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Occupational exposure in brick kiln: Evaluating cytogenetic and oxidative-stress related damage

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Abstract

Background: In the developing nations, the growing demand for infrastructure necessitates a steady production of bricks, rendering brick kilns indispensable. Despite their economic relevance, the pollutants emitted from these kilns contribute substantially to environmental degradation and pose serious health hazards to both workers and nearby residents.Workers in these environments are frequently exposed to high concentrations of gaseous and particulate pollutants, which are known to produce reactive oxygen species (ROS) and damage DNA.Prolonged exposure to these pollutants can overwhelm the body's natural defense systems, leading to oxidative stress, which may lead to DNA damage and increased genotoxicity.**Objectives:** The purpose of this study was to investigate the levels of oxidative stress and the potential for genotoxic effects in people exposed to pollutants while working in brick kilns.**Material & Methodology:** Samples of peripheral blood were collected from 110 workers exposed to brick kiln environments and 110 non-exposed individuals (controls).Superoxide dismutase, catalase, carbonylated proteins, and the thiobarbituric acid assay were used to measure oxidative indicators.Genotoxicity was measured through micronucleus (MN) assay .**Results:** The exposed group showed increased MDA, nitrite levels. In contrast, antioxidant defense markers such as reduced glutathione (GSH) and catalase showed a declining trend, indicating depletion of the cellular antioxidant system under continuous exposure to pollutants. The evidence of chromosomal damage and genomic instability was confirmed by the micronucleus assay results, which showed a considerably higher MN frequency in exposed workers compared to controls.**Conclusion:** The results show that, in comparison to controls, those who work in brick kiln conditions have considerably greater levels of oxidative stress and increased DNA damage.

1.INTRODUCTION

In recent years, brick kiln industries have expanded considerably in developing countries, particularly in suburban and rural regions. Brick kiln workers represent a large segment of the informal labour force in developing nations, where they engage in physically demanding work under unsafe and polluted conditions. These workers are exposed to various occupational hazards due to high temperatures, chronic contact with dust, fumes, and heavy metals, and strenuous manual labor, putting them at increased risk of multiple health problems including respiratory disorders, musculoskeletal pain, skin diseases, and infectious illnesses (Karanikas, N., et al 2021). Continue inhalation of fumes due to the combustion of diesel, coal, rice husk , rubber tyres and many other industrial by products increases the risk of cardiovascular diseases, cytotoxic ,genotoxic, ROS, RNS and DNA damaging in addition to being carcinogenic. Suspended particulate matter , respirable suspended particulate matter (RSPM), silica content in the soil, as well as elevated levels of humidity and noise within the work environment, in addition to the presence of heavy metals, contribute to a complex mixture of chemical, physical, biological, psychosocial, and ergonomic hazards in brick kiln

industries(Golbabaei, F 2019).These environmental contaminants are known to cause oxidative stress, which produces reactive oxygen species that can overpower the body's antioxidant defenses and harm cellular macromolecules,including DNA ((Wąsowicz et al. 2001).One of the key mechanism behind these negative health impacts is oxidative stress, which happens when the body's antioxidant defense systems are unable to keep up with the production of reactive oxygen species (ROS) due to environmental variables (Sabuncuoglu, S 2023). Particulate matter, particularly fine and ultrafine particles, can penetrate deep into the respiratory tract, carrying transition metals and polycyclic aromatic hydrocarbons (PAHs) that trigger the formation of superoxide radicals and hydroxyl radicals (Montano, L.,et al 2025). Among these, oxidative stress is essential to the development of diseases like carcinogenicity and genotoxicity (García-Lestón, J et al 2012).Oxidative stress occurs when several reactive oxygenspecies(ROS)areproducedsuchashydroxylradicals, superoxide anion, hydrogen peroxide, singlet oxygen (Surdu et al. 2013; Kitchin and Conolly 2010) during inorganic arsenic metabolism in living cells, and can directly or indirectly induce cellular DNA damage, and can react with cellular ele ments like lipids and thiols, inhibits DNA repair enzymes and alters antioxidant defense system (Jomova et al. 2011; Wang and Huang 1994; Liu et al. 2001; Shi et al. 2004). Genotoxicity and oxidative stress provides a valuable, non-specific tool for assessing the cumulative burden of occupational health risk in this vulnerable population(Frazzoli, C et al 2010). Continuously breathing in the atmosphere of a brick kiln leads to prolonged exposure to pollutants generated by combustion, resulting in persistent oxidative stress and severe genotoxic effects. In these circumstances, the "Reactive Oxygen Species" (can directly ROS) that are produced harm DNA structure, possibly leading to point mutations, chromosomal aberrations, and breaks in DNA strands Ahluwalia, M., & Kaur, A. (2021).These Genotoxic damage is capable of inducing mutations (mutagenesis), which play a significant role in carcinogenesis and other chronic diseases (Luzhna, Let al., 2013). The micronucleus assay, which quantifies small, extranuclear chromatin bodies formed from chromosomal fragments or whole chromosomes lagging during anaphase, serves as a well-established and widely used biomarker for assessing genotoxic damage and chromosomal instability in human populations (Luzhna, L et al 2013). Its utility has been demonstrated in numerous studies evaluating occupational exposure to genotoxic agents. Oxidative stress contributes to a number of chronic illnesses by causing damage to essential macromolecules like lipids, proteins, and DNA.Biomarkers such as malondialdehyde (MDA), protein carbonyls and nitrite, are widely used to assess oxidative damage, while antioxidant parameters including glutathione (GSH), catalase (CAT) and total antioxidant status (TAS) reflect the body's defense capacity(Kadiiska, M., et al 2004). Although oxidative stress has been studied in populations exposed to urban air pollution and industrial hazards, limited data are available on brick kiln workers, who are among the most vulnerable occupational groups due to poor workplace safety and lack of protective equipment Assessing oxidative stress levels in this population is critical for understanding the biological consequences of such exposure. The purpose of this study was to evaluate the oxidative stress and genotoxic levels in employees exposed to a brick kiln environment. The findings provide important insights into the occupational health risks associated with brick kiln exposure.Therefore, the present study was designed with the objective of evaluating the environmental health effects on workers employed in brick kilns, for which selected oxidative stress markers and genotoxicity were assessed.

