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The role of KRAS SNP rs61764370 and p53 expression in preeclampsia pathophysiology among pregnant women

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We examined the relationship between p53 gene expression levels and the KRAS single nucleotide polymorphism (SNP) rs61764370 in pregnant women. From January 23, 2024 until June 13, 2024, Babylon Teaching Hospital for Maternity and Children carried out a case-control research. There were 130 participants in all, 65 of whom had been diagnosed with third-trimester preeclampsia and 65 of whom were healthy controls. Blood samples were obtained for quantitative real-time PCR (qRT-PCR) evaluation of p53 gene expression and KRAS SNP analysis. *Distribution of T/G SNPs in KRAS* . Both groups had a high prevalence of the TT genotype, and the distribution of genotypes did not differ statistically significantly between the groups. *TP53 gene expression.* The sick group's p53 expression levels were noticeably higher than those of the controls, suggesting that p53 may play a part in the pathophysiology of preeclampsia. Compared to controls, patients had a considerably larger median fold change in p53 expression. These results suggest that p53 overexpression might play a crucial role in the pathophysiological mechanisms underlying preeclampsia, potentially by promoting oxidative stress and endothelial dysfunction. More research on these features is necessary since the lack of a significant association with the KRAS SNP raises the possibility that preeclampsia is driven by other genetic or environmental variables.

Keywords: preeclampsia; KRAS SNP rs61764370; p53 gene expression; pregnancy complications.

Introduction

Preeclampsia is a notable hypertension condition that occurs during pregnancy, impacting between 2% to 8% of pregnancies worldwide. It is characterized by the onset of hypertension and proteinuria after the 20th week of gestation, often leading to severe maternal and fetal complications (Ives et al., 2020). The pathogenesis of preeclampsia is intricate, encompassing aberrant placentation, endothelial dysfunction, and systemic inflammatory responses. The syndrome arises from insufficient trophoblast invasion and remodeling of uterine spiral arteries, leading to placental ischemia and the subsequent production of antiangiogenic factors, including soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) (Bisson et al., 2023). Endothelial dysfunction, vasoconstriction, and immunological dysregulation are caused by these variables, which ultimately affect the development of the fetus and the organ systems of the mother (Burton et al., 2019).

Preeclampsia's genetic foundations are becoming more widely acknowledged as being essential to comprehending its pathophysiology. Single nucleotide polymorphisms (SNPs) in genes like KRAS and p53 have attracted attention among the genetic variants examined because of their possible implications in regulating the risk and severity of preeclampsia (Burton et al., 2019). The KRAS gene has been linked to a number of cancers and may potentially have an impact on placental development. It encodes a protein that is involved in cell signaling pathways that regulate cell proliferation and differentiation (Ferretti et al., 2007). The KRAS gene's SNP rs61764370 has been linked to changed gene expression, which may have an impact on trophoblast function and vascular remodeling during pregnancy (Olcum et al., 2022).

The p53 gene is essential for controlling cell cycle progression, apoptosis, and genomic stability. Increased trophoblastic cell death in the placenta has been connected to aberrant p53 expression in preeclampsia. The clinical signs of preeclampsia may be exacerbated by this increased apoptosis, which might result in insufficient placental development and function. Research has indicated that fetal growth limitation, which frequently coexists with preeclampsia, is linked to increased p53 levels (Sharp et al., 2014). The relationship between trophoblast apoptosis and p53 expression implies that modifying this pathway may have therapeutic benefits for the treatment of preeclampsia. Individual susceptibility to preeclampsia may be revealed by the interaction between genetic variants like KRAS SNP rs61764370 and p53 expression, underscoring the need of comprehending these genetic determinants in the larger framework of disease pathogenesis. Deciphering the genetic relationship between p53 expression and KRAS SNP rs61764370 is essential to understanding the processes behind preeclampsia.

This study aims to explore the genetic determinants linked to preeclampsia by analyzing the association between pregnant women's p53 gene expression levels and the KRAS single nucleotide polymorphism (SNP) rs61764370. This study specifically aims to clarify the potential roles that changes in p53 expression and variants in the KRAS gene may play in the pathophysiological processes that underlie preeclampsia.

