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Assessment of Silent Information Regulator of Transcription Polymorphism Frequency among Female Hypertensive Patients: A Cross-Sectional Study.

Ibrahim Munaf Ahmed [1], Muhammad Saad Hafeez [2], Syeda Sadia Fatima[3*]

[1]*Department of Primary Care and Population Health, Stanford Healthcare, California, USA*
[2] *Department of Surgery, Henry Ford St John Hospital, Detroit, MI, USA*
[3]*Department of Biological and Biomedical Sciences, Aga Khan University, Karachi*

Abstract: The prevalence of Silent Information Regulator of Transcription Polymorphism (SIRT) variants about obesity and hypertension susceptibility remains a subject of interest. This study evaluated the frequency of the SIRT1 rs369274325 polymorphism among hypertensive pregnant females. In this cross-sectional study at Aga Khan University from March 2017 to May 2020, 120 pregnant women aged 18-45 were grouped into Normal Weight Hypertensive, Obese Hypertensive, Normal Weight Normotensive, and Obese Normotensive categories. Excluding individuals with pre-existing diabetes, gestational diabetes, inflammatory conditions, or using oral contraception/hormonal support, venous blood (3ml) was collected for DNA extraction. Tetra arms polymerase chain reaction (T-arms PCR) targeted SIRT1 rs369274325 polymorphism, with subsequent agarose gel electrophoresis for PCR product analysis. While age matching was achieved across groups (p= >0.05), differences were seen for BMI, systolic, and diastolic blood pressure among the groups (p= <0.05). No disparities in allelic and genotypic distributions of the SIRT1 polymorphism were detected across the four groups, with a minor allele frequency (MAF) of 0 (p=1.00). The findings suggest that the SIRT1 rs369274325 GG genotype prevails within the local population and may not exhibit a direct association with hypertension.

Key words: Hypertension; Obesity; Pregnancy; SIRT1; sirtuins

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***Correspondence:** Syeda Sadia Fatima, Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, 74800, Pakistan. Phone: +9221-34864147, Email: sadia.fatima@aku.edu, ORCID ID: 0000-0002-3164-0225

Introduction

Obese individuals suffer many detrimental impacts on their lifestyle as well as a raised health risk, including dyslipidaemia, hypertension, coronary heart disorders, stroke, type 2 diabetes, shortness of breath, and sometimes early death [1]. The current pathophysiology of hypertension is poorly understood while the role of gene polymorphism and over/under expression is still poorly understood [2].

Silent Information Regulator of Transcription polymorphism (SIRT) (10q21.3) is involved in a vast array of physiological actions through its role as a nicotinamide adenine dinucleotide-dependant histone deacetylase. It regulates smooth muscle cell functions and vascular disorders [3, 4]. Moreover, SIRT1 is associated with inherited disorders of one-carbon metabolism, autophagy-related endocrine disorders, neurological and hepatic diseases [5-8]. Seven sirtuins are found in humans,

represented by the genes SIRT1 to SIRT7. SIRT1 is being newly termed as a master metabolic regulator due to its influence and/or control over numerous intermediaries important in metabolic homeostasis in different organs, which translates to metabolic influence over the entire body [9]. Some processes that are known to be influenced by SIRT1 include gluconeogenesis, fat metabolism and release, insulin release, and fat loss during low caloric intake (Figure 1).