2. MATERIALS AND METHODS

The study was conducted at the Centre for Medical Biotechnology, Maharshi Dayanand University, Rohtak. Ethical clearance was obtained from the Institutional Human Ethics Committee (HEC/2023/37).Written informed consent was secured from all participating subjects prior to their inclusion in the study, guaranteeing voluntary participation and adherence to ethical research practices.

Study population

The study was conducted on 110 brick kiln workers and 110 participants from control group who were not exposed to brick kiln work.Brick kiln workers recruited were between the ages of 18 and 55, and had a minimum of two years of experience working in a brick kiln. Pregnant women and individuals with chronic illnesses were excluded. Controls were matched for socioeconomic status and were recruited from nearby colonies. This matching minimized potential confounding factors related to economic conditions and living standards, ensuring a more accurate evaluation of the health and occupational outcomes.

Data collection involved administering a structured questionnaire by research staff during field visits and direct interactions with the workers. The questionnaire captured detailed information on demographics, medical history, healthcare habits, and occupational exposures. A structured performa was completed for each participant, followed by comprehensive clinical assessments including pulse measurement, blood pressure, and random sugar level anthropometric measurements—such as weight, height, and body mass index (BMI)—that were recorded. Height was measured without shoes to the nearest centimeter, and weight was recorded using a weighing scale accurate to the nearest kilogram.

Blood sample Collection:

Peripheral blood samples were collected from each participant by trained technicians. Approximately 2 mL of blood was collected in red-capped clot Vacutainers for serum separation, and 2 mL of blood was collected in green -capped heparinized tubes for micronucleus (MN) culture.

Assessment of brick kiln exposure

Exposure refers to the ability of a brick kiln environment to cause toxic effects over an extended period of time, typically after continuous exposure that may last the exposed organism's entire life. The exposure index of brick kiln workers was calculated as follows:

Chronic exposure level = log 10 {[Yx D] + 1}/ age -18

Y is the number of years of occupational exposure to the brick kiln environment, and D is the most recent estimate of the number of days worked in the brick kiln per year. Index values from 2.000377 to 3.1007 (median to highest value) were classified as high chronic exposure, while those from 2.000377 to 1.4642 (median to lowest value) were classified as mild chronic exposure.(Tsai, C. Y., et al 2023).

Estimation of oxidative stress:

Protein carbonyl: The protein carbonyl spectrophotometric assay was used as a marker of oxidative protein damage (Levine et al., 1990). The formation of a light-absorbing and stable compound known as 2,4-dinitrophenylhydrazone (DNP-hydrazone) occurs after the chemical reaction between the carbonyl group of the sample and the reagent 2,4-Dinitrophenylhydrazine (DNPH). 200 µl of sample in two tubes, with one containing 800µl of DNPH and the other 800 µl of 2.5

M HCl, incubated for one hour in a dark place with brief vortexing. The derivatized protein was precipitated with Trichloroacetic acid (TCA). Afterward, the pellet was washed with a 1:1 Ethanol/Ethyl Acetate mixture to remove any interfering, unreacted free DNPH. Finally, the washed, DNP-adduct-containing pellet was dissolved in a strong denaturing solvent, Guanidine Hydrochloride. The concentration of protein carbonyls was determined at 375 nm, using a plate reader and the accepted molar extinction coefficient.

Lipid estimation: Lipid peroxidation is a free-radical-mediated process that damages polyunsaturated fatty acids in membranes, producing unstable lipid hydroperoxides that decompose into toxic by-products like malondialdehyde (MDA) and 4-hydroxy-2-nonenal(wills 1966). MDA content, measured as TBARS (Thiobarbituric Acid Reactive Substances), was determined as follows: 500 µl of serum were mixed with 500 µl of Tris-HCl and incubated at 37 °C for two hours. Then, 1 ml of ice-cold 10% TCA was added and the mixture was centrifuged at 5000 x g for 10 minutes. The supernatant was mixed with 1 ml of 0.67% TBA and placed in boiling water for 10 minutes. After 10 minutes, it was allowed to cool, and the absorbance was measured at 540 nm.

Determine the concentration of serum nitrite: The serum nitrite assay was performed according to the method of Green et al., (1982), in which 100 µl of serum were mixed with 500 µl of Griess reagent, and the absorbance was measured at 540 nm. The concentration was calculated from a standard curve, with units of mmol/mL. This was a spectrophotometric assay in which nitrite compounds present in the serum react with aromatic amines to form colored diazonium compounds, which were measured at 540 nm.

Estimation of total antioxidant status: Reactive oxygen species present in biological systems are highly unstable and difficult to measure. Total oxidant status provides a combined measure of the effects of all oxidative stress biomarkers. A colorimetric method developed by Erel (2005) involves incubating 10 µl of serum with 100 µl of 0.01 M N,N-diethyl-p-phenylenediamine and 0.01 M acetic acid buffer for 75 minutes at 37 degrees Celsius, and then taking a reading at 490 nm. The concentration was measured according to a standard curve, and the units were expressed as mmol/ml of H2O2.