Materials and methods

Specimen collection. From January 23, 2024 until June 13, 2024, Babylon Teaching Hospital for Maternity and Children conducted a case-control study. A total of 130 people participated in the study, including 65 patients who had been diagnosed with third-trimester preeclampsia and an equal number of healthy expectant mothers for comparison. Blood samples were collected using EDTA tubes containing 3 mL of whole blood. For the analysis of p53 gene expression, 1 mL of TRIzol was added to 1 ml of whole blood transferred to Eppendorf tubes. The remaining 2 mL of whole blood in the EDTA tube was designated for DNA extraction for KRAS SNP analysis. Both sets of samples were subsequently stored in a deep freezer until molecular testing could be conducted.

Requirements for inclusion in patient groups. Gathering of demographic information, including occupation, gender, and age. recording any past pregnancies in which preeclampsia occurred.

Evaluation of the family's preeclampsia history. People with a history of infertility or pregnancy difficulties are excluded.

Detection of KRAS (rs61764370 T>G) SNP. The KRAS rs61764370 T>G single nucleotide polymorphism (SNP) is found using fragment length polymorphism (RFLP) study. This method amplifies the target region using specific primers designed for the KRAS gene from (Al-Haddad et al. (2020). 5'-GTGTCAGAGTCTCGCT CTTGTC-3' for the forward primer and 5'-AGACCACACTAGCA CTACCTAAGGA-3' for the reverse primer, an amplicon length of 376 base pairs (bp) will be expected.

The restriction enzyme HinfI is used to digest the final product following PCR amplification. Depending on the allele present, the digestion produces distinct fragment patterns: T allele: after digestion, the T allele is broken up into pieces that are 80 bp, 135 bp, and 161 bp in size. G allele: the G allele produces 80 bp and 296 bp fragments (Fig. 1).

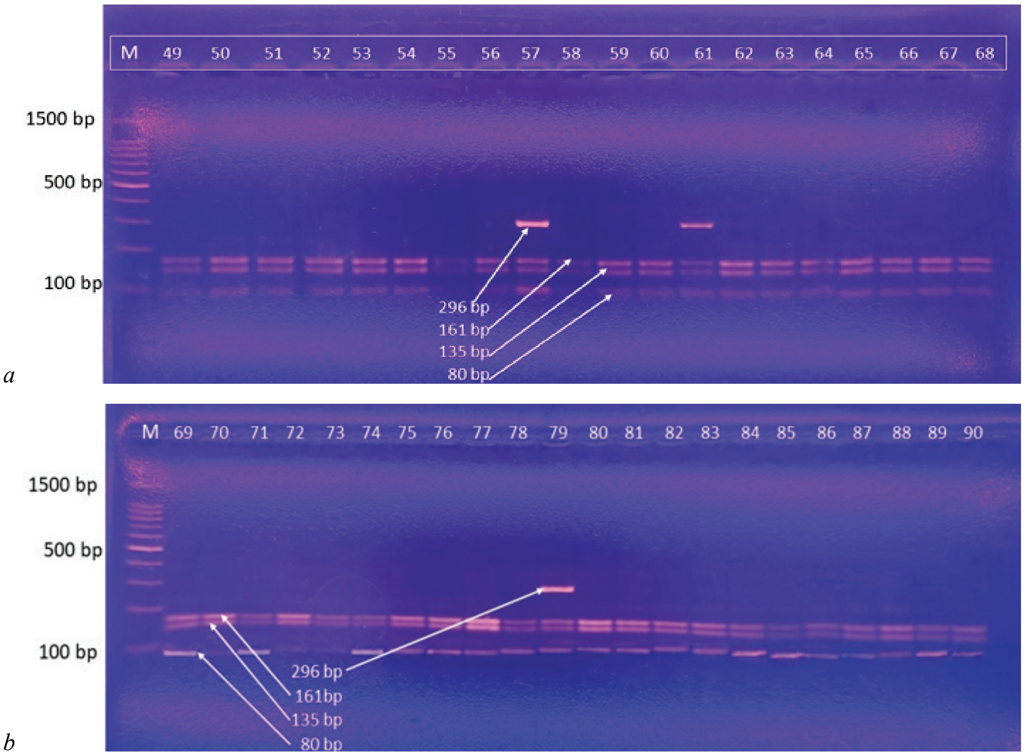

Fig. 1. Electrophoresis of samples on agarose gel by using RFLP-PCR assay: the KRAS rs61764370 T>G locus genotyping resolved on a 1.5% agarose gel stained with ethidium bromide (90 min, 75 Volts, 1X TBE buffer); 376 bp bands were amplicon; allele-specific bands T and G were (161, 135, 80 bp) and 296 bp amplicons, respectively, display four digested products (296, 161, 135, and 80 bp) for people with the TG genotype; three digested products (161, 135, and 80 bp) for participants with the TT genotype; and two digested products (296 and 80 bp) for participants with the GG genotype: a – patient's samples; *b* – control samples

