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Association of miRNA-31 & miRNA 192 with Nrf2/NF-κB Axis : A Novel Molecular Framework for Assessing Occupational Lead Toxicity

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Research Article

Keywords: Lead Toxicity, miRNA-31, Nrf2, NF-κB, Oxidative Stress, Epigenetics

Posted Date: June 16th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9929504/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Biological Trace Element Research on September 3rd, 2026. See the published version at https://doi.org/10.1007/s12011-026-05318-9.

Abstract

Background

Lead (Pb) exposure is a global health threat characterized by oxidative stress. While Nrf2 and NF-κB pathways are known mediators of systemic toxicity, the epigenetic modulation of these defenses via microRNAs (miRNAs) in human cohorts remains to be fully elucidated. This study investigates the association between blood lead levels (BLL) and a potential molecular axis involving miRNA-31, miRNA-192, Nrf2, and NF-κB.

Materials and Methods

In this cross-sectional study, 80 occupationally lead-exposed healthy workers and 80 age-gender matched healthy controls were enrolled. BLL was estimated using Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Relative expressions of miRNA-31Nrf2, and NF-κB were quantified via RT-PCR, while serum protein levels were measured using commercially available sandwich ELISA kits.

Results

The exposed group exhibited significantly higher median BLL compared to controls ($p < 0.001$). A significant downregulation was observed in Nrf2 mRNA expression (fold change 0.58) and serum protein levels ($p < 0.001$) in occupationally exposed group. Additionly NF-κB showed a non-significant increasing trend at the transcriptional level, while serum protein levels demonstrated a significant positive correlation with BLL. Among the miRNAs, only miRNA-31 was significantly upregulated (fold change 3.95). Correlation analysis revealed a potential molecular axis: BLL showed a significant negative correlation with Nrf2 protein levels ($r = -0.704$) and a strong positive correlation with miRNA-31 ($r = 0.815$). Notably, miRNA-31 expression was inversely correlated with Nrf2 serum levels ($r = -0.729$), suggesting a repressive role in antioxidant defences.

Conclusion

This research characterizes a miRNA-31/Nrf2 axis, revealing that lead-induced deactivation of systemic antioxidant defence is linked to dose-dependent miRNA-31 upregulation. These findings position miRNA-31, Nrf2, and NF-κB as integrated biomarkers to assess the cumulative biological stress and severity of heavy metal toxicity.

INTRODUCTION

Heavy metals are naturally occurring elements found in the earth's crust which have been extensively used in various industrial and manufacturing activities; resulting in their high concentrations in the

environment than natural occurrence levels, thus making them a prominent environmental pollutant; and their toxic effect, a matter of global concern [1]. Lead is one of the oldest known and widely used toxic heavy metals in occupational and environmental settings [2]. Despite stringent public health interventions, lead poisoning remains a critical issue, particularly in low- and middle-income countries. According to the 2019 report of Institute for Health Metric and Evaluation (IHME), Pb exposure accounted for 9,00,000 deaths and is a major driver of hypertensive heart disease and intellectual developmental disabilities, with no known safe blood lead levels [3]. India accounts for 26% of global deaths due to lead exposure every year [4] and 0.6% of the global burden of disease [5].

