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Genetic variation in *GSTP1* and *TBXA2R* genes: influence of Badoush cement factory pollutant

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Ethical considerations: the study has been reviewed and approved by Badoosh Cement Factory 2nd Extension which is part of the Northern Cement Associate before data collection commenced, ensuring that all ethical standards have been met. The Association Between Genetic Variation and Exposure to Cement Industry Pollutants Among Factory Workers,' received ethics approval from the University of Mosul, College of Environmental Sciences, under protocol number 2882. All procedures were conducted in accordance with the approved guidelines and regulations. The confidentiality of all personal and sensitive information has been strictly maintained. Measures have been implemented to ensure that participants are protected from any potential harm arising from their involvement in this study. Informed consent was obtained from all participants prior to their involvement in the research. The purpose, nature, and potential risks of the study were clearly explained, and participants were given the opportunity to ask questions and withdraw at any time without penalty.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Abstract

Industrial advancements, while driving economic growth, have simultaneously intensified environmental pollution levels, posing significant health risks to populations residing near industrial hubs. The release of a complex mixture of pollutants, including heavy metals, particulate matter, and volatile organic compounds, has been linked to a range of adverse health outcomes, including respiratory diseases, cardiovascular complications, and cancer. The Badoush Cement Factor in Mosul city, Nineveh, Iraq, a major industrial facility, exemplifies this challenge, emitting substantial quantities of air pollutants capable of long-range transport. This study, therefore, investigated the potential gene-environment interactions focusing on *TBXA2R* and *GSTP1* genes and contributing to respiratory health disparities among 64 employees of the Badoush Cement Factory compared to 10 control subjects. Specifically, the study employed the tetra-primer amplification refractory mutation system-polymerase chain (ARMS-PCR) reaction technique to analyze the role of the *TBXA2R* gene, known for its involvement in bronchoconstriction and susceptibility to asthma, and the *GSTP1* gene, encoding a crucial antioxidant enzyme protecting lung tissue from oxidative stress. By examining these genetic markers within the context of occupational exposure at the Badoush Cement Factory, this research aimed to elucidate the intricate interplay between genetic predisposition and environmental factors in determining respiratory health outcomes. The results indicated that there was no direct correlation between exposure to pollutants and genetic variation in the *TBXA2R* gene. However, prolonged exposure to pollutants may increase the risk of developing respiratory diseases. No significant association was found between pollutants emitted from the cement plant and genetic variation in the *GSTP1* gene.

Introduction

Air pollution, a common issue today, is mainly due to industrial development and the release of harmful emissions. These emissions significantly threaten public health, especially the respiratory system. Inhaling polluted air with industrial and vehicular contaminants can harm the respiratory tract, with the impact depending on exposure duration and particulate size. Long-term exposure to smaller particles increases the risk of respiratory issues, ranging from mild allergies and bronchitis to severe conditions like asthma.[1]

Fine Particulate Matter (PM), a complex mixture of solid and liquid particles suspended in air, constitutes the primary component of air pollution. The size and chemical composition of PM exhibit significant spatial and temporal variability.[2] Human activities were found to be the dominant source of fine PM, which was categorized alongside natural sources and secondary particulate matter.[3] In addition to particulate matter, gaseous pollutants like Ozone (O_3), Volatile Organic Compounds (VOCs), Carbon Monoxide (CO), and Nitrogen Oxides (NO_x) significantly contribute to air pollution. These gases can induce respiratory inflammation and pose serious health risks.[4]

The cement industry is vital to the global economy, supporting infrastructure development, urbanization, and construction. Cement production involves quarrying, crushing, additive mixing, milling, high-temperature kiln processing, cooling, grinding, and packaging.[5] However, fine dust generated during production poses risks. Dust particles (0.05 to 5 μm) can settle in the lungs, causing pulmonary complications. Components like chromium can trigger immunologic responses, leading to delayed hypersensitivity, chronic inflammation, and epithelial damage. According to statistics, cement plants contribute significantly to air pollution by releasing approximately 500,000 tons of Sulfur Dioxide (SO_2), Nitrogen Oxides (NO_x), and Carbon Monoxide (CO) annually.[6] Over time, these reactions impair lung function and can cause asthma, bronchitis, and other chronic respiratory conditions.[5]

