

Association of CYP1A2*1C (Rs 2069514) Genetic Polymorphism With CRP and Total Protein Levels in Brick Kiln Workers

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ABSTRACT

Background & Aim: Workers in brick kiln environments characterized by smoke, dust, intense heat, and arid conditions face a heightened risk of various ailments, including inflammation, sunburn, and skin diseases. Furthermore, genetic polymorphisms exacerbate the risk of serious conditions such as cancer. Additionally, a decline in the levels of naturally occurring bodily biochemical's can trigger or aggravate illnesses. This study investigated association between variants of Cytochrome P450 enzymes, which play a crucial role in the metabolism of xenobiotic compounds and total protein and CRP levels. **Materials & Methodology:** Blood samples from 75 brick kiln workers and controls were obtained for this study. These samples were analyzed to assess total protein and CRP levels. CYP1A2*1C alleles were analyzed using PCR-RFLP, followed by statistical analysis of the data. **Results:** In the exposed group, value for total protein and the inflammatory biomarker CRP were found to be significantly higher compared to the control group. CRP levels were observed to be highest in the AA genotype (0.93 ± 0.40) and total protein was found to be lowest 5.86 ± 34 . **Conclusion:** This study demonstrates the impact of the brick kiln environment on both genetic polymorphisms and biochemical parameters, as well as the combined effect of these factors on workers health and the associated health risks they face. To mitigate these health risks, regular health check-ups, the use of protective measures, awareness programs, and modifications to dietary habits are essential.

Keywords: Genetics polymorphism, Cytochrome p450, Cyp1A2*1C, Biochemical tests, CRP, Brick kiln.

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INTRODUCTION:

Brick kilns are prevalent across Asian nations, including India, Pakistan, Bangladesh, and Nepal. As these are all developing countries, fostering infrastructure—such as residential complexes, buildings, hospitals, and educational and commercial facilities—are essential for their national development (Thirupathi and Anthonisamy, 2015). Consequently, the demand for building materials is rising daily, which in turn, drives both the demand for and the production of bricks. In India, the brick kiln industry stands as one of the largest, oldest, most traditional, and most labor-intensive sectors, employing approximately 10 million people (Bhat et al., 2014). India is the world's second-largest manufacturer of bricks, and the sector boosts the GDP of the nation by 2.3 trillion rupees (Baum, 2010). Despite being economically significant, the brick-kiln industry is largely unorganized, operates on debt, and relies on migrant labor. The industry is also associated with various environmental and occupational health hazards, as workers are exposed to dust, smoke, heat, and toxic pollutants. Living conditions are often substandard, characterized by inadequate housing, a lack of toilet facilities, and a scarcity of clean drinking water. Due to prolonged exposure to these conditions, these individuals become highly vulnerable to various diseases. Many dangerous pollutants, including carbon monoxide (CO), nitrogen oxides (NOx), sulfur oxides (SOx), and particulate matter, are produced during the brick-making process. These pollutants cause oxidative stress, genetic polymorphisms, and inflammation in the body (Golbabaei, F 2019). Cytochrome P450 enzymes play a major role in metabolizing a wide range of endogenous and exogenous substances, including drugs and xenobiotics (Zanger, U. M., & Schwab, M. (2013). There are eighteen CYP families in humans, and CYP1A2 is a significant Phase I drug-metabolizing enzyme that is involved in the biotransformation of many xenobiotics, hazardous chemicals, and environmental contaminants (Ahmed, S. et al. (2016). The CYP1A2 gene consists of 7 exons and 6 introns, located on chromosome 15q24.1. CYP1A2 is a hepatic enzyme that is also highly polymorphic, exhibiting numerous variants; among these, CYP1A2*1C is particularly significant in the metabolic response to environmental toxicants