The pathogenesis of lead toxicity is multifactorial, but oxidative stress resulting from an imbalance between pro-oxidant reactive oxygen species (ROS) and cellular antioxidant defences is a primary hypothesized mechanism [2, 6]. When the ROS levels transcend the antioxidant ability to defend against oxidized molecules, oxidative stress occurs resulting in cell damage [7]. This disbalance in turn activates a variety of transcription factors to prevent oxidative damage [8]. The nuclear factor (erythroid-derived 2) – like 2 (Nrf2) and Nuclear factor – kappa B (NF-κB) are two key redox sensitive transcription factors that are found to regulate cellular redox homeostasis and inflammation in response to intracellular and environmental signals [9]. Nuclear factor erythroid 2–related factor (Nrf2)/antioxidant response element (ARE) signalling pathway is a key protective mechanism found to regulate cellular redox status in response to external and internal stimuli. NRF2 regulates phase 2 antioxidant gene transcription required for ROS mitigation and eventually oxidative stress [10]. NF-κB Nuclear Factor kappa B, another transcription factor activated by ROS further modulates various cellular processes ranging from inflammation, cell growth and survival [11, 12].The levels of ROS determine the cell outcome by regulating these redox responsive transcription factors with the consequent cellular response aiming to prevent further oxidative damage and maintain cell survival [13]. Recent advances reported the effects of Pb on these two TFs and the downstream pathways either in vivo or in experimental animals which is yet to be explored in humans [14–16].

Recent advancements in toxicoepigenomics have highlighted lead's role as an epigenetic modifier[17, 18]. Lead exposure can alter gene expression through non-coding RNAs, particularly microRNAs (miRNAs), which regulate post-transcriptional gene silencing. MiRNAs are highly conserved and pleiotropic, making them ideal candidates for stable biomarkers of environmental exposure[10, 19, 20]. miRNA-31 and miRNA-192 were selected for this study due to their established roles in regulating oxidative stress and inflammatory pathways, which are key mechanisms underlying lead toxicity. [19, 21–23]. miRNA-31 is known to be regulated by NF-κB and is upregulated under oxidative stress conditions[24, 25]. Likewise, miRNA-192 has been associated with redox imbalance and inflammation, partly through modulation of NOD2 and NF-κB signaling pathways[19, 21, 26]. While some in vitro and animal models suggest heavy metals can deregulate these miRNAs, human data regarding their association with Nrf2 and NF-κB under occupational lead exposure remains sparse and often contradictory[19, 20, 26–28].

In context to our previous studies, wherein individuals occupationally exposed to Pb with a significant increased BLL showed redox disbalance [29] and an altered inflammatory status [29–31], we aimed to find how Pb exposure is affecting the master regulators (Nrf2 and NF-κB) of these signalling pathways and their association to expression of miRNAs. The results obtained from this study will aid in understanding the underlying mechanism of Pb toxicity within the body and the regulation of redox stress caused by it.

MATERIALS AND METHODS

This cross-sectional study consisted of 80 healthy individuals working in metal and wood handicraft factories of the industrial area of Jodhpur, Rajasthan, India and 80 healthy age-gender matched controls. Informed consent was taken from each individual. Individuals (exposed and non-exposed) having any history of medical illnesses like diabetes, hypertension, inflammatory disease, cancer, and cardiovascular diseases were excluded from the study. Socio-demographic profile, lifestyle, and body mass index (BMI), working hours, medical history, tobacco and alcohol consumption were taken using a series of self-developed questionnaires. Ethical clearance was provided by the Institutional Ethics Committee (IEC) (AIIMS/IEC/2022/3939).

Sample collection

Venous blood was collected under aseptic conditions in EDTA and plain vacutainers for Pb levels, Nrf2 &NF-κB gene expression and protein estimation. Samples collected in plain vacutainers were then centrifuged at 3000 rpm for 10 minutes to separate the serum. EDTA samples and the separated serum were then aliquoted and stored at -20 ^{0}C till further processing.

Estimation of Blood Lead Levels (BLL):

Blood Pb levels were measured using Graphite Furnace Atomic Absorption Spectrometry (GFAAS) with Zeeman correction using iCE 3500 AAS (Thermo Fischer Scientific, Waltham, USA). Combination of 0.5% Triton X-100, 0.1% $NH_4H_2PO_4$ (ammonium dihydrogen orthophosphate) and 0.2% HNO_3 was used as matrix modifier for blood Pb assessment. Quality control was ensured using CLIN Check whole blood metal controls (Recipe, Germany). Pb level were quantified in duplicate using absorbance at 283.3 nm. 5-step program was used for analysis (130°C/20 s, 200°C/30 s, 600°C/10 s, 2000°C/3 s, 2450°C/3 s). Standards were acquired from Thermo Fischer Scientific. Different standard solutions were made using 1000 ppm Pb stock solution for calibration. Calibration curve of 95% fit was considered satisfactory. All glassware and plasticware used for estimation were kept in acid (5% HNO3) overnight, washed with distilled water and dried before use. The results were expressed as µg/dL for Pb.