Researchers have suggested a link between air pollution and genetic variations in the *GSTP1* and *TBXA2R* genes.[7,8] *GSTP1*, the most abundant subtype of the glutathione S-transferase family, is located on chromosome 11q13, spans about 3.2 kb, and contains nine exons.[9] Predominantly expressed in the human lung, *GSTP1* plays a crucial role in detoxifying byproducts of lipid and DNA oxidation, protecting lung tissue from oxidative damage caused by pollutants. Studying

genetic variations in *GSTP1* and their interaction with air pollution could provide insights into respiratory diseases and aid in developing targeted interventions.[10]

Thromboxane A2 (*TXA2*) is crucial in allergic inflammation and asthma by binding to its receptor, *TBXA2R*. *TBXA2R* is abundantly expressed in bronchial and vascular smooth muscle, epithelium, endothelium, and various tissues. The *TBXA2R* gene, located on chromosome 19p13.3, spans about 15 kb and has four exons. It encodes a G protein-coupled receptor essential for various physiological responses. Understanding the *TXA2*-*TBXA2R* interaction is key to grasping asthma's pathophysiology and other allergic conditions. Research into *TBXA2R*'s genetic and molecular mechanisms can reveal how pollutants affect respiratory diseases, leading to more effective therapies.[8]

The tetra-primer amplification refractory mutation system-polymerase chain (ARMS-PCR) reaction method uses four primers in a single PCR reaction to determine genotypes with high specificity.[11] Two non-allele-specific outer primers amplify the region with the Single Nucleotide Polymorphism (SNP), creating a fragment that serves as a template for the two allele-specific inner primers, which produce allele-specific fragments. By positioning the outer primers at different distances from the polymorphic nucleotide, the allele-specific fragments can be distinguished by size during agarose gel electrophoresis. This precise allele discrimination is valuable for genetic studies, particularly those exploring the relationship between genetic variations and environmental factors.[12]

In a Pakistani study investigating the association of 16 polymorphisms in 10 genes linked to asthma, including *TBXA2R*, it was found that the minor allele (A) of the *TBXA2R* gene at the rs1131882 polymorphism was more prevalent in the control group (healthy individuals). When using the odds ratio to assess the relationship between the A allele and the likelihood of developing asthma, the ratio was 0.73. This indicates that the presence of the A allele is associated with a reduced risk of asthma compared to the major allele, as individuals carrying the A allele have 27% lower odds of developing asthma compared to those who do not, assuming that the more common allele is associated with a higher risk of asthma.[13] In another study,[14] a meta-analysis was conducted to investigate the association between a single nucleotide polymorphism at rs1138272 in the *GSTP1* gene and the risk of Chronic Obstructive Pulmonary Disease (COPD), The results suggested a potential association between the SNP at rs1138272 in

the *GSTP1* gene and the risk of COPD, particularly among Caucasians carrying the homozygous genotype.

The aims of this study are to establish a relationship between genetic predisposition to respiratory diseases and harmful environmental factors, such as air pollution, and to screen for substitution mutations in *GSTP1* and *TBXA2R* using the tetra-primer ARMS-PCR technique. By achieving these objectives, the study seeks to enhance the understanding of how genetic variations interact with environmental pollutants to influence the development and progression of respiratory conditions such as asthma.

Materials and Methods

Primer design

Tetra-primer ARMS technique was employed to detect the *TBXA2R* rs1131882 polymorphism and the *GSTP1* rs1138272 polymorphism. This method utilized four primers from Oligo factory, United States, each designed to represent the forward inner and its corresponding reverse outer, as well as the forward outer and its corresponding reverse inner,[11] with a specific primer length, expressed as a number of nucleotides, referred to as MER and sequences detailed in Table 1.