Total Antioxidant Status (TAS): Total Antioxidant Status was calculated in accordance with Erel (2004). Antioxidants in the sample prevent the development of a colored dianisidyl radical produced by the Fenton reaction. The antioxidant capacity, measured in mmol ascorbic acid equivalents/L/ L is indicated by the drop in colour intensity at 505 nm.

Reduced Glutathione: The reduced glutathione assay is based on the principle that Ellman's reagent reacts with GSH present in the serum, producing a yellow-colored TNB chromophore, which is measured at 412 nm. According to Ellman's method, serum and 4% sulfosalicylic acid were mixed in a 1:1 ratio and incubated at 41°C for one hour, followed by centrifugation at 1200 g for 15 minutes. The supernatant was separated, and 2.7 ml of phosphate buffer and 0.2 ml of DTNB were added to it. The absorbance was then measured at 412 nm.

Catalase: Catalase (CAT) activity was estimated according to the method described by Luck (1971) with slight modifications. The assay is based on the spectrophotometric determination of residual hydrogen peroxide (H_2O_2), which forms a stable yellow complex with ammonium molybdate, .The decrease in H_2O_2 concentration, reflected by the intensity of this color, is proportional to catalase activity in the sample. One unit of catalase is defined as the amount of enzyme that decomposes 1 µmol of H_2O_2 per minute under assay conditions, and absorbance was recorded at 405 nm against a reagent blank. Under assay conditions, one unit of CAT activity is defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 per minute. Results were expressed as kilo units per liter (kU/L).

Superoxide dismutase Assay: The activity of SOD was measured with minor modifications to the original method by Marklund and Marklund (1974). (SOD) activity is based on the enzyme's ability to catalyse the dismutation of superoxide anions (O_2^-) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). In this assay, SOD's action is monitored by its ability to inhibit the autoxidation of pyrogallol, a compound that spontaneously oxidizes in the presence of oxygen. The reaction was carried out in alkaline pH (8.2) using Tris-EDTA buffer with a 0.2 mM pyrogallol solution prepared in 50 mL of 10 mM HCl. The reaction mixture consisted of 750 µL of serum sample, 2 mL of Tris-EDTA buffer, and 500 µL of pyrogallol solution. After gentle mixing, the change in absorbance was recorded at 420 nm using a kinetic program.

Cytokinesis - block micronucleus cytome assay: Peripheral blood (0.4 mL) collected in a heparinized tube was cultured in 5 mL of RPMI-1640 medium and incubated at 37°C in a 5% CO_2 incubator. To arrest cytokinesis, Cytochalasin-B was added 44 hours after incubation. After 72 hours, the cells were harvested by centrifugation at 1500 rpm for 15 minutes. The supernatant was discarded, and the cell pellet was treated with 8–10 mL of 0.075 M KCl hypotonic solution and incubated at 37°C for 7–9 minutes. Subsequently, 1 mL of fixative was added, and the sample was centrifuged again at 1500 rpm for 15 minutes. The cells were washed several times with fixative, and the volume of the solution was gradually reduced after each wash. Once a clean pellet was obtained, an appropriate amount of fixative was added to prepare a cell suspension. The suspension was dropped onto a clean slide, air-dried, and stained with 2% Giemsa for 15 minutes. The slide was rinsed with distilled water and examined under a microscope at 100× magnification for micronucleus scoring(Fenech M. (2007).

Statistical analysis:

The statistical analysis was done in Microsoft Excel. Data were presented as mean ± SD. The Student's t-test and , One way ANOVA, Shapiro–Wilk test were used for analysis. (P-Value)was used to assess differences across groups. Statistical significance was defined as p-values less than 0.05 ($p < 0.05$), while $p < 0.01$ was regarded extremely significant.

3. RESULTS

Table 1: Extended Socio-Demographic, Lifestyle and Occupational Profile of Study Participants

Variables	Categories	Exposed (Brick kiln workers) n (%)	Controls n (%)	p-value
Sample size		110 (100%)	110 (100%)	-

Age group (years)	18-30	47(42)	28 (25.45)	
	31-40	31(28.18)	37(33.63)	
	>40	32(29.09)	45(40.90)	
Mean ± SD		34.74± 9.69	37.86± 10.07	0.0201
Gender	Male	90 (81)	56 (50)	
	Female	20 (27)	54(29)	
Body Mass Index (BMI)	Underweight	42 (38.18)	12 (10)	
	Normal	63 (57.27)	70 (63.63)	
	Overweight	4(3.63)	30 (27)	
Mean ± SD		18.1±2.96	23.43±1.55	<0.0001
Random blood sugar level	Normal Range	108(98.18%)	73(66.36%)	
	Prediabetes	2(1.81%)	24(21.81%)	
	Diabetes	0	13(11.81%)	
Blood Pressure	Low blood Pressure Below **90/60 mmHg**	20(18.18%)	8(7.27%)	
	Normal range 90/60 to 120/80 mmHg	36(32.72%)	36(32.72%)	
	High blood pressure Above **130/80 mmHg**	52(47.27%)	59(53.63%)	
Pulse Rate	Low Pulse Rate (Bradycardia)	1(0.90%)	4(3.6%)	
	Normal Pulse Rate	99(90%)	95(86.36%)	
	High Pulse Rate (Tachycardia)	100(90.9%)	11(10%)	
Sleep duration	<6 h/day	0	6	0.0231
	≥ 6h/day	110	104	
Education status	Illiterate	39(35.45%)	29(26.36%)	
	Primary	35(31.81%)	32(29.09%)	
	Secondary+	36(32.72%)	49(44.54%)	
Socio-economic status	Low	110	110	
Daily working hours	≤8 h	0	18	=0.1653 (not significant)
	>8 h	10	92	
Diet type	Vegetarian	0	31	<0.0001