Detection of TP53 gene expression. Quantitative real-time PCR (qRT-PCR) was used in the study to assess the TP53 gene expression levels in blood samples taken from preeclamptic and healthy pregnancies. The TRIzol® reagent, a dependable technique for separating high-quality RNA appropriate for use in subsequent processes, was used to complete the RNA extraction.

In accordance with Bong et al. (2006), two sets of primers were used in this analysis: one set was made to amplify the TP53 gene, while the other set was made to amplify the endogenous control gene, GAPDH. The particular oligonucleotide sequences that were employyed were as follows: 5'-AGA GTC TAT AGG CCC ACC CC-3' was the forward primer for TP53, and 5'-GCT CGA CGC TAG GAT CTG AC-3' was the reverse primer. 5'-CAT GGG GAA GGT GAA GGT CGG A-3' was the forward primer for GAPDH, while 5'-TTG GCT CCC CCC TGC AAA TGA G-3' was the reverse primer. Each sample analyzed in real time with SYBR Green I technology is shown in Figure 2.

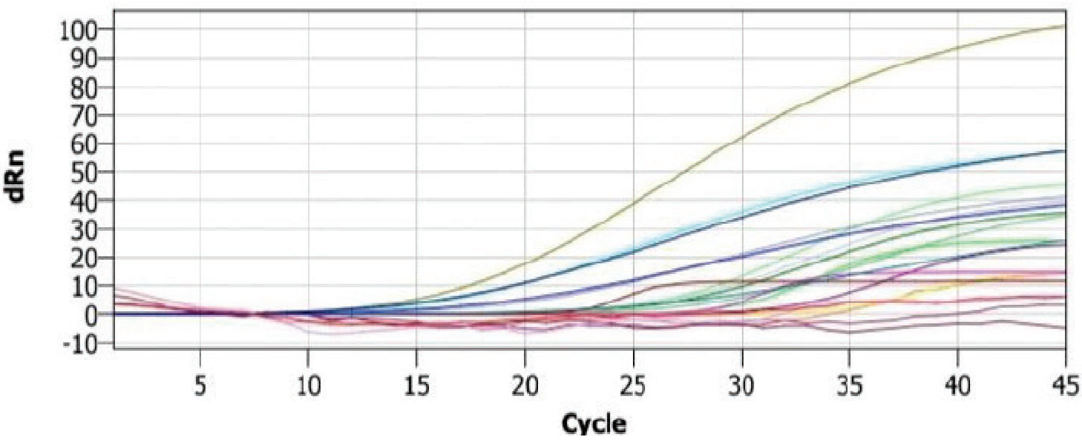

Fig. 2. Real time PCR image show Ct value of TP53 gene expression

Statistical analysis. Using SPSS statistical software (version 26), the data were examined, and the findings were shown as means and standard deviations. The criteria for performing independent t-tests and Pearson correlation analysis were followed in evaluating the normality of the variable data, which was then used to identify significant differences between groups. For genetic analysis, the Chi-square (χ^2) test was also used. A statistically significant P-value was defined as less than 0.05.

Ethical issues. In terms of ethics, this study complied with the standards established by Babylon University's College of Medicine's Department of Community Medicine. Every individual taking part in the study gave their informed consent.

Results and discussion

Demographic characteristics. Both the preeclampsia group and the control group had their demographic traits, such as mean age, body mass index (BMI), and gestational age, evaluated. The findings showed that there were no appreciable differences between the two matched groups (P > 0.05). As shown in Table 1, this matching was necessary to account for any possible differences in results that could be attributed to these parameters.