RNA Isolation and Gene Expression Analysis

The total cellular RNA including miRNAs was extracted from whole blood using TRI Reagent BD (Sigma-Aldrich, USA) and reverse transcription was carried out using iScript™ cDNA Synthesis Kit (BioRad). Quantitative RT-PCR was performed using miRCURY LNA SYBR® Green PCR Kit (Qiagen). For mRNA analysis, specific primers for Nrf2 and NF-κB were used, with GAPDH serving as the endogenous control for normalization. For miRNA analysis, miRCURY LNA miRNA PCR Assays for miRNA-31 and miRNA-192 were normalized with SnU6 RNA as control. The primers of Nrf2, NF-κB and GAPDH utilised in the current study are given in Table 1. The RT- PCR data was analyzed by means of the relative gene expression method. 2^-ΔΔCT method was used to calculate the fold change of target genes in the chosen population.

Table 1
Primer sequence used in the present study.

Gene name	Primers	Sequences	Amplicon size (bp)	Accession number
Nrf2	Forward	5'-CACATCCAGTCAGAAACCAGTGG-3'	112	NM_006164
	Reverse	5'- GGAATGTCTGCGCCAAAAGCTG-3'		
NF- κB	Forward	5'- GCAGCACTACTTCTTGACCACC-3'	130	NM_047415744
	Reverse	5'-TCTGCTCCTGAGCATTGACGTC – 3'		
GAPDH	Forward	5'-GGATTTGGTCGTATTGGG-3'	151	NM_001357943
	Reverse	5'-GGAAGATGGTGATGGGATT-3'		

NF-κB and NRF2 serum level quantification:

NF-κB and NRF2 serum levels were estimated in duplicate by ELISA (ABKINE Biosystems). The optical density was taken by using a microplate reader (EON BioTek) at 450 nm.

Statistical Analysis

The data collected was analysed using Microsoft Excel and GraphPad Prism 8.0 for Windows. Shapiro–Wilk test (p value > 0.05) was utilised to assess the normality of the data. Mean ± SD and median with range was used to represent continuous variables with normal and skewed distribution. Non-parametric data was compared with the help of Mann Whitney U test and Spearman rho was tested to determine the correlation between different variables. P value < 0.05 was considered to be statistically significant.

RESULTS

The mean age of the occupationally Pb exposed subjects (n = 80) was 34.3 $\pm$ 12.2 years (range 18–65 years) with the Mean $\pm$ Standard Deviation (SD) of their BMI as 22.5 ± 5.11 kg/m^2. The male to female

ratio was 7:1. Among the total subjects, 60% individuals consumed vegetarian diet and 40% were non vegetarians. In the exposed group 16 individuals consumed alcohol on a regular basis and 32 participants consumed alcohol. In total, 80 seemingly healthy subjects without any history of occupational exposure to metals were enlisted in the study as controls with their age in the range of 21–50 year. The demographic characteristics of the study subjects are shown in Table 2. No statistically relevant difference was observed between the different characteristics in cases and controls. Lead (Pb) level was measured in the whole blood by Atomic Absorption Spectrometry (AAS) and expressed as μg/dL. The result showed significant increased BLL in the exposed population with the mean ± SD BLL of 6.52 ± 4.13 μg/dL as compared to the healthy individuals (Table 3). On associating the various demographic parameters with BLL in cases and control groups, males showed a significant higher trend with median BLL of 6.54 μg/dL as compared to median BLL of 3.62 μg/dL in females. No significant difference in BLL was noted in exposed as well as non-exposed population in relation to alcohol and tobacco usage.