	flexitarians	110	79	
Occuaption work	Baker	40(36.36%)	-	
	Modular	50(45.45%)	-	
	Loader	20(18.18%)	-	
Smoking Habits	Current Smoker	73	26	<0.0001
	Non smoker	37	**84**	
Tea Drinking	Non-drinker	0	0	
	Occasional	27(24.54%)	36(32.72%)	
	Regular	83(75.45%)	74(67.27%)	
	>200ml	45(40.90%)	3(2.72%)	
Alcohol Drinking				
	Rare	23(20.9%)	12(10.90%)	
	No	42(38.18%)	95(86.36%)	
Tobacco chewing	Non-user	20(18.18%)	104(94.54%)	
	Low exposure ≤ 1 pack/day	7(6.36%)	6(5.45%)	
	Moderate exposure 2-3 packs/day	72(65.45%)	-	
	High Exposure ≥ 4 packs/day	10(9.09%)	-	
Work duration (years)	<5	9	-	
	5-10	79	-	
	>10	22	-	

Table 2. Oxidative Stress Biomarkers and micronucleus (mean ± SD)

Parameter	**Control (n=110)**	**Exposed (n=110)**	**p-value**
Protein carbonyl(nmol/ml)	2.51 ±1.55	6.054 ±3.17	<0.0001
Lipid Peroxidation(mg/mL)	1.47 ±0.84	2.25 ±1.62	<0.0001
Serum Nitrite(mmol/ml)	3.012 ±1.399	4.23 ±1.13	<0.0001
Total Oxidant status(CARR U)	277.36 ±70.16	318.16 ±93.72	=0.0003
Total antioxidant status(mmol ascorbic acid)	1.89 ±0.26	1.63 ±0.09	<0.0001
GSH (µmol/L)	25.71±5.64	19.48 ±7.392	<0.0001
Catalase(ku/l)	107.18 ±23.20	22.82 ±11.76	<0.0001
SOD(U/ml)	43.53 ±13.78	29.22 ±15.84	<0.0001

MN	3.63±1.077	6.95±1.42	<0.0001

Table3: Effect of Duration of Exposure on Oxidative Stress & Genotoxicity (Workers Only)

Exposure Duration (years) / Parameters	**≤5 years(n=29)**	**6-10 years (n=59)**	**≥ 10 years(22)**
Protein carbonyl(nmol/ml)	5.80±2.83	6.33±2.30	5.41±2.94
Lipid Peroxidation(mg/mL)	2.18±1.60	2.30±1.69	2.18±1.50
Serum Nitrite(mmol/ml)	9.15±9.25	21.46±35.47	25.21±40.30
Total Oxidant status(CARR U)	289.31±96.61	274.99±61.31	267.96±49.424
Total antioxidant status(mmol ascorbic acid)	1.62±0.07	1.63±0.09	1.64±0.12
GSH (µmol/L)	20.49±10.18	19.30±6.36	18.65±5.58
Catalase(ku/l)	22.25±11.75	22.94±12.01	24.02±11.84
SOD(U/ml)	26.88±17.88	29.99±14.89	30.30±15.87
MN frequency	7±1.38	6.86±1.45	7.13±1.45

Table4: Age-wise Distribution of Oxidative Stress Markers and Micronucleus Frequency

Age Groups	18-30years			31-40years			41-50years			51-60years		
Parameters	Control (n=28)	Worker (n=47)	P-Value	Control (n=37)	Worker (n=31)	P-Value	Control (n=35)	Worker (n=28)	P-value	Control (n=10)	Worker (n=4)	P-value
Protein carbonyl(nmol/ml)	2.62±1.65	5.47±2.82	<0.0001	2.56±1.60	6.87±3.18	<0.0001	2.35±1.35	6.24±3.58	<0.0001	2.55±1.96	3.92±2.49	0.2929
Lipid Peroxidation(mg/mL)	1.78±1.14	2.38±1.68	0.0988	1.12±0.73	2.16±1.17	<0.0001	1.72±0.78	1.94±1.65	0.4840	1.39±1.07	3.48±1.58	0.0108
Serum Nitrite(mmol/ml)	2.83±1.42	4.21±1.18	<0.0001	3.09±1.37	4.28±1.17	0.00003	3.22±1.52	4.20±1.09	0.00056	2.72±1.28	4.06±1.01	0.0876
Total Oxidant status(CARR U)	263.71±56.59	312.82±76.11	0.0042	285.07±63.62	333.46±131.6	0.0519	294.11±87.47	302.7±63.77	0.6651	248.7±43.15	370±69.32	0.0017
Total antioxida	2.001±0.37	1.63±0.07	<0.0	1.92±0.2	1.63±0.	<0.0	1.87±0.2	1.63±0.1	<0.0	1.95±0.3	1.61±0.1	0.08

nt status(mmol ascorbic acid)			001	7	096	001	4	20	001	5	3	85
GSH (µmol/L)	25.82±4.700	19.93±8.77	0.00015	25.08±5.99	18.70±5.66	<0.001	27.11±5.18	20.21±7.09	<0.001	26.81±5.915	15.60±1.86	0.00034
Catalase(ku/l)	106.70±24.63	22.85±11.82	<0.001	110.12±24.24	24.26±13.35	<0.001	103.88±24.31	21.94±10.45	<0.001	110.7±17.60	21.44±10.30	<0.001
SOD(U/ml)	49.14±20.40	43.39±12.84	0.21 92	42.85±12.31	42.40±10.97	0.8752	41.61±11.73	45.45±18.72	0.3238	47.87±25.05	41.08±3.82	0.6078
MN frequency	3.32±0.944	7.31±1.57	<0.001	3.64±1.03	6.62±1.33	<0.001	3.88±1.24	6.74±1.22	<0.001	3.6±0.84	6.75±0.95	<0.001