DNA extraction

Genomic DNA was extracted from 64 blood samples collected from workers in the cement factory and 10 control samples from volunteers, all preserved in Ethylenediaminetetraacetic Acid (EDTA) tubes. The extraction process was conducted using the gSYNC DNA Extraction Kit GS100 from Geneaid Biotech Ltd, New Taipei City, Taiwan, following the manufacturer's instructions meticulously to ensure the integrity and quality of the extracted DNA. The summary of statistics of workers and controls are shown in table 2 accordingly.

Study design

In total, 74 participants were enrolled in the study; 64 of them were workers at the Badoush Cement Factory and 10 were included as a control group. The control group consisted of subjects who were not affiliated with the Badoush Cement Factory and had not been exposed to airborne pollutants on a regular basis. Blood samples were collected solely for DNA analysis

from all participants at the medical department of the Badoush Cement Factory for a two-month period from November 2023 to January 2024. EDTA tubes were used to store the blood samples. We created a questionnaire using Google Forms, which was used to identify respiratory diseases experienced by workers at the Badoush Cement Factory by asking them about their medical history. The workers filled out this questionnaire, which highlighted the worsening of their conditions after prolonged exposure to emitted pollutants. A significant portion of the workforce at the Badoush Cement Factory reported a marked increase in respiratory allergies, characterized by symptoms such as wheezing, coughing, and difficulty of breathing, and most of them reported shortness of breath, indicating impaired respiratory function. Additionally, many workers experienced ocular irritation and suffered from high blood pressure and diabetes. These health issues are directly linked to prolonged exposure to industrial pollutants in the factory environment.

The pollutants emitted from the factory were not measured in our study due to the existence of other studies that focused on this aspect indicating the hazards posed by emissions from the Badoush Cement Factory. Harmful gases are released during the production process as a result of reactions between raw materials and fuels used in manufacturing. A study recorded the highest concentration of sulfur dioxide (SO_2) at the Badoush Expansion Plant (1.0250 ppm), exceeding European standards by 0.02 ppm. While carbon dioxide (CO_2) concentrations were within international standards, the results showed a significant increase in the concentration of particulate matter (PM2.5 and PM10) and SO_2 in the crushing, bagging, and kiln units [15]. These concentrations were found to exceed safe limits and were significantly higher than European standards. The current research concentrated on investigating the environmental-genetic interactions.

Gel electrophoresis

Gel Electrophoresis was employed to verify the accuracy of the DNA extraction, utilizing Tris-Borate-EDTA (TBE) Buffer solution from Cleaver Scientific, Glossop, Derbyshire, England, and agarose powder from Bio-Helix Co., Ltd., New Taipei City, Taiwan. In this procedure, 5 µL of the extracted DNA were combined with 2 µL of loading dye for visualization. Additionally, 6 µL of diamond nucleic acid dye were incorporated into the gel prior to its solidification within the casting box. The electrophoresis was conducted for one hour at a constant voltage of 70V.

Following the run, the results were examined using a UV transilluminator (Cleaver Scientific, Warwickshire, United Kingdom) to assess the presence and quality of the DNA bands.

PCR technique

The PCR technique was employed to amplify the DNA strand, using tetra-primer ARMS technology to detect the presence or absence of bands indicative of genetic mutations among the workers. The PCR components are DNA Template, Master mix from Bioneer, nuclease-free water, Primers. The separation technique was utilized to identify the *TBXA2R* rs1131882 polymorphism, as the mixing technique did not yield clear results in this case. Conversely, the mixing technique proved successful in providing accurate and reliable results for the *GSTP1* gene, specifically in detecting the rs1138272 polymorphism. This approach ensured precise identification of genetic variations, facilitating a comprehensive analysis of the genetic mutations present among the workers. Table 3, and Figure 1 show the size of the bands for all alleles of each gene. While table 4 shows the temperatures, duration, and the number of cycles for each step of the PCR reaction for both the *GSTP1* and *TBXA2R* genes. The first columns in the figure 1 contain the DNA ladder, which is used to estimate the approximate size of the DNA fragments separated in the gel by comparing the distance traveled by each fragment in the gel to the distance traveled by the known-size fragments of the ladder. The remaining columns contain the DNA samples that are the subject of the study.