Table 2
Demographic characteristics of study population

Characteristics	Exposed (n = 80)	Non-Exposed (n = 80)	P value
	n	n	
Gender			
Male	70	62	0.63
Female	10	18	1.0
BMI (kg/m^2)			
Mean ± (SD)	22.5 ±5.11	24.0±3.29	0.12
Age (years)			
Mean ± (SD)	34.3±12.2	30.5 ±5.9	0.06
Duration of exposure (years)			
< 5	32	-	-
> 5	48	-	-
Tobacco			0.09
Yes	16	33	
Alcohol consumption			0.34
Yes	32	30	
Food habits			
Veg	48	44	0.59
Non veg	32	36	0.65

Data is presented as Mean± SD and Median. Mann–Whitney test of significance was used for comparison. p<0.05 is considered significant.

Gene expression of NF- κB and Nrf2 genes

mRNA levels of NF- κB and Nrf2 were determined by using RT-PCR using specific primers (Table 1) for the included genes. Normalisation was achieved by utilising GAPDH as an endogenous control. The relative gene expression of the target genes in the study population was quantified by $2^{-\Delta\Delta CT}$ method.

The Nrf2 gene expression was significantly downregulated (fold change 0.53, p value = 0.0061) in cases. Increasing trend in NF- κB was noted in the exposed group (fold change 1.76, p value 0.09) in comparison to control (Fig. 1). The Spearman correlation analysis (Fig. 2) revealed negative significant association between Nrf2 expression and BLL (Spearman r = -0.4680 p value < 0.0001), while a weak positive albeit non-significant correlation was found between expression of NF- κB gene and BLL (r = 0.1485, p value = 0.16) (Fig. 2).

Serum Nrf2 and NF- κB level

Serum levels of Nrf2 and NF- κB were estimated by commercially available ELISA kits. Exposed population had significantly lower Nrf2 levels 25.41 $\pm$ 8.5 pg/mL (p value < 0.001) and an increased trend in the serum NF- κB levels was noted (Fig. 3, Table 3). A significant negative asociation was found between Nrf2 levels and BLL (r = -0.703, p value < 0.001). Spearman correlation analysis showing a significant positive association between BLL and serum NF-κB (ng/L) levels (r = 0.3661, p = 0.001), suggesting that increasing lead burden may trigger inflammatory signalling (Fig. 4).

Table 3
Comparison of BLL and serum levels of proteins in study population.

	Exposed (n = 80)		**Non-exposed (n = 80)**		**p-value**
	Mean ± SD	**Median (Range)**	**Mean ± SD**	**Median (Range)**	
Blood lead levels (µg/dL)	6.52±4.13	5.15 (3.26–16.57)	1.92±1.13	1.67 (0.41–5.11)	**< 0.001***
Nrf2 (pg/mL)	25.41±8.51	24.87 (19.0-44.4)	62.4±52.2	41.2 (19.1–264)	**< 0.001***
NF- κB (ng/L)	222.6 ± 114.8	193.4 (101.2–669.6)	198.4 ± 50.63	181.8 (136-367.6)	**0.61**

Data is presented as Mean± SD and Median. Mann–Whitney test of significance was used for comparison. p<0.05 is considered significant.

Analysis of circulating miRNAs

The circulating levels of miRNA-31 and miRNA-192 were assessed in total RNA isolated from whole blood. To normalize the expression data, SnU6 RNA was utilized as the endogenous control. Analysis of the results revealed a significant upregulation of miRNA-31 in the occupationally exposed group, which

exhibited a relative fold change of 3.95 compared to 1.01 in the control group (p = < 0.001). In contrast, the expression levels of miRNA-192 did not show a significant relative fold change in the cases, with a fold change of 1.01 compared to 1.00 in the controls (p = 0.61).