Sulem, P. et al. (2011). The variant influence detoxification processes and modulate susceptibility to the toxic effects associated with occupational exposure. The CYP1A21C polymorphism (rs2069514) is characterized by a G>A substitution at the -3860 position in the promoter region in intron 1 of the CYP1A2 gene. Polymorphism in CYP1A2*1C is associated with reduced enzymatic activity (Carrascal-Laso, L. et al. (2021). Furthermore, within a brick kiln environment, numerous xenobiotic compounds enter the body through inhalation, dermal contact, and ingestion. This leads to alterations in the body's biochemical parameters. contributes to inflammatory responses. Impairment of liver function triggers an inflammatory response within the body and activates the cytokine production environment. Consequently, CRP is recognized as an inflammatory biomarker that is closely linked to this physiological environment. The present study was conducted to examine the combined impact of genetic polymorphisms and biochemical parameters—specifically CYP1A2 variant, CRP and total protein level in both control and exposed groups. The findings of this study aim to elucidate the role of genetic polymorphisms in modulating toxic responses among workers.

MATERIALS AND METHODOLOGY:

Subject & Sampling:

The studied population comprised 75 brick kiln workers and 75 control subjects—healthy volunteers who were not engaged in the brick kiln industry and whose socioeconomic status was matched to that of the exposed group. Adhering to the study's inclusion and exclusion criteria, participants were fully informed about the nature of the study in advance and were required to complete a consent form. Those who voluntarily agreed to participate completed a questionnaire designed to collect data regarding their demographic characteristics, dietary habits, drinking and chewing habits, and anthropometric measurements. Ethical approval for this study was obtained from the Institutional Human Ethics Committee of Maharshi Dayanand University, Rohtak

Blood Sampling:

Blood samples were collected by a trained technician; this involved drawing 3 mL of peripheral blood—1 mL into a pre-sterilized purple-top (EDTA) Vacutainer for DNA extraction, and 2 mL into a red-top plain Vacutainer (clot) for serum separation. The samples were transported from the collection site to the laboratory in an icebox maintained at a temperature of 4°C.

DNA Isolation & Genotyping:

Genomic DNA extraction was performed using the PCI method Guha, P. et al. (2018). A NanoDrop spectrophotometer was used to measure the quantity and quality of the DNA; a 260/280 ratio of 1.8 was regarded as a sign of high purity. The samples were then subjected to agarose gel electrophoresis analysis. PCR amplification was carried out using a reaction volume of 25 µL, comprising 1 µL of DNA template, 1 µL each of forward and reverse primers, 12.5 µL of master mix, and 9.5 µL of nuclease-free water added to bring the volume to the required to complete the volume. For the PCR-RFLP, 10 µL of PCR product was taken; 1 µL of enzyme, 2 µL of buffer, and 17 µL of nuclease-free water were added. After mixing thoroughly, the mixture was incubated at 37°C for 4 hours, followed by gel electrophoresis.

Table 1: PCR Conditions for CYP1A2*1C gene.

Rs Id's	Primer sequences	PCR Cycle	Product Size	Enzyme
CYP1A2*1C (rs2069514)	F5'GCTACACATGATCGAGCTATAC3' R5'CAGGTCTCTTCACTGTAAATTA3'	5 Min at 94 °C, 94 °C for 45 second, zones (49,52,53,54,55,56) for 1 min 20 second 72 °C for 2 min, 72 °C for 5 min .	598 bp	DdeI

Serum Separation:

After keeping the blood in the red-top vacutainer at room temperature for half an hour, it was centrifuged at 3000 RPM for ten minutes. This caused the serum to separate to the top while the red clot settled at the bottom; the serum was carefully separated, transferred into an Eppendorf tube, and divided into aliquots, which were then stored at -20°C until needed.

Biochemical analysis:

Total Protein level was analyzed using their respective kit, following the protocols provided by the kit manufacturer, ERBA. (Transasia Bio-Medicals Ltd.). The level of CRP in the serum was measured using the ERBA kit provided by Transasia Bio-Medicals Ltd.

Statistical Analysis:

Statistical analysis was performed using Microsoft Excel, including mean and standard deviation, percentages, Student's t-test, and one-way ANOVA. P-values were used to assess differences between groups. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant, while $p < 0.01$ was considered highly significant.