Table5: Effect of oxidative stress and micronuclei on brick kiln workers categorised on the basis of their job profile

Occuaption	Protein Carbonyl	Lipid Peroxidation	Serum Nitrite	TOS	TAS	GSH	Catalase	SOD	MN Frequency
Baker(n=40)	5.62±2.84	2.51±1.69	4.21±1.14	332.94±78.75	1.63±0.1	17.92±8.49	28.35±13.86	26.01±17.3	6.77±1.38
P-Value	.579	.021	< .001	.004	.895	< .001	.029	.079	.003
	Normal distribution	Not normal distribution	Not normal distribution	Not normal distribution	Normal distribution	Not normal distribution	Not normal distribution	normal distribution	Not normal distribution
Loader(n=20)	6.92±3.61	1.95±1.66	4.22±1.23	316.72±79.37	1.64±0.12	23.07±7.2	22.24±10.12	32.74±11.88	6.81±1.23
P-Value	.831	.015	.004	< .001	.107	.116	.050	.460	.182
	Normal distribution	Not normal distribution	Not normal distribution	Not normal distribution	Normal distribution	Not normal distribution	Normal distribution	Normal distribution	Normal distribution
Modular(n=50)	5.72±3.21	2.15±1.54	4.25±1.12	307.25±108.27	1.63±0.09	19.31±6.08	19.03±8.68	30.47±15.21	7.16±1.53
P-Value	.147	.007	< .001	< .001	.087	.003	< .001	.574	.018

	Normal distribution	Not normal distribution	Not normal distribution	Not normal distribution	Normal distribution	Not normal distribution	Not normal distribution	Normal distribution	Not normal distribution
One way ANOVA (P-Value)	0.435 (Not Significant)	0.321 (Not Significant)	0.932 (Not Significant)	0.073 (Not Significant)	0.757 (Not Significant)	0.005 (Significant)	0.005 (Significant)	0.250 (Not significant)	0.464 (Not Significant)

Normality of data was assessed using the Shapiro-Wilk test. Variables with $p > 0.05$ were considered normally distributed, while those with $p \leq 0.05$ were treated as non-normally distributed."

Results:

This study included 110 brick kiln workers who were directly exposed to the environment, while 110 individuals were includedto a 'control group.' The age distribution revealed that 42% of the exposed individuals fell within the 18-30 age group, followed by 28.18% in the 31-40 age group, and 29.09% who were over 40 years of age. In contrast, the control group comprised 25.45%, 33.63%, and 40.90% of individuals belonging to these respective age groups. The mean age of the exposed group (34.74 ± 9.69 years) was significantly lower than that of the control group (37.86 ± 10.07 years) ($p = 0.0201$). Based on BMI categorization—divided into underweight, normal, and overweight—the exposed group consists of 38.18% underweight, 57.27% normal, and 3.63% overweight individuals; whereas the control group consists of 63.63% normal, 10% underweight, and 27% overweight individuals (expressed as percentages). This difference was associated with a p-value < 0.0001, indicating a highly significant difference. Both groups had a high prevalence of individuals with high blood pressure—47.27% in the exposed group and 53.63% in the control group. Regarding pulse rate, 90% of individuals in the exposed group had a normal pulse rate, whereas this figure stood at 86.36% in the control group. Sleep duration (≥ 6 hours/day) was comparable in both groups. In terms of random blood sugar levels, 21.81% of individuals in the control group were pre-diabetic, compared to 1.81% in the exposed group. The prevalence of smoking, alcohol consumption, and tobacco chewing was higher in the exposed group—at 66.36%, 40.90%, and 65.45%, respectively—exceeding the rates observed in the control group. Regarding dietary habits, all individuals in the exposed group were 'flexitarians'; conversely, in the control group, 71.81% were flexitarians, while 28.18% were vegetarians.

Biochemical results:

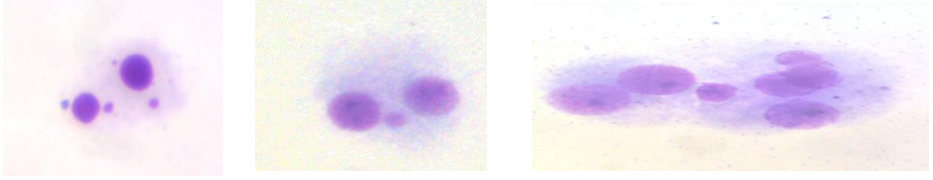
The levels of oxidative stress biomarkers showed notable differences between brick kiln workers and controls, indicating an imbalance between oxidant and antioxidant systems.The level of malondialdehyde (MDA), a marker of lipid peroxidation, was significantly higher in the exposed group (2.25±1.62) as compared to controls (1.47±0.84) ($p < 0.0001$), indicating enhanced oxidative damage. whereas protein carbonyl a marker of protein oxidation and serum nitrite were higher in exposed (6.0.54±3.17) and (4.23±1.13) respectively and their values in controls were (2.51±1.55) and 3.012±1.39). In contrast, antioxidant parameters such as reduced glutathione (GSH) and catalase were significantly decreased in exposed individuals (GSH: 19.48 ± 7.392).