Correlation Analysis of Lead Exposure and Molecular Markers

Spearman correlation analysis was conducted to evaluate the relationships between blood lead levels (BLL), transcriptional expression, and circulating protein markers in the study population. A robust and significant negative correlation was established between BLL and the Nrf2 signaling axis, where increasing lead concentrations were inversely associated with both Nrf2 mRNA expression ($r = -0.471$, $p < 0.001$) and serum Nrf2 protein levels ($r = -0.704$, $p < 0.001$). Conversely, a significant and strong positive correlation was observed between BLL and the relative fold change of circulating miRNA-31 ($r = 0.815$, $p < 0.001$), suggesting a dose-dependent upregulation of this microRNA in response to increasing lead exposure. Regarding the inflammatory marker NF-κB, systemic lead levels showed a significant positive association with serum protein levels ($r = 0.342$, $p = 0.001$). The mRNA expression of NF-κB did show an increasing trend in exposed population its correlation with BLL was not significant ($r = 0.131$, $p = 0.225$).

Furthermore, miRNA-31 expression exhibited a significant negative correlation with both Nrf2 mRNA fold change ($r = -0.487$, $p = 0.012$) and serum Nrf2 levels ($r = -0.729$, $p < 0.001$), implying that the upregulation of miRNA-31 may contribute to the repression of the Nrf2-mediated antioxidant response. Analysis of crosstalk between the two transcription factors revealed a significant positive correlation between NF-κB mRNA and Nrf2 mRNA fold changes ($r = 0.227$, $p = 0.035$). However, at the systemic protein level, a significant negative correlation was noted between serum Nrf2 and NF-κB levels ($r = -0.228$, $p = 0.040$), reflecting the antagonistic relationship between antioxidant and inflammatory pathways during lead-induced stress. The collective associations, including the central role of lead-induced Nrf2 suppression and concurrent dose-dependent increases in miRNA-31, are summarized in the Spearman correlation matrix (Fig. 6).

DISCUSSION

Exposure to Pb, both occupational and environmental has been proven to be profoundly damaging to the human body and no level of exposure to Pb is known to be without harmful effects [32]. Oxidative stress is one of the most commonly hypothesized underlying mechanism responsible for these effects which can further regulate expression of many transcription factors and hence signalling pathways[7, 10, 12]. In the present study, BLL of occupationally Pb exposed individuals working in metal and wood handicraft factories was assessed and found to be significantly high with a mean BLL of 6.52 ± 4.13 µg/dL respectively with 36 individuals showing elevated BLL > 5 µg/dL. Our previous studies conducted in the

same occupational population also reported similar BLL findings whereas other studies conducted in Indian population had reported much higher BLL which could be attributed to the difference in the chosen industry where exposure could have occurred[33, 34].

To further discern the molecular mechanism involved in Pb toxicity, the expression and levels of two redox sensitive transcription factors: Nrf2 and NF-κB were measured. These two transcription factors get activated under oxidative stress and together, modulate the cellular redox homeostasis [14]. A significant downregulation was noted in the mRNA expression of Nrf2 (fold change 0.53, p value = 0.0061) in the exposed population, which was also reflected in the serum (p value < 0.001). Our findings establish a robust and significant negative correlation between BLL and the Nrf2 signalling axis; as lead concentrations rise, both Nrf2 mRNA expression (r = -0.471) and serum Nrf2 protein levels (r = -0.704) decrease significantly.Nrf2 is a master regulator of transcription of various antioxidant enzymes in response to oxidative stress. The potential relationship between Pb and Nrf2 signalling has been suggested in previous in vivo and in vitro experimental studies wherein conditions which cause increase in oxidative stress has been noted to cause downregulation of both Nrf2 gene and protein at different doses and duration of Pb exposure [15, 35–37]. Albarakati A. et al. demonstrated a significant decrease in the expression of Nrf2 in Pb treated rats and high levels of malondialdehyde (MDA), which is the marker for oxidative stress [15]. Thus, this decreasing trend of Nrf2 in our study indicates elevated levels of ROS within the body. The expression and levels of Nrf2 has been used as a readout of oxidative stress in various disease outcome because of Nrf2's role as an upstream modulator of key antioxidant enzymes [38]. Therefore, the findings of current study support the explanation for the lowered antioxidant defence system observed in association with the Pb toxicity [39, 40]. There are many observational studies, evaluating Nrf2 expression in PBMCs (peripheral blood mononuclear cells) in different disease contexts wherein imbalance in antioxidant reserve plays an important role in pathogenesis [38, 41–43]. To the best of our knowledge, this is among the first human cohort studies to assess the mRNA expression and levels of Nrf2 in association with Pb in healthy human cohort implying the role of Nrf2 in modulating oxidative stress induced by Pb exposure.