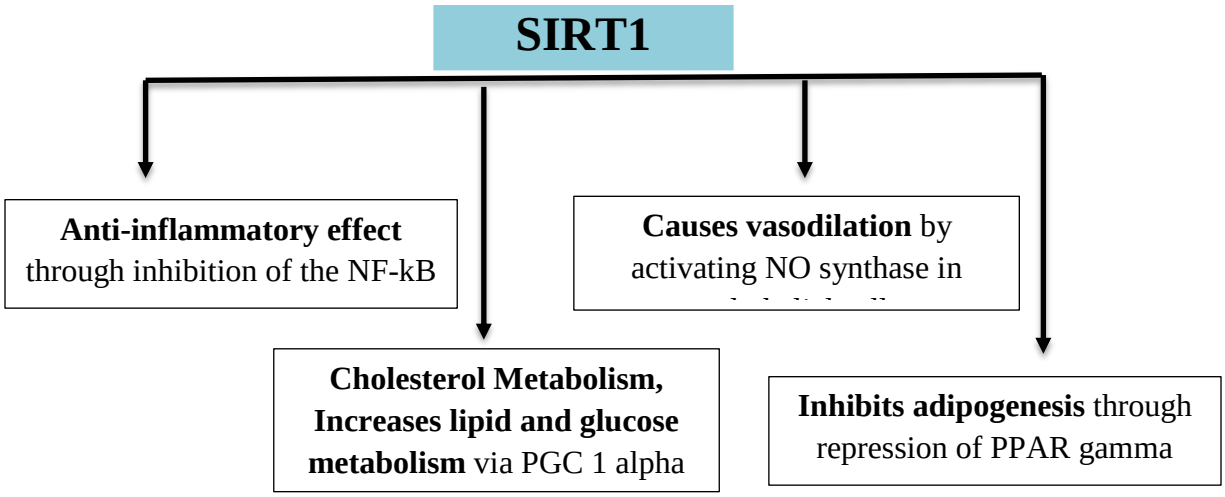

Figure1: Schematic representation for SIRT 1 functions

Where: PGC 1 alpha= Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPAR gamma= Peroxisome proliferator-activated receptor gamma; NO= Nitric Oxide; NF-kB= Nuclear Factor kB.

**Note that most of the metabolic effects shown above occur through SIRT1 induced repression or expression of genes.*

Studies in twins have demonstrated that SIRT1 and SIRT3 expressions are inversely correlated with different measures of adiposity which inversely vary with inflammatory states. Thus, a decrease in the NAD+/SIRT pathway is associated with obesity, insulin resistance, and inflammation [10]. Hypertensive disorders of pregnancy, comprising gestational hypertension and pre-eclampsia, affect approximately 10% of pregnancies and pose a significant health concern [11]. Women who experience hypertensive disorders of pregnancy encounter a doubled risk of developing cardiovascular disease later in life compared to those with normotensive pregnancies [12]. Given that cardiovascular disease represents a leading cause of mortality globally, with women typically developing cardiovascular disease 10-15 years later than men, early intervention strategies are paramount [13].

Through this study, we hypothesized that SIRT1 promoter region variant rs369274325 G/T polymorphisms near the 5' end of the gene may account for differential SIRT1 expression, rendering individuals susceptible to certain pathologies such as obesity and hypertension. Therefore, we assessed the frequency of SIRT1 rs369274325 polymorphism and relate genotypes across BMI categories in hypertensive pregnant females visiting a tertiary care hospital.

Methods

Study Design and Population

This cross-sectional study comprising 120 pregnant women aged 18-45 years was conducted at Aga Khan University from March 2017 to May 2020. The sample size was calculated using "Open-Epi website" [14] based on a 95% confidence level, 80% power, a minimum odds ratio of 2, and an estimated prevalence of pregnancy hypertension at 8% from previously published data sources [15]. The calculated minimum sample size was determined as n=106. Ethical approval was obtained from the institutional ethics committee (Ref # 4523-BBS-ERC-16 and 2019-1309-3708). All participants provided written informed consent after receiving full information about the study's purpose, procedures, risks, and benefits, in line with Declaration principles.

Sample Collection

Three milliliters of blood was collected after an overnight fast. Weight and body mass index (BMI) assessments followed South Asian criteria for BMI values: normal weight (BMI 18-22.9 kg/m²) and obese (BMI ≥26 kg/m²) [16]. Blood pressure (BP) was assessed according to the latest "European Society of Hypertension (ESH) task force guidelines" [17] (Normal BP <139/85 mmHg and hypertension >139/85 mmHg).

Individuals with pre-existing diabetes, gestational diabetes, inflammatory conditions, or using oral contraception/hormonal support were excluded. Subjects were categorized into groups based on readings: A. Normal Weight Hypertensive (n= 30), B. Obese Hypertensive (n=30), C. Normal Weight Normotensive (n=30), D. Obese Normotensive (n=30)

DNA Extraction

DNA was extracted using a Qiagen DNA extraction kit (Cat. #51185, Valencia, CA, USA). Tetra arms polymerase chain reaction (PCR) using Ruby Taq PCR Master mix 2X (Cat. #71191, Affymetrix, USA) was performed as per the manufacturer's instructions. PCR products were electrophoresed in a 2% agarose gel. Genotyping quality control included duplicate checking (>99% concordance rate).