Catalase: (22.82±11.76) compared to controls (GSH: 25.71±5.64) Catalase: (107.18±23.20) Catalase and GSH levels were significantly reduced in the exposed group compared to controls ($p < 0.0001$), indicating a highly significant depletion of antioxidant defense.Superoxide dismutase (SOD) levels showed a decrease in the exposed group (29.22 ± 15.84) as compared to controls (43.53 ± 13.78) ($p < 0.0001$). The observed decrease may indicate oxidative stress-induced depletion of antioxidant activity.Furthermore, total oxidants were significantly elevated in exposed individuals (318.16 ± 93.72) compared to controls (277.36 ± 70.16) ($p < 0.0001$), reflecting increased oxidative stress. Total antioxidant levels were higher in controls compared to exposed individuals (1.89 ± 0.26) and in unexposed individuals (1.09 ± 0.09).Overall, these findings suggest that occupational exposure to brick kiln emissions leads to increased oxidative stress and compromised antioxidant defense mechanisms.

Micronucleus results:

Compared to the control group, the frequency of micronuclei (MN) in the lymphocytes of the exposed group was significantly higher, indicating greater genotoxic damage. The mean frequency of MN in the exposed individuals was (6.95 ± 1.42), whereas in the control group, it was (3.63 ± 1.077). This difference was found to be statistically significant ($p < 0.0001$).

Among the exposed subgroups—[Bakers (n=40), Loaders (n=20), and Modulors (n=50)]—the Modulors exhibited the highest frequency of MN, indicating a greater extent of DNA damage associated with the exposure. Overall, the elevated frequency of MN in the exposed workers reflects increased chromosomal damage resulting from occupational exposure to brick kiln pollutants.



(A) (B) (C)

Figures1: A, B, and C represent the results of the micronucleus assay.

4. DISCUSSION

The present study was conducted on individuals working in brick kilns to assess oxidative stress and genotoxicity. India is a developing nation where construction activities are constantly underway, creating a persistent demand for bricks. In India, traditional-style brick kilns remain the predominant method of production. The environment within these brick kilns is characterized by the combustion of various fuels—such as coal and husks—which releases toxic fumes and smoke; inhaling this contaminated air poses significant health risks, thereby increasing the likelihood of oxidative stress and genotoxicity. For this study, 110 brick kiln workers and 110 control subjects were selected, with participants in both groups matched based on their socioeconomic status. Anthropometric data for the participants were collected through questionnaires and physical measurements conducted directly at the study site. Regarding age distribution, the age profiles of both groups were comparable. Based on BMI assessments, a higher proportion of underweight individuals was observed among the brick kiln workers compared to the control group; however, blood pressure levels were found to be similar in both groups. The workers typically worked shifts ranging from 6 to 7 hours. They did not utilize any form of protective equipment, leaving them highly exposed to sunlight, dust, heat, and the risk of sunburn. In this study, we performed assessments for oxidative stress and conducted micronucleus assays. This study assessed various parameters—including lipid peroxidation (Wills, 1966), protein carbonyl (Levine et al., 1990), reduced glutathione (Ellman, 1959), catalase (Luck, 1971), serum nitrite concentration (Green et al., 1982), TAS (Rail, 2004), TOS (Halliwell and Gutteridge, 2015; Leffler, 1993), SOD (Marklund and Marklund, 1974), and micronuclei assay (Fenech and Morley, 1985)—to understand the effects of a stressful occupational environment on oxidative stress parameters among workers. Oxidative stress markers—specifically MDA, protein carbonyl, serum nitrite, and TOS—were found to be significantly higher in the exposed group compared to the control group; conversely, antioxidant levels—such as catalase, GSH, SOD, and TAS—were higher in the control group than in the exposed workers. The increase in ROS and RNS levels, coupled with a decline in antioxidants, leads to cellular damage within the body, specifically causing damage to lipids and proteins (Valko et al., 2006). Furthermore, micronuclei (MN) frequency was higher in the exposed group, indicating an increase in genotoxicity—a finding consistent with other studies on oxidative stress in workers (Kaushik et al., 2012). Continuous exposure to brick kiln emissions—containing particulate matter, toxic gases, fumes, and other environmental pollutants generated during brick manufacturing—may lead to increased oxidative stress among workers, thereby damaging cellular components, including DNA (Gómez-Oliván et al., 2012). We distribute workers into three distinct catergories (baker, modulars, loaders) to know the effect of job type. Levels of MDA, protein carbonyl, and serum nitrite across all groups were comparable, suggesting a similar degree of oxidative stress—possibly attributable to the shared working environment within the brick kiln industry. Alterations in antioxidant enzyme activities have been found to be associated with various metabolic disorders (Birben et al., 2012). Among whom only the bakers were observed wearing any form of facial covering (typically a small cloth wrapped around the mouth). Oxidative Stress (TOS) was highest in the baker group. This indicates that compared to those in other occupations, bakers have a greater oxidative burden. Because they are constantly exposed to high temperatures, furnace heat, smoke, and pollution from burning objects—all of which can enhance the generation of reactive oxygen species (ROS)—bakers may have elevated TOS levels. Despite being exposed to heat, dust, and pollutants during work, workers' antioxidant systems are generally stable, as seen by the stable TAS levels. MN frequencies are same in all Groups in Baker. The findings of the present study are supported by previous reports demonstrating increased oxidative stress and genotoxicity among workers exposed to brick kiln emissions and other occupational hazards (Kaushik et al., 2012; Jahan et al., 2016; Alghazeer et al., 2018; David et al., 2022) also provided similar results. The study conducted by Maryam Raza et al. (2016) demonstrated that occupational exposure to toxic substances significantly increased oxidative stress and DNA damage among industrial workers. The findings revealed that brick kiln workers exhibited the highest level of oxidative stress (77%), followed by furniture workers (67%), paint industry workers (67%), and welding workers (62%). In terms of , Lipid peroxidation levels were comparatively higher in 6-10 years exposure group, but no significant variation was observed indicating that oxidative stress was elevated irrespective of the exposure duration. No differences between groups may be attributed to continuous occupational exposure which affects workers similarly irrespective of duration of employment. In terms of the antioxidant status, the relatively higher levels of GSH, Catalase and SOD in workers having more than 10 years of exposure may be indicative of an adaptive or compensatory mechanism developed by the body against prolonged oxidative stress. The long-term exposure may stimulate the endogenous antioxidant defense mechanisms to compensate for the overproduction of ROS upto certain limit. The frequency of micronucleus was almost similar in all three groups indicating that genotoxic damage may occur at the beginning of occupational exposure and last throughout the work tenure without remarkable progression. Overall, the combined increase in oxidative stress markers along with depletion of antioxidant defenses suggests that long-term occupational exposure in brick kiln workers may disturb the oxidant-antioxidant balance, leading to continuous cellular stress and possible long-term health complications. Although overall genetic damage appeared similar across groups, loaders may experience marginally higher chromosomal damage due to greater occupational burden. The findings of the present study are supported by previous reports demonstrating increased oxidative stress and genotoxicity among workers exposed to brick kiln emissions and other occupational hazards. Based on the findings of this study, it can be suggested that there is an need for modernization of traditional brick-making methods, considering the large number of workers exposed to harsh brick kiln environment and other health hazards factors . Adoption of modern and efficient brick kiln technologies may help reduce health risk .Additionally, timely determination of oxidative stress biomarkers is crucial for the early detection of the toxic effects of the brick kiln environment on workers, thereby enabling their