The other candidate transcription factor assessed in this study was NF-κB which has been associated with both oxidative stress and various cellular pathways [44]. The experimental studies highlight the role of NF- κB in generation of inflammatory cytokines under Pb exposure [45–48].These inflammatory cytokines are suggested to participate in generation of oxidative stress along with negative association with antioxidant enzymes [29, 30, 49]. Increased oxidative stress is reported to cause an accentuated production of inflammatory cytokines with NF- κB having a major role in transcription of these cytokines (35). These findings are suggestive of role of NF- κB signalling pathway in Pb induced inflammation and oxidative stress. While experimental models often show significant upregulation of NF-kappa B under high lead concentrations, our results showed an increasing trend in mRNA expression (fold change) and protein levels. Interestingly, our correlation analysis revealed a significant positive association between BLL and serum NF- κB levels (r = 0.3661, p = 0.001). This suggests that while transcriptional changes may be subtle at current exposure levels, rising lead burden does trigger systemic inflammatory signalling. This could be attributed to the lower Pb exposure in the cases in comparison to experimental

studies as well as the other environmental factors such as psychological stress, sleep deprivation, age, diet, consumption of alcohol and tobacco, environmental pollutants like organics dust that can contribute to upregulated expression and levels of the protein [50–57].

There is some general agreement that diverse biological responses are noted under different level of ROS which dictates the cellular outcome by modulating different redox-receptive transcription factors like Nrf2 and NF-κB [12]. Low ROS levels have been found to induce Nrf2, however under moderate increase in ROS levels, NF-κB was found to get activated [13]. On the contrary when there is persistent ROS accumulation in the cell, apoptosis is triggered through certain atypical NF-κB pathways [58]. The correct cellular response against the oxidative stress, is determined by the levels of ROS. NF- κB target genes are typically known to promote cellular survival, and therefore would try to attenuate ROS to promote survival [44]. Consequently, activation of NF-κB-associated pathways may represent a compensatory cellular response to oxidative stress through the induction of various target genes, including antioxidant enzymes. This is further evidenced by the significant negative correlation between serum Nrf2 and NF-κB levels ($r = -0.228$), reflecting an antagonistic relationship between antioxidant and inflammatory pathways at the systemic protein level.

In the present study, miRNA-31 and miRNA-192 were selected as primary candidates for investigation due to their documented involvement in modulating cellular responses to oxidative stress and inflammation, which are central to the pathophysiology of lead toxicity. Scientific evidence suggests that miRNA-31 is universally expressed and is directly regulated by NF-κB, which acts as an upstream enhancer to trigger its transcription[24, 25, 28]. Beyond its inflammatory role, miRNA-31 has a potential link to oxidative stress; experimental models have demonstrated that its expression increases under oxidative conditions, where it may disrupt mitochondrial activity and target antioxidant enzymes like GPX8[59]. Similarly, miRNA-192 was chosen because it is frequently reported in diseases associated with redox imbalance and has been shown to decrease the expression of NOD2, a protein involved in activating NF-κB signaling [21, 60]. Previous in vitro studies had also reported the upregulation of miRNA-192 following exposure to other heavy metals like cadmium and manganese[61, 62].