Primers used for SIRT1 rs369274325 gene polymorphism amplification were: Forward and Reverse Outer (336bp): TAGGTTCCATACCCCATGAAG; CATTACTCTTAGCTGCTTGGTC Forward Inner (G allele 229bp): GAATTGTGTCATAGGTTAGGAGG, Reverse Inner (T allele 152bp): ACAGCAAAGTTTGGCATATTGAA

Quantitative PCR and Statistical analysis

Statistical analysis was performed using IBM SPSS version 21. Descriptive statistics were conducted. Genotype and allele frequencies were determined using chi-squared statistics, considering $p < 0.05$ as significant in all analyses.

Results

The study subjects were of similar age group ($p>0.05$). Significant differences were seen for BMI, which were higher in obese individuals (groups B and D) as compared to normal weight subjects (groups A and C) ($p<0.05$). Blood pressures among the group C and D were significantly higher than the normotensive group A and B, irrespective of the BMI values ($p<0.05$) (Table 1).

Figure 2 show the gel electrophoresis pattern of the PCR genotyping data of the study subjects where all samples showed the GG genotype. Therefore, it can be observed that the genotypic distributions of the SIRT1 polymorphism at position rs369274325 G/T were not different among the groups. Furthermore, the minor allele frequency (MAF) of 0 was reported in all four groups in this study ($p=1.00$) (Table 2).

Table1: Demographics of study participants

Variable	Group A (Normal Weight with HTN)	Group B (Obese with HTN)	Group C (Normal Weight Normotensive)	Group D (Obese Normotensive)
	(n=30)	(n=30)	(n=30)	(n=30)
Age (year)	27.50 ± 3.53	28.27± 6.54	23.61± 5.29	28.12± 4.23
Weight (kg)	51.0± 1.22	81.72 ± 15.55*	53.66± 4.78	75.40± 8.60*
BMI (kg/m^2)	19.96± 0.34	31.21 ± 5.71*	20.48± 1.27	29.81 ± 3.38*
SBP (mmHg)	145.00±21.21*	146.11±18.19*	106.19± 10.23	113.33± 10.61
DBP (mmHg)	100.00 ± 14.14*	99.44± .98*	67.00 ± 8.64	71.33 ± 8.60
FBG (mg/dl)	103.50± 3.53	85.61± 1.37	93.33 ± 25.56	97.00± 28.21

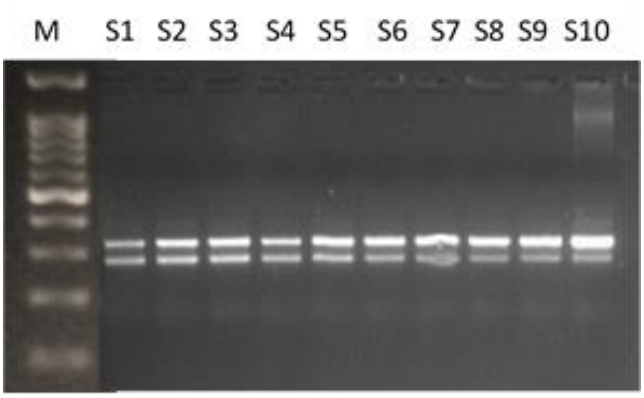

Figure 2: Gel electrophoresis image showing from left to right 100bp marker; samples from group A and C labelled as 1 through 5; samples from group B and D labelled as 6 through 10. Control 336bp; G allele 229bp; T allele 152bp

Table 2: Genotype and Allele Frequency of Study Subjects

Genotype and Allele Frequency		
SIRT Genotype	**Hypertensive Subjects**	**Normotensive Subjects**
	n=60	**n=60**
GG	60	60
TT	0	0
TG	0	0
Data Presented as mean ± SD and absolute numbers. It should be noted that diabetic patients are not included.		
*p<0.05 All samples showed G alleles only. Odds ratio= 1; p value =1		

Discussion

Silent information regulator 1 (SIRT1) serves as a highly conserved NAD-dependent deacetylase, acting as a cellular regulator that influences energy and metabolism by modulating gene expression through histone deacetylation, transcription factors, and modifications to lysine residues in other proteins [3]. This study indicates that there was no observable difference in the genotype distribution of the SIRT1 promoter region, leading to an inability to establish an association between the SIRT1 gene and hypertension. The primary reason for this outcome could be the predominant presence of the wild-type or 'GG' genotype of the SIRT gene within the entire study cohort. Another contributing factor could be the relatively smaller sample size and the specific demographic attending a single hospital. However, a comprehensive analysis involving a more extensive dataset from the South Asian population could yield a more conclusive statement.