management through the administration of antioxidant tablets or dietary modifications and proper use of personal protective equipment (PPE), improvement in workplace safety measures reduces occupational health risks among workers and inclusion of antioxidant-rich dietary supplements may help reduce oxidative stress and Work primarily affects the bakers, the tasks should be performed on a rotational basis to allow sufficient time to manage the impact and enable the bakers to recover. Regular medical examinations and health monitoring programs for workers are also recommended to improve occupational health outcomes and overall quality of life. To prevent health hazards in brick kiln workers, determination of oxidative stress biomarkers is very important for the early detection of toxic effect of heavy metals due to brick kiln pollutants. Protective measure should be taken to avoid any health hazards.

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Compliance with Ethical Standards

Conflict of Interest : The authors declare no competing interests.

5. REFERENCES

- Karanikas, N., Steele, S., Bruschi, K., Robertson, C., Kass, J., Popovich, A., & MacFadyen, C. (2021). Occupational health hazards and risks in the wind industry. *Energy Reports*, *7*, 3750-3759.
- Golbabaei, F., Gholami, A., Teimori-Boghsani, G., Yaseri, M., & Kianmehr, M. (2019). Evaluation of occupational exposure to silica dust in mining workers in eastern Iran. *The Open Environmental Research Journal*, *12*(1), 1-6.
- WąsowiczW,GromadzińskaJ,RydzyńskiK(2001)Bloodconcentration of essential trace elements and heavy metals in workers exposed to lead and cadmium. Int J Occup Med Environ Health
- Sabuncuoglu, S., Yalcinkaya, A., & Blumenberg, M. (2023). *Importance of oxidative stress and antioxidant system in health and disease*. BoD–Books on Demand.
- Montano, L., Baldini, G. M., Piscopo, M., Liguori, G., Lombardi, R., Ricciardi, M., ... & Motta, O. (2025). Polycyclic aromatic hydrocarbons (PAHs) in the environment: occupational exposure, health risks and fertility implications. *Toxics*, *13*(3), 151.
- García-Lestón, J., Roma-Torres, J., Vilares, M., Pinto, R., Prista, J., Teixeira, J. P., ... & Laffon, B. (2012). Genotoxic effects of occupational exposure to lead and influence of polymorphisms in genes involved in lead toxicokinetics and in DNA repair. *Environment international*, *43*, 29-36.
- Surdu S, Fitzgerald E, Bloom MS, Boscoe FP, Carpenter DO, Hasse RF, Gurzau E (2013) Occupational exposure to arsenic and risk of nonmelanoma skin cancer in a multinational European study. Int J Cancer 133:2182–2191
- Kitchin KT, Conolly R (2010) Arsenic-induced carcinogenesis oxidative stress as a possible mode of action and future research needs for more biologically based risk assessment. Chem Res Toxicol 23: 327–335
- Jomova K, Jenisova Z, Feszterova M, Baros S, Liska J, Hudecova D, Valko M (2011) Arsenic: toxicity, oxidative stress and human disease. J App Toxicol 31(2):95–107
- Wang TS, Huang H (1994) Active oxygen species are involved in the induction of micronuclei in XRS-5 cells. Mutagenesis 9:253–257
- Liu SX, Athar M, Lippai I, Waldren C, Hei TK (2001) Induction of oxyradicals by arsenic: implication for mechanism of genotoxicity. Proc Natl Acad Sci USA 98:1643–1648
- Shi H, Shi X, Liu KJ (2004) Oxidative mechanism of arsenic toxicity and carcinogenesis. Mol Cell Biochem 255:67–78
- Frazzoli, C., Orisakwe, O. E., Dragone, R., & Mantovani, A. (2010). Diagnostic health risk assessment of electronic waste on the general population in developing countries' scenarios. *Environmental Impact Assessment Review*, *30*(6), 388-399. https://doi.org/10.1016/j.eiar.2009.12.004
- Ahluwalia, M., & Kaur, A. (2021). Assessment of chromosomal aberrations among agricultural workers exposed to pesticides in Punjab, India. *Journal of biochemical and molecular toxicology*, *35*(2), e22646. https://doi.org/10.1002/jbt.22646
- Luzhna, L., Kathiria, P., & Kovalchuk, O. (2013). Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. *Frontiers in Genetics*, *4*, 131. https://doi.org/10.3389/fgene.2013.00131
- Kadiiska, M., Gladen, B., Baird, D., Germolec, D., Graham, L., Parker, C., Nyska, A., Wachsman, J., Ames, B., Basu, S., Brot, N., FitzGerald, G., Floyd, R., George, M., Heinecke, J., Hatch, G., Hensley, K., Lawson, J., Marnett, L., . . . Barrett, J. (2004). Biomarkers of Oxidative Stress Study II: Are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? *Free Radical Biology and Medicine*, *38*(6), 698–710. https://doi.org/10.1016/j.freeradbiomed.2004.09.017
- Tsai, C. Y., Su, C. L., Wang, Y. H., Wu, S. M., Liu, W. T., Hsu, W. H., ... & Tseng, C. H. (2023). Impact of lifetime air pollution exposure patterns on the risk of chronic disease. *Environmental Research*, *229*, 115957.
- Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B. W., Shaltiel, S., & Stadtman, E. R. (1990). Determination of carbonyl content in oxidatively modified proteins. *Methods in enzymology*, *186*, 464-478. https://doi.org/10.1016/0076-6879(90)86141-h
- Wills E. D. (1966). Mechanisms of lipid peroxide formation in animal tissues. *The Biochemical journal*, *99*(3), 667-676. https://doi.org/10.1042/bj0990667
- Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Analytical biochemistry*, *126*(1), 131-138.
- Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. Clinical biochemistry, 38(12), 1103-1111.
- Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical biochemistry, 37(4), 277-285.
- ELLMAN GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959 May;82(1):70-7. doi: 10.1016/0003-9861(59)90090-6. PMID: 13650640.
- Goth L., A simple method for determination of serum catalase activity and revisionof reference range. Clinica Chimica Acta, 1991, 196: 143 – 152
- Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European journal of biochemistry*, *47*(3), 469-474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