RESULTS:

In the present study, 75 controls and 75 exposed people were taken in which gender was equally distributed (19 females and 56 males). Table 2 contains anthropometric and demographic data in which age difference is not significant in both groups with p value = 0.1197. A preponderance of individuals with a normal BMI was observed in both groups (56% in the affected group and 61.33% in the control group); this difference between the two groups was statistically significant ($p < 0.0001$). With respect to random blood sugar levels and blood pressure, the majority of individuals in both groups fell within the normal range. All individuals in the affected group reported sleeping for six hours or more, and in both groups, individuals typically worked for more than eight hours. All workers followed a flexitarian diet, whereas in the control group, 28% were vegetarian and the remainder were flexitarian. Compared to the control group, the prevalence of tobacco chewing (64%), smoking (62.66%), and alcohol consumption (42.66%) was higher among participants in the affected group. Among the 75 workers—regarding the distribution of roles such as bakers, molders, and loaders—bakers constituted the largest subgroup (40%), and the majority of individuals in the affected group (70.66%) possessed 5 to 10 years of work experience. Gender-wise and complete data on biochemical and inflammatory parameter (Table 3). Total protein levels were lower in exposed males (6.59 ± 3.7) compared to the control group (8.65 ± 4.3), whereas no difference was observed within the female groups (exposed and control). CRP levels were higher in the exposed group (males and females). Table 4 details the genotype distribution of CYP1A2*1C in relation to CRP and total protein levels. Among the control group, CRP levels are highest in the AA genotype (0.93 ± 0.40), whereas in the exposed group, they are lower (0.78 ± 0.62); conversely, the GA genotype in the exposed group shows a level of 1.71 ± 1.29 . Regarding total protein, the AA genotype in the exposed group measures 5.86 ± 3.47 , while the GG genotype shows the highest value at 7.625 ± 3.91 ; in the control group, the levels for GA and GG genotypes are largely similar. Overall, these results indicate that the brick-kiln environment can induce genotype-dependent changes in inflammation and biochemical responses; evidence for this is provided by the differences observed in CRP and total protein levels associated with the CYP1A2*1C genotype.

Table2: The anthropometric characteristics and general health profile of the study participants.

Variables	Categories	Exposed (n=75)	Controls (n=75)
Age		43.93±27.75	38.58±10.32P-Value =0.1197(not significant)
Gender	Male	56 (74.66%)	56 (74.66%)
	Female	19(25.33%)	19(25.33%)
Body Mass Index (BMI)	Underweight(<18.5)	31(41.33%)	5 (6.66%)
	Normal(18.5-24.9)	42(56%)	46 (61.33%)
	Overweight(25.0-29.9)	2(2.66%)	25(33.33%)
Mean age ± SD		27.2±3.28	<0.0001(significant)
Random blood sugar level	Normal Range(<140 mg/dl)	75(100%)	52(69.33%)

	Prediabetes(140-199 mg/dl)	0	15(20%)
	Diabetes(>200mg/dl)	0	7(9.33%)
Blood Pressure	Low blood Pressure(Below 90/60 mmHg)	6(8%)	3(4%)
	Normal range (90/60 to 120/80 mmHg)	35(46.66%)	39(52%)
	High blood pressure (Above 130/80 mmHg)	33(44%)	33(44%)
Pulse Rate	Low Pulse Rate (Bradycardia)<60 heartbeat/minute	0	4
	Normal Pulse Rate(60-100 heartbeat/minute)	68 (90.66%)	62(82.66%)
	High Pulse Rate (Tachycardia)>101 heartbeat/minute)	7(9.33%)	9(12%)
Sleep duration	<6 h/day	0	4(5.33%)
	≥ 6h/day	75(100%)	71(94.66%)
Education status	Illiterate	30(40%)	13(17.33%)
	Primary	25(33.33%)	26(34.66%)
	Secondary+	20(26.66%)	36(48%)
Socio-economic status	Low	75(100%)	75(100%)
Diet type	Vegetarian	0	21(28%)
	flexitarians	75(100%)	54(72%)
Smoking Habits	Current Smoker	47(62.66%)	26(34.66%)
	Non Smoker	28(37.33%)	26(34.66%)
Alcohol Drinking	yes	32(42.66%)	3(4%)
	Rare	15(20%)	12(16%)
	No	28(37.33%)	60(80%)
Tobacco chewing	Low exposure (≤ 1 pack/day)	4(5.33%)	
	Moderate exposure(2-3 Packs/ day)	48(64%)	10(13.33%)
	High Exposure(≥ 4 packs/day)	9(12%)	0
Work duration (years)	<5 years	6(8%)	
	5–10 years	53(70.66%)	