Statistical analysis

The Hardy-Weinberg equilibrium was applied to calculate the frequencies of the three genotypes and their corresponding alleles, ensuring that the population's genetic distribution aligns with expected values. To further evaluate the association between genetic variants and health outcomes, the odds ratio and confidence interval were calculated using MedCalc software, providing insight into the strength and precision of the observed associations. According to conventional statistical standards, a p-value less than 0.05 is considered indicative of a statistically significant relationship between the variables under investigation.

Results

To assess the potential influence of the *TBXA2R* gene on asthma risk in cement factory workers, we analyzed genotype frequencies using the Hardy-Weinberg 2-Allele Calculator. The Hardy-Weinberg calculator predicts genotype frequencies in a population assuming no evolution. We input allele frequencies, and it calculated the expected genotype frequencies. Table 5 reveals that the AA recessive genotype (284-bp fragment) was less common, while the GG dominant genotype (201-bp fragment) was more prevalent. Interestingly, the heterozygous GA genotype displayed a higher frequency than either homozygous genotype, suggesting no immediate association between genetic variation and environmental pollutants such as particulate matter, NO_x, SO_x, and CO_2 during the study period. However, continued exposure to pollutants from the cement industry could potentially interact with this genotype and influence gene expression. Comparing our findings to a control group, we observed a higher frequency of both recessive (AA) and co-dominant genotypes (GA) in the control group compared to the cases. Table 5 shows that the p-value for Hardy-Weinberg equilibrium was 0.0005, the odds ratio was 0.6154 with a confidence interval ranging from 0.1191 to 3.1791. A confidence interval is a range of values that is likely to include the true value of a population parameter. In simpler terms, it is a way to estimate how accurate a particular statistic is. The p-value for the risk ratio assessment was >0.658.

Looking at the *GSTP1* gene, Table 5 shows that the dominant CC genotype (264-bp fragment) was more common than both the heterozygous genotype CT and the recessive TT genotype (340-bp fragment). This high frequency of the CC genotype suggests that environmental pollution may not significantly impact the antioxidant function of *GSTP1*.

When comparing the allele frequency distributions of the three genotypes between the workers and the control group, it was found that the heterozygous CT and homozygous recessive TT genotypes were significantly higher in the control group compared to the workers. Table 5 also shows that the p-value for Hardy-Weinberg equilibrium was 0.0143, the odds ratio was 0.556 with a confidence interval ranging from 0.0105 to 0.2938, while the p-value for the risk ratio assessment was >0.102.

Finally, we compared the outcomes between smokers and non-smokers and did not find any significant effect of smoking on the outcomes in our study.

Discussion

While previous research has linked *TBXA2R* to asthma susceptibility[16] and airway hyper-responsiveness (rs8113232),[17] and also Suzuki *et al.*[18] indicated that *TBXA2R* may contribute to the development of idiopathic pulmonary fibrosis, our study did not demonstrate a direct association between *TBXA2R* polymorphisms and asthma in cement workers. However, the high frequency of the GA genotype in the cases warrants further investigation. This suggests that the *TBXA2R* (rs1131882) polymorphism, specifically GA genotype, may be expressed in the future if prolonged exposure to factory emissions continues. In light of the fact that this SNP in this gene is linked to the narrowing of the airways' smooth muscles, leading to respiratory difficulties, it may contribute to the development of respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) and help clinicians in identifying individuals at increased risk for respiratory problems.[13] Therefore, understanding the link between this SNP in the *TBXA2R* gene and the constriction of smooth muscles in the airways can be considered a crucial step in developing novel therapies for inflammatory respiratory diseases. Individuals carrying this polymorphism are at a higher risk of developing respiratory disorders.

This finding contradicts several studies, like the one by Hamzah *et al.*,[19] which reported a gene-environment interaction influencing the activity of the *GSTP1* enzyme. In another study, our findings were contradicted, and an association was found between the presence of the minor allele at SNP rs1138272 and an increased risk of asthma and wheeze in individuals exposed to high levels of NO_2 air pollution, with an odds ratio of 1.61 for current asthma and wheeze.[20] Yang *et al.*[14] reported that the single nucleotide polymorphism in this gene was associated with an increased risk of COPD. Therefore, further studies are needed in the future to investigate this gene and its relationship to respiratory diseases caused by inhaling pollutants from cement factories.