- Fenech M. (2007). Cytokinesis-block micronucleus cytome assay. *Nature protocols*, *2*(5), 1084–1104. https://doi.org/10.1038/nprot.2007.77
- Halliwell, B., & Gutteridge, J. M. (2015). Free radicals in biology and medicine. Oxford University Press, USA.
- Fenech, M., & Morley, A. A. (1985). Measurement of micronuclei in lymphocytes. *Mutation research*, *147*(1-2), 29-36. https://doi.org/10.1016/0165-1161(85)90015-9
- Valko M, Morris H, Cronin MT (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12:1161–1208
- Kaushik, R., Khaliq, F., Subramaneyaan, M., & Ahmed, R. S. (2012) Pulmonary dysfunctions, oxidative stress and DNA damage in brick kiln workers. Human and Experimental Toxicology. 31(11), 1083-1091.
- Oxidative stress in brickmakers of Juárez City, Chihuahua, México: Case-control study Gómez-Oliván, L. M., Corral-Avitia, A. Y., Carrasco-Urrutia, K. A., González-Granados, N. A., Galar-Martínez, M., Neri-Cruz, N., Islas-Flores, H., & Dublan-García, O. (2012). *Oxidative stress in brickmakers of Juárez City, Chihuahua, México: Case-control study. Advances in Bioscience and Biotechnology, 3*(7), 1051–1059. https://doi.org/10.4236/abb.2012.327128
- Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S. and Kalayci, O. (2012) Oxidative stress and antioxidant defense. World Allergy Organization Journal. 5(1), 9-19.
- Jahan, S., Falah, S., Ullah, H., Ullah, A. and Rauf, N. (2016) Antioxidant enzymes status and reproductive health of adult male workers exposed to brick kiln pollutants in Pakistan. Environmental Science and Pollution Research. 23, 12932-12940.
- Alghazeer, R., Alghazir, N., Awayn, N., Ahtiwesh, O. and Elgahmasi, S. (2018) Biomarkers of oxidative stress and antioxidant defense in patients with type 1 diabetes mellitus. Ibnosina Journal of Medicine and Biomedical Sciences. 10(06), 198-204. doi:10.4103/ijmbs.ijmbs_59_18
- David, M., Jahan, S., Hussain, J., Rehman, H., Cloete, K.J., Afsar, T., Almajwal, A., Alruwaili, N.W. and Razak, S. (2022) Biochemical and reproductive biomarker analysis to study the consequences of heavy metal burden on health profile of male brick kiln workers. Scientific Reports. 12(1), 7172.
- Raza, M., Mahjabeen, I., Fahim, M., Malik, W. A., Khan, A. U., Kayani, M. A., Khan, A., & Akram, Z. (2018). Redox balance and DNA fragmentation in arsenic-exposed occupational workers from different industries of Pakistan. *Environmental science and pollution research international*, *25*(33), 33381–33390. https://doi.org/10.1007/s11356-018-3274-6