Recent research has demonstrated the downregulation of Sirtuin 7 (SIRT7) and Kruppel-like factor 15 (KLF15) levels in hypertensive mice experiencing renal injury. Interestingly, the administration of recombinant adeno-associated virus-SIRT7 or the ferroptosis inhibitor ferrostatin-1 effectively mitigates renal ferroptosis, interstitial fibrosis, and renal injury in hypertensive mice [18]. A recent study highlighted an association between genetic variation in SIRT1, ketogenesis and management of visceral obesity, [19] concluded that ketogenic diet may augment SIRT1 activation in people affected by obesity, yet the cause and effect still need to be researched. Additionally, an investigation into common single nucleotide polymorphisms of the SIRT1 rs7069102 revealed that patients who possessed the CC genotype showed a higher likelihood of developing diabetic nephropathy in T2DM [20]. In the CORDIOPREV study involving patients with coronary heart disease (CHD), the interaction between SIRT1 genotypes (specifically rs7069102) and long-term consumption of low-fat (LF) or Mediterranean (Med) diets was investigated. Regardless of diet type, individuals carrying the GG genotype for rs7069102 exhibited maintained leucocyte telomere length (LTL) and experienced improvements in oxidative stress (OxS) and inflammation markers over the four-year follow-up period. The LF diet intervention further stabilized LTL in GG-carriers, suggesting potential benefits of healthy diets in mitigating aging-related processes and associated diseases in CHD patients with this genotype.

In the CORDIOPREV study, researchers examined how SIRT1 genotypes, particularly rs7069102, interacted with long-term consumption of low-fat or Mediterranean diets in coronary heart disease patients. GG genotype carriers for rs7069102 showed improvement in improvements in oxidative stress and inflammation markers regardless of diet [21]. Another study conducted in Saudi Arabia investigated the prevalence of two common functional single nucleotide polymorphisms in the promoter region of SIRT1, rs12778366 and rs3758391 and their potential association with type 2 diabetes mellitus. Despite genotypic variations, logistic regression analysis revealed no significant relationship between these SNPs and T2DM risk, suggesting that these variants may not contribute substantially to T2DM susceptibility in this population [22]. These findings are like the findings of this paper.

In studies concerning SIRT1 gene polymorphisms, the rs7069102 C allele was notably higher in chronic kidney disease (CKD) patients with cardiovascular disease (CVD) compared to the control group and CKD patients without CVD in the Egyptian population [23]. In a separate study focused on nephropathy, the average serum SIRT1 protein levels exhibited a marked increase in the diabetic nephropathy group compared to both diabetics without nephropathy and the control group. However, the disparity in SIRT1 protein levels between diabetics without DN and the control group did not reach statistical significance [24]. On the other hand, Resveratrol, an activator of SIRT1, has demonstrated its ability to modify signalling pathways, inhibit apoptosis, and negatively regulate angiogenesis. It can increase the production of anti-angiogenic factors or inhibit pro-angiogenic

factors, particularly in pulmonary hypertension [25]. This study has some limitations. The cross-sectional design does not allow conclusions about causal relationships. The relatively small sample size may limit how widely the findings can be applied. Being a single centred study, the results may not fully represent other populations. Also, only one genotype was observed in our study, further investigation is warranted regarding the association and mechanism of action between hypertension and SIRT. It's crucial to note that single nucleotide polymorphisms have been the primary focus thus far, and these variations can differ significantly among populations worldwide [26].

Conclusion

This study indicates that the SIRT1 rs369274325 GG genotype prevails prominently within the local population and may not demonstrate a direct correlation with hypertension.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was received from the Ethics Review Committee at Aga Khan University, Pakistan. Informed consent was obtained from all individual participants included in the study.

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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CONFLICT OF INTEREST

The author of the study has no financial or non-financial conflicts of interest to declare.

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