Table 1
Mean demographic characteristics of studied subject s

Characteristics	Patient group	Control group	P-value
Age, years	28.3 ± 6.5	28.2± 4.9	0.952^{NS}
BMI, kg/m^2	31.9 ± 4.5	31.3± 4.3	0.457^{NS}
Gestational age, weeks	34.2 ± 2.9	33.6 ± 2.5	0.401^{NS}

The characteristics of the patient and control groups were compared across several parameters, including age, body mass index (BMI), and gestational age. The average age of the patient group was recorded as 28.3 ± 6.5 years, while the control group exhibited a similar mean age of 28.2 ± 4.9 years, with a P-value of 0.952 indicating no significant difference between the two groups. In terms of BMI, the patient group had an average of 31.9 ± 4.5 kg/m², compared to the control group's average of 31.3 ± 4.3 kg/m², yielding a P-value of 0.457, which also suggests no significant difference. Lastly, the gestational age for the patient group was noted to be 34.2 ± 2.9 weeks, whereas the control group had a mean gestational age of 33.6 ± 2.5 weeks, resulting in a P-value of 0.401, further supporting the absence of significant differences between the groups in this characteristic as well.

Distribution of KRAS T/G SNP. Table 2 illustrates the distribution of alleles and genotypes across the patient and control groups. The homozygote TT genotype is much more common in both populations (93.8% in patients compared to 92.3% in controls), whereas the heterozygote TG genotype is less common (6.2% in patients compared to 7.7% in controls), while GG genotype (0.0%). Additionally, the allele frequencies show that the T allele is more prevalent than the G allele (3.1% in patients vs. 3.9% in controls), with 96.9% of patients and 96.2% of controls having the T allele. The genotype distribution did not differ statistically significantlybetween the preeclampsia patient group and the normal pregnant control group.

Table 2
Distribution of KRAS T/G SNP (rs61764370) genotypes and alleles

rs61764370		Study groups		P	Odds ratio (CI)
		patient	control		
Genotype	TT	61	60	1	none
	%	93.8	92.3		
	TG	4	5		
	%	6.2	7.7		
Allele	T allele	126	125	1	none
	%	96.9	96.2		
	G allele	4	5		
	%	3.1	3.9		

The KRAS gene, part of the Ras gene family, is a critical oncogene involved in the regulation of cellular signaling pathways that control cell proliferation, differentiation, and survival. Mutations in KRAS are pivotal in the pathogenesis of various solid tumors, particularly colorectal cancer (CRC), pancreatic cancer, and lung cancer (László et al., 2021).

The KRAS gene encodes a GTPase protein that functions as a molecular switch within the RAS/MAPK signaling pathway. This pathway relays external signals to the nucleus, facilitating cell growth and division. When bound to GTP, KRAS is active; conversely, it becomes inactive upon hydrolyzing GTP to GDP. Mutations often lead to a constitutively active KRAS protein that cannot effectively switch off, resulting in uncontrolled cell proliferation and tumorigenesis (Jancík et al., 2010).

MicroRNAs (miRNAs), particularly let-7, which targets the 3′-untranslated region (3′-UTR) of KRAS mRNA, post-transcriptionally regulate the expression of KRAS. KRAS expression levels can be impacted by variations in the let-7 complementary binding sites, which can impact cancer susceptibility (Jancík et al., 2010). A pro-tumorigenic environment is also significantly influenced by other elements, such as inflammatory cytokines (like IL-6) produced by oncogenic KRAS (Hamarsheh et al., 2020).

Tightly controlled RAS/MAPK signaling throughout development is changed as a result of these consequences, which cause the K-Ras protein to become activated for an extended period of time. The many symptoms are caused by the altered signals interfering with the development of organs and tissues throughout the body (Weber & Carroll, 2021).

Expression distribution of the TP53 gene. The current study's findings show that the patient and control groups' p53 gene expression differ significantly. The patient group's median fold change was 4.16, which is much higher than the control group's median of 1. This suggests that patients had significantly higher levels of p53 expression than healthy controls.

This conclusion is further supported by the confidence intervals; the control group shows a much narrower range of 0.92 to 1.12, indicating minimal variability around a baseline expression level, whereas the 95% CI for patients ranges from 3.7 to 4.88, suggesting a strong increase in p53 expression.

Furthermore, the range of p53 expression levels varies significantly between patients and controls, with the former displaying values between 2.58 and 12.71 and the latter between 0.39 and 2.15. This wide range of variation among patients emphasizes the potential importance of p53 in preeclampsia as well as the variety of p53 expression in various tumor settings (Table 3 and Fig. 3).

