diseases such as Type 2 diabetes mellitus (T2DM), hypertension and dyslipidemia which leads to the increased risk of developing cardiovascular diseases (Da Silva *et al.*, 2017). The 2019 National Health and Morbidity Survey (NHMS) found that 50.1% of Malaysian adults were overweight or obese, and 52.6% were centrally obese. Compared to the results from 2011 and 2015, Malaysia's trends for overweight and obesity are still on the rise (NHMS 2019). In addition to that, the cost of treating obesity and its related complications pose a significant economic burden. Hence, this underscores the importance of determining the risks for obesity in order to initiate lifestyle changes much earlier to prevent its development and its subsequent complications.

Mutation of the aquaglyceroporin 7 gene (AQP7) has been linked to the pathogenesis of obesity. (Lebeck *et al., 2014;* Maeda *et al, 2012;* Hibuse *et a., 2005*). Glycerol outflow from adipose tissue into the bloodstream is facilitated by AQP7 channel protein mainly found in adipocytes. The glycerol is then taken up into the liver as an important source for gluconeogenesis and energy substrate to produce ATP (Maeda *et al,* 2012). The predisposition to obesity thru abnormal glycerol metabolism has been linked with dysfunctional AQP7 expression and associated polymorphisms, where changes in AQP7 expression are determined by several factors such as fasting–refeeding state, exercise, insulin levels and others (Kishida *et al.*, 2000). Studies have shown that AQP7-knockout (AQP7-KO) mice exhibited a significant rise in fat mass and adipocyte hypertrophy as a result of their adipocytes' buildup of glycerol and triglycerides (TG), resulting in abnormal glycerol release leading to obesity, insulin resistance and impaired plasma glycerol (Maeda *et a.*, 2004; Hara-Chikuma *et al*, 2005; Hibuse *et a.*, 2005; de Luis *et al*, 2017; Iena *et a.*, 2018).

Numerous studies have suggested a link between *AQP7* expression or polymorphism and obesity in humans, which is consistent with AQP7's function in controlling adipocyte metabolism. Prudente et al. were the first to identify a polymorphism common to Caucasians, A953G (rs2989924), which is positioned in the promoter region of AQP7 and is postulated to be linked to obesity. (Prudente *et al.*, 2007). A similar study involving 400 obese patients also showed an association with *AQP7* A953G mutation within the Chinese Han population (Wang *et al.*, 2018). However, there was also conflicting findings where one study showed no association between single nucleotide polymorphisms (SNPs) of *AQP7* variants [G264V (rs62542743), V59L (rs4008659) and R12C (rs777690481)] and obesity among Japanese cohort (Ceperuelo-Mallafre *et al*, 2007), whilst another study by de Luis et al. found that obese Spanish subjects' adipocytes had higher levels of AQP7 (*AQP7* upregulation) than their non-obese counterparts. (de Luis *et al.* ,2017). These conflicting reports and limited studies on obesity-related genetic mutation

among Asians prompts for further investigation. To the best of our knowledge, no Malaysian cohort has been recruited for similar studies. In addition, *AQP7* genetic studies have been limited to cell culture and animal model experiments which may or may not translate accurately to humans. These research gaps warrant further exploration to determine if, in fact, there is any relationship between *AQP7* expression and plasma glycerol levels among obese subjects and whether population differences may influence such expressions.

We hypothesized that there is a predominant genotype within *AQP7* polymorphisms among overweight/obese subjects in our Malay population from Malaysia, giving rise to abnormal plasma glycerol. This study's objective was to determine the genotype distribution of the AQP7 genes V59L (rs4008659) and A953G (rs2989924) and to explore their association with plasma glycerol, among normal and overweight/obese subjects.

METHODOLOGY

Study population and design

Between November 2020 and December 2021, a comparative cross-sectional study was conducted at the Hospital Universiti Teknologi MARA (HUiTM) in Sungai Buloh, Selangor, Malaysia. From 100 subjects who were recruited in this study, they were divided into normal and overweight/obese groups based on their body mass index (BMI). Subjects in the normal group were recruited from health screening events whereas those in the overweight/obese group were recruited from the Endocrine and Lipid follow-up clinics. Based on Malaysia's 19.7% obesity prevalence, the sample size was calculated using the Sample Size Calculator (Calculator.net) (NHMS, MOH, 2019), considering the error of margin at 10% and power of study at 90% which derived a minimum sample size of 44 in each group. This study adheres to the Declaration of Helsinki and was approved by the Research Ethics Committee of Universiti Teknologi MARA (REC/06/2021(FB/32). Recruitment of participants and collection of any sample or data was only done upon prior written informed consent.

Inclusion and Exclusion Criteria

For the normal group, we included adult males or females aged between 18 and 65 years with BMI between 18.5 - 22.9 kg/m2, based on the BMI cut-off point for adult classification by WHO Expert Consultation and Malaysia Ministry of Health (MOH) Clinical Practice Guideline on Management of Obesity (WHO, 2004, MOH, 2004). For the overweight/obese group, we included adult males or females aged between 18 and 65 with BMI > 22.9 kg/m^2. We excluded participants in either group who had 1) Diabetes mellitus, 2) other diseases causing obesity (such as hypothyroidism, Cushing's syndrome or polycystic ovarian

syndrome), 3) being on oral contraceptives or other medications, 4) having a malignancy, 5) being pregnant, or 6) being mentally impaired that informed consent cannot be obtained.

Data and sample collection

Demographic data and Biochemical analysis

The physician interviewed the participants during the consultation to complete a structured clinical report form which includes details on demographic background, lifestyle information, medical and family history. All subjects had their blood pressure (BP) and anthropometric measurements such as weight, height, waist, and hip circumference taken. Using a standardized measuring tape wrapped around the abdomen at the level of the iliac crest, the measurement of waist circumference was done at the end of a normal expiration. (NHLBI, 2000). By dividing the body weight (kg) and squares of height (m^2), the body mass index (BMI) was calculated (kg/m^2). BP was measured with the subject sitting comfortably and using the manual sphygmomanometer for overweight/ obese patients recruited from the Lipid Clinic. The OMRON BP set was used for healthy subjects recruited during health screening programs. The final blood pressure reading was calculated as the average of three consecutive readings.

Blood samples were collected into a plain and EDTA tube following an overnight fast. A total of 10 mL sample was withdrawn by standard venipuncture. Prior to storing at -80°C and sample analysis, serum and plasma were separated by centrifugation at 4000 rpm for 7 minutes. Colorimetric analysis was applied to measure the plasma glycerol level, using a free glycerol assay kit by Cell Biolab's Inc Free Glycerol Assay Kit (San Diego, USA). Fasting plasma glucose and lipid profile results from overweight/obese subjects were extracted from the hospital information system (HIS) within the last six months. In addition, utilizing an automated Roche Cobas c501 platform (Mannheim, Germany), plasma glucose and lipid profile (total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and triglyceride (TG)) were analysed in normal subjects. To determine low-density lipoprotein cholesterol, the Friedewald equation was applied (LDL-c) (Knopfholz et al., 2014).

Study variables

Two primary independent variables included in this study were two SNPs of the *AQP7* V59L (rs4008659) and A953G (rs2989924). The dependent variable was glycerol which is further classified into normal glycerol level (0.4-1.2 mg/dL) and abnormal glycerol level (<0.4 mg/dL or >1.2 mg/dL). Based on the Framingham Risk Score, eleven parameters of confounding variables were noted to be frequently linked to cardiovascular risk (MOH, 2017; D'Agostino et al., 2008) which are age (years),