In the results, the lack of significant change in miRNA-192 expression can be justified by several factors unique to this human cohort. While miRNA-31 showed a significant dose-dependent upregulation ($r = 0.815$), the absence of a fold change in miRNA-192 may be attributed to the relatively lower blood lead levels observed in this study compared to the high concentrations used in experimental cell line and animal models[17, 63]. Furthermore, because the change in NF-κB levels in this study did not reach statistical significance, and miRNA-192 is extensively linked to the NF-κB signaling pathway, the stable status of the transcription factor likely explains the lack of miRNA-192 modulation. The differential response between these two miRNAs highlights that miRNA-31 may be a more sensitive epigenetic marker for moderate, occupational lead exposure, whereas miRNA-192 might only be altered under more severe toxic insult.

Limitations of the Study

One of the primary limitations of the present study is that lead was the only heavy metal estimated. Assessing other toxic heavy metals to which the population might have been exposed would have provided a more comprehensive view of the cumulative effect of metal exposure on gene expression. Furthermore, blood lead level (BLL) serves as an indicator of recent exposure, whereas most accumulated lead is stored in the bones; therefore, bone lead assessment is preferred for evaluating chronic exposure. The relatively small sample size also serves as a constraint, as a larger cohort might have been helpful in presenting a more definitive BLL trend and verifying the mechanistic link between lead exposure and altered miRNA expression.

Additionally, the expression and levels of transcription factors like NF-κB are influenced by various confounding factors, such as psychological stress, sleep deprivation, age, diet, and the consumption of alcohol or tobacco[64, 65]. In the present study, individuals with tobacco and alcohol consumption habits were present in both the exposed and control groups. Although no significant correlation was observed between tobacco/alcohol consumption and NF-κB levels, these factors may still have contributed to elevated baseline NF-κB expression in the control population, thereby potentially masking significant intergroup differences. Finally, the study could be further strengthened by assessing specific oxidative stress markers and the levels of other downstream proteins involved in the Nrf2 and NF-κB signaling pathways to provide a more holistic picture of the molecular mechanisms involved in lead-induced toxicity.

Conclusion

Occupational and environmental exposure to Lead (Pb) remains a critical global health concern, as no level of exposure is considered safe for the human body. The findings of this study demonstrate that individuals working handicraft industry exhibit significantly elevated blood lead levels, which serve as a catalyst for systemic molecular alterations. While the role of oxidative stress in lead pathogenesis is well established, this study offers a novel contribution by identifying a specific molecular axis between epigenetic modifiers and redox-sensitive transcription factors in a seemingly healthy human cohort. The novelty of this research lies in its characterization of a specific miRNA-31/Nrf2 molecular axis, revealing that lead-induced deactivation of the systemic antioxidant defense system is intrinsically linked to a significant, dose-dependent upregulation of this microRNA.

Establishing this molecular axis is key to understanding the early pathophysiological changes that occur during occupational exposure. The identified relationship between miRNA-31, Nrf2, and NF-κB provides a potential template for clinical application, serving as a set of sensitive readout markers to assess the level of oxidative stress and the severity of heavy metal toxicity before clinical disease states manifest. By demonstrating how these pathways modulate in a dose-dependent manner, this study offers a more holistic and predictive view of how systemic lead burden progresses into a disease state within the human body.

Declarations

Compliance with Ethical Standards

Conflict of Interest : The authors declare that they have no conflict of interest.

Funding Information

Institution support, All India Institute of Medical Sciences Jodhpur.