Our study had certain limitations, including a small sample size, which should be increased in future studies. However, a strength of the study was its focus on both *GSTP1* and *TBXA2R* genes, which have well-established roles in respiratory diseases.[18,21]

Conclusions

The current research suggests a strong need for further investigation into the potential association between specific genetic markers, namely *GSTP1* and *TBXA2R*, and the development of respiratory problems like hypersensitivity, airway infections, and bronchial asthma. This

investigation is particularly important in the context of industrial emissions, with a specific focus on the cement industry. The previously proposed studies should delve deeper into the mechanisms by which emissions from various industrial sources, including cement production, might influence the expression and activity of *GSTP1* and *TBXA2R.* The mechanisms include oxidative stress, free radical generation, change in protein conformation and activation or inhibition of transcription factors.

Understanding these mechanisms could reveal critical insights into how environmental exposure, genetic predisposition, and respiratory health are interconnected. Future research should focus on how genetic differences affect responses to environmental pollutants and the long-term impact of such exposures on respiratory health. Findings could guide personalized health interventions and inform policies to improve worker safety and minimize health risks based on genetic and environmental factors.

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Table 1. Tetra-primer ARMS-PCR for the detection of mutation in *GSTP1* gene rs1138272 and *TBXA2R* gene rs1131882 for human.

Name	Sequences	Length	Temperature
***GSTP1* gene rs1138272**			
Forward Inner (FI)	5-GATGATACATGGTGGTGTCTGGCAGGATGT-3	30MER	67.7°C
Reverse Inner (RI)	5-CAGTGCCTTCACATAGTCATCCTTGCACG-3	29MER	69.2°C
Forward Outer (FO)	5-GCCAGCTCTAAAGCTTTTGCAATCATCTGG-3	30MER	64.8°C
Reverse Outer (RO)	5-TCCAGCAGGTTGTAGTCAGCGAAGGAGA-3	28MER	66.7°C
***TBXA2R* gene rs1131882**			
Forward Inner (FI)	5-GCGGGTTTCGCAGCACTGTCTGGTCA-3	26MER	71.5°C
Reverse Inner (RI)	5-TGACAGCTCTCCCCTTTGCAGGTCTTCCTC-3	30MER	72.1°C

Forward Outer (FO)	5-GGCGCTCTGTCCACTTCCTACTGCAGCC-3	28MER	72.8°C
Reverse Outer (RO)	5-CTGAGATCGCACCACTGCACTGCACTCCAGCCT-3	28MER	72.2°C

Table 2. Summary of data forworkers and controls

Characteristic	**Workers Value**	**Controls Value**
Total Participants	64	10
Age Range (years)	20-64	22-46
Gender	Male (89.1%), Female (10.9%)	Male (50%), Female (50%)
Smoking Status	Smoker (53.1%), Non-Smoker (46.9%)	Smoker (20%), Non-Smoker (80%)
Mean Years of Employment	16.28	-
Common Diseases	High blood pressure (14.1%), Diabetes (7.8%), Blood lipids (1.6%), Heart diseases (1.6%), Diabetes & High blood pressure (1.6%)	No Diseases

Table 3. Size of bands in *TBXA2R* and *GSTP1*.

Band	**Size**
TBXA2R	
Product size for A allele	201 bp
Product size for G allele	284 bp
Product size for two outer primers	429 bp
GSTP1	
Product size for T allele	340 bp
Product size for C allele	264 bp

Product size for two outer primers	545 bp

Table 4. Temperatures, duration, and number of cycles for each step of the PCR reaction for the *TBXA2R* and *GSTP1* genes using the Forward Outer Primer and Reverse Inner Primer.