Table 3
Expression distribution of the TP53 gene between study groups

p53 gene expression (fold change)	Study groups	
	patient	control
Median	4.16	1.00
5% CI	3.70	0.92
95% CI	4.88	1.12
Minimum	2.58	0.39
Maximum	12.71	2.15
P-value	<0.001**	

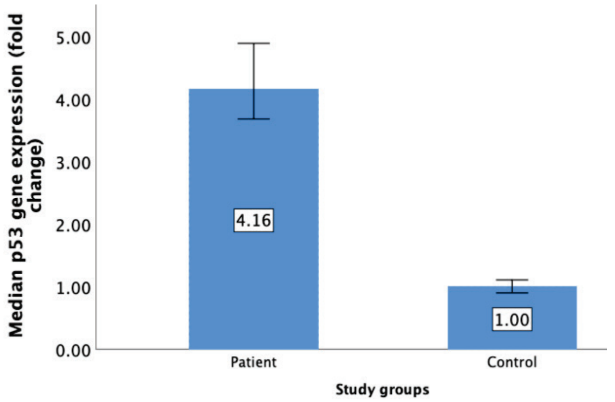

Fig. 3. The fold change difference of p53 expression

Known as the "guardian of the genome," the p53 gene is essential for controlling apoptosis, DNA repair, and cellular reactions to stress. Its increase in preeclampsia points to important roles for DNA damage and cellular stress in the disease's pathophysiology. The following are some consequences of this upregulation:

– *oxidative stress*: one of the main characteristics of preeclampsia is elevated oxidative stress. Oxidative stress can cause p53 activation, which can result in cellular malfunction and damage. This reaction is crucial because p53 controls the expression of genes that lessen oxidative damage, which affects the pathways leading to cellular survival and apoptosis (Moulder et al., 2018; Jaiswal et al., 2021);

– *endothelial dysfunction*: oxidative stress is especially harmful to endothelial cells, which border the blood arteries. One important aspect of preeclampsia is endothelial dysfunction, which may be exacerbated by the overexpression of p53 in these cells. This malfunction can worsen the illness by impairing vascular responses and raising blood pressure (Moulder et al., 2018; Abbas et al., 2024);

– *placental dysfunction*: the development of preeclampsia is mostly dependent on the placenta, which is essential during pregnancy. Reduced nutrition and oxygen exchange between the mother and fetus could arise from impaired function of placental cells due to increased p53 expression. Fetal growth limitation is one of the negative pregnancy outcomes that could result from this impairment (Al-Gazally et al., 2016a; Abbas et al., 2024);

– *immune response*: p53 also affects the immune system, which is important when preeclampsia is present. The immune system's interactions with trophoblasts and other placental tissues may be impacted by its function in controlling apoptosis, which could have an impact on the overall immunological tolerance required for a healthy pregnancy (Moulder et al., 2018).

The fact that p53 is upregulated in preeclampsia highlights its complex function in modulating cellular stress responses, especially in situations when endothelial dysfunction and oxidative stress are present. Our findings are consistent with earlier research. According to a study by Sharp et al. (2014), preeclampsia is linked to elevated p53 and its downstream protein production, which causes villous trophoblasts to undergo excessive apoptosis. According to research published previously, miR-495 inhibits preeclampsia by focusing on the p53/PUMA axis (Al-Gazally et al., 2016b). The studies identified higher levels of p53 in placental tissues from preeclamptic patients, suggesting that p53 plays a substantial role in the pathophysiology of preeclampsia through its participation in trophoblast cell behavior and apoptosis (Abdul-Mohaymen et al., 2011; Zhao et al., 2022).

Conclusion

The relationship between the KRAS SNP rs61764370 and p53 gene expression in pregnant women is the main subject of this work, which explores the genetic foundations of preeclampsia. The results show that p53 expression levels are much higher in the preeclamptic group than in healthy controls, even if the KRAS SNP rs61764370 does not significantly correlate with preeclampsia susceptibility.

These findings imply that p53 overexpression may be essential to the pathophysiological processes that underlie preeclampsia, possibly by contributing to endothelial dysfunction and oxidative stress. The lack of a substantial correlation with the KRAS SNP suggests that preeclampsia may be caused by additional genetic or environmental factors, which calls for more research on these aspects.

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No potential conflict of interest relevant to this article was reported.

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