Author Contribution

Kanishka Kumar and Sudha Anjali contributed equally to this work and share first authorship. Kanishka Kumar and Sudha Anjali were involved in study conceptualization, methodology, data acquisition, laboratory investigations, data analysis, interpretation of results, and manuscript drafting. Shailja Sharma conceived and supervised the study, secured resources, guided data interpretation, and critically revised the manuscript. Shweta Rana contributed to data analysis and interpretation of findings. Purvi Purohit, Mithu Banerjee, Dharmveer Yadav, and Praveen Sharma assisted in sample collection, experimental procedures, data curation, and manuscript review. All authors read, reviewed, and approved the final manuscript.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Roy S, Gupta SK, Prakash J, Habib G, Baudh K, Nasr M (2019) Ecological and human health risk assessment of heavy metal contamination in road dust in the National Capital Territory (NCT) of Delhi, India. Environ Sci Pollut Res 26(29):30413–30425
2. Mitra P, Sharma S, Purohit P, Sharma P (2017) Clinical and molecular aspects of lead toxicity: An update. Crit Rev Clin Lab Sci 54(7–8):506–528
3. Lead poisoning [Internet] [cited 2022 Apr 11]. Available from: https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health
4. Experts call for urgent state and national level action plans to tackle lead poisoning in India [Internet]. [cited 2023 Jan 24]. Available from: https://www.teriin.org/press-release/experts-call-urgent-state-and-national-level-action-plans-tackle-lead-poisoning-india
5. Lead poisoning | National Health Portal Of India [Internet] [cited 2022 May 16]. Available from: https://www.nhp.gov.in/disease/non-communicable-disease/lead-poisoning

6. Engedal N, Žerovnik E, Rudov A, Galli F, Olivieri F, Procopio AD et al (2018) From Oxidative Stress Damage to Pathways, Networks, and Autophagy via MicroRNAs. Oxid Med Cell Longev 2018:4968321
7. Lopes ACBA, Peixe TS, Mesas AE, Paoliello MMB (2016) Lead Exposure and Oxidative Stress: A Systematic Review. In: de Voogt P (ed) Reviews of Environmental Contamination and Toxicology Volume 236. Springer International Publishing, Cham, pp 193–238
8. Halliwell B, Cross CE (1994) Oxygen-derived species: their relation to human disease and environmental stress. Environ Health Perspect 102(Suppl 10):5–12
9. Bellezza I, Mierla AL, Minelli A (2010) Nrf2 and NF-κB and Their Concerted Modulation in Cancer Pathogenesis and Progression. Cancers (Basel) 2(2):483–497
10. Buha A, Baralić K, Djukic-Cosic D, Bulat Z, Tinkov A, Panieri E et al (2021) The Role of Toxic Metals and Metalloids in Nrf2 Signaling. Antioxid (Basel) 10(5):630
11. Park MH, Hong JT (2016) Roles of NF-κB in Cancer and Inflammatory Diseases and Their Therapeutic Approaches. Cells 5(2):15
12. Lingappan K (2018) NF-κB in Oxidative Stress. Curr Opin Toxicol 7:81–86
13. Morgan MJ, Liu Z (2011) Crosstalk of reactive oxygen species and NF-κB signaling. Cell Res 21(1):103–115
14. Aglan HS, Gebremedhn S, Salilew-Wondim D, Neuhof C, Tholen E, Holker M et al (2020) Regulation of Nrf2 and NF-κB during lead toxicity in bovine granulosa cells. Cell Tissue Res 380(3):643–655
15. Albarakati AJA, Baty RS, Aljoudi AM, Habotta OA, Elmahallawy EK, Kassab RB et al (2020) Luteolin protects against lead acetate-induced nephrotoxicity through antioxidant, anti-inflammatory, anti-apoptotic, and Nrf2/HO-1 signaling pathways. Mol Biol Rep 47(4):2591–2603
16. Zhang L, Bo J, Chen W, Li S, Wang Y, Yan L et al (2021) The Role of Nrf2 on the Cognitive Dysfunction of