PCR Steps	Temperature	Time	Repeat cycle
TBXA2R			
Initial Denaturation	94°C	7:00m	1
Denaturation	94°C	0:45s	35 Cycles
Annealing	68°C	0:45s	
Extension	72°C	0:45s	
Final Extension	72°C	7:00m	1
GSTP1			
Initial Denaturation	95°C	5:00m	1
Denaturation	95°C	0:50s	35 Cycles
Annealing	58°C	1:00m	
Extension	72°C	1:00m	
Final Extension	72°C	7:00m	1

Table 5. Genotype Hardy-Weinberg 2-Allele Calculator and Odds ratio (OR) and Confidence Interval (CI) of rs1131882 in *TBXA2R* gene and rs1138272 in *GSTP1* gene.

Genotypes	Workers H-W freq.%		Control group H-W freq.%		P-Value H-W	OR	OR (95%Cl)	P-Value for the risk Ratio Assessment
rs1131882 in *TBXA2R* gene								
GG	20.34	28.65%	2.5	25%	0.0005	0.6154	0.1191 - 3.1791	>0.658
GA	35.32	49.75%	5	50%				
AA	15.34	21.6%	2.5	25%				
rs1138272 in *GSTP1* gene								
CC	42.1	66.82%	1.22	12.25%	0.0143	0.0556	0.0105 - 0.2938	>0.102
CT	18.8	29.84%	4.55	45.5%				
TT	2.1	3.33%	4.23	42.25%				

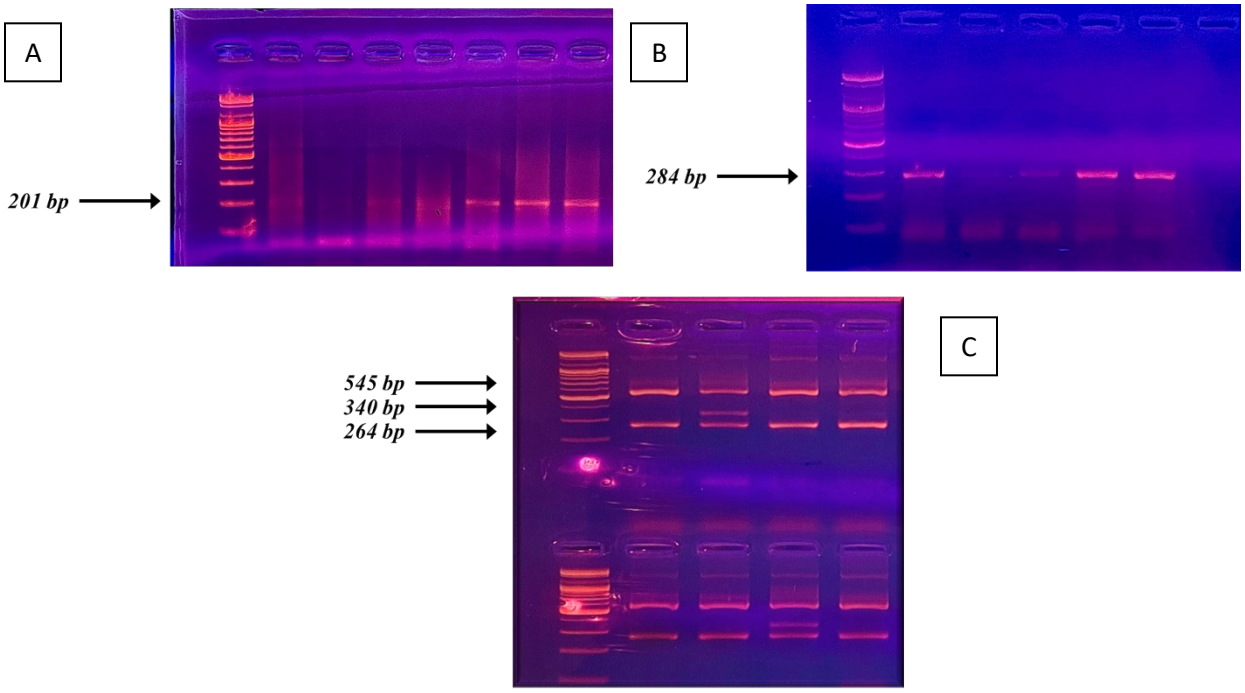

Figure 1. Patterns of DNA bands under UV light. A and B: *TBXA2R*. C: *GSTP1*.