	>10 years	16(21.33%)	
Daily working hours	≤8 h	0	11(14.66%)
	>8 h	75(100%)	64(85.33%)
Occupation work	Baker	25(33.33%)	
	Modular	30(40%)	
	Loader	20(26.66%)	

n= number of Subjects.*P<0.05 is statistically significant

Table 3: Comparative analysis of biochemical & inflammatory parameters:.

	Exposed	Control	P Value	Exposed Male	Control Male	P value	Exposed Female	Control Female	P value
Total Protein(g/dl)	7.07 ±4.01	8.54 ±4.31	=0.0322 (significant)	6.59 ±3.7	8.65 ±4.3	=0.0076 (significant)	8.21 ±4.63	8.21 ±4.26	=1.0000(Not Significant)
CRP(mg/l)	1.45 ±1.11	0.77 ±0.42	<0.0001 (significant)	1.36 ±1.10	0.77 ±0.43	=0.0003 (significant)	1.53 ±0.799	0.74 ±0.40	0.087(Not significant)

Table 4: Genotype-wise Distribution of CYP1A2*1C (rs2069514) and Its Association with CRP Levels among Brick Kiln Workers

Gene Polymorphism	Genotype	CONTR OL (n=75)	EXPOS ED (n=75)	CRP level (Contr ol)	CRP level(Expo sed)	P- valu e	Total Protein(Con trol)	Total Protein (Expose d)	P- Val ue
CYP1A2*1C (rs2069514)	GG	59 (78.66%)	51 (68%)	0.74±0.41	1.51±1.10	<0.001	8.58±4.58	7.625±3.91	0.241
	GA	10 (13.33%)	14 (18.66%)	0.81±0.51	1.71±1.29	0.029	8.71±4.01	5.91±4.53	0.125
	AA	6 (8%)	10 (13.33%)	0.93±0.40	0.78±0.62	0.566	6.9±0.98	5.86±3.47	0.392

DISCUSSION:

India is home to numerous brick kilns where a large workforce is employed; these workers continuously inhale in the kiln environment, thereby indirectly ingesting dust and smoke. particulate matter, polycyclic aromatic hydrocarbons (PAHs), silica dust, sulfur dioxide, nitrogen oxides, carbon monoxide, fluoride compounds, and other toxic substances that adversely affect human health (Joshi & Dudani, 2008; Ahmad et al., 2012; Waseem et al., 2019; Liu et al., 2021).which leads to increased oxidative stress, inflammation, and a heightened risk of cancer. This exposure exerts adverse effects on various biochemical parameters (Zuskin et al., 1998; Rahman et al., 2022). For this study, a cohort comprising 75 control subjects of same socioeconomic status and 75 exposed subjects was recruited, from whom peripheral blood samples were collected following a physical examination to assess vital signs. Questionnaires were administered to record the participants' anthropometric and demographic characteristics, after which all requisite laboratory tests were conducted. Genetic polymorphism for CYP1A2*1C were analyzed using the PCR-RFLP technique, while assays for total Protein and CRP levels were performed using specific diagnostic kits. The same age and gender groups (male and female) were represented in the study cohort.. The majority of individuals in both groups fell within the normal range for Body Mass Index (BMI); however, a higher proportion of underweight individuals was observed in the exposed group, whereas overweight individuals were more prevalent in the control group. Within the exposed group, 62.66% of participants were smokers, 64% were tobacco chewers, and 42.66% were alcohol consumers. Previous research also found a high prevalence of smoking among brick kiln workers (Raza, A. & Ali, Z 2012).These conditions cause lung infection and cause genotoxicity. Cytochrome P450 1A2 (CYP1A2) is an important xenobiotic-metabolizing enzyme involved in the metabolism of PAHs and numerous other environmental contaminants commonly present in brick kiln emissions Guo, J. et al. (2021).. Genetic polymorphisms within the CYP1A2 gene can alter enzyme expression and catalytic activity,resulting in significant inter individual variability in toxicant metabolism (Wooding, S. P. et al. (2002).). Total protein levels were lower in the exposed group compared to the control group; a significant difference was observed between exposed and control males, whereas the reduction in protein levels among exposed females compared to control females was only marginal. However, CRP levels were higher in the exposed group—specifically in both the exposed male and female subgroups—compared to the controls. Thus, the simultaneous decrease in total protein and increase in CRP among exposed workers collectively suggest that occupational exposure to brick kiln emissions is associated with altered protein metabolism, systemic inflammation, and an increased burden of oxidative stress David, M. et al. (2022).. Present study examined the association of CRP levels and total protein with the CYP1A2*1C (rs2069514) polymorphism among brick kiln workers and a control group. The GG genotype was the most prevalent in both the control (78.66%) and exposed (68%) groups, followed by the GA and AA genotypes. Regarding CRP levels, individuals with GG and GA genotypes in the exposed group exhibited significantly higher mean CRP levels compared to their counterparts in the control group. For the GG genotype, the mean CRP level rose from 0.74 ± 0.41 in the control group to 1.51 ± 1.10 in the exposed workers, while for the GA genotype, it increased from 0.81 ± 0.51 to 1.71 ± 1.29 . Significant changes were observed in the GG and GA genotypes, whereas minimal changes were seen in the AA genotype regarding serum total protein levels. These results suggest that exposure to brick kiln emissions may enhance the systemic inflammatory response in individuals with the GG and GA genotypes of CYP1A2*1C. In contrast, individuals with the AA genotype showed lower mean CRP levels in the exposed group (0.78 ± 0.62) compared to the control group (0.93 ± 0.40). This finding may indicate a variation in inflammatory response based on genetic background. However, given the small number of individuals with the AA genotype in both groups (control = 6; exposed = 10), this result should be interpreted with caution and does not necessarily indicate a protective effect of the AA genotype. Further studies with larger sample sizes are required to confirm this association. Regarding total protein levels, the exposed group showed lower mean values across all genotypes compared to their respective control groups. Among individuals with the GG genotype, total protein levels dropped from 8.58 ± 4.58 in the control group to 7.62 ± 3.91 in exposed workers. Similarly, levels decreased from 8.71 ± 4.01 to 5.91 ± 4.53 in GA carriers, and from 6.90 ± 0.98 to 5.86 ± 3.47 in AA carriers. The reduction in total protein levels among workers continuously exposed to brick kiln smoke suggests a potential impact on protein metabolism, nutritional status, or liver function. This increased their health risks by exposing them to numerous toxins by inhaling, digesting or direct skin contact. The findings of this study revealed reduction in total protein in the exposed workers. Similarly, Mazumdar & Goswami (2014) reported similar outcomes in Indian plastic industry workers. Hassan et al 2025 study found a reduction in albumin and protein levels in the brick kiln working group. Our findings are supported by Al Salhen (2014) where cement industry workers' of Libya plasma concentrations of total protein, albumin and globulin were found to be significantly lower. Overall, the findings indicate that occupational exposure to brick kiln smoke can lead to systemic inflammation and alterations in biochemical parameters. The CYP1A21C (rs2069514) genotype may influence the severity of these

effects. Higher CRP levels were observed in workers with GG and GA genotypes, suggesting that genetic variations can affect susceptibility to inflammation caused by occupational exposure. However, due to the small sample size, genotype-based differences should be interpreted with caution, particularly regarding the AA genotype. Further research involving larger populations and robust statistical analysis is required to clarify the role of the CYP1A21C polymorphism in determining susceptibility to the adverse health effects of brick kiln smoke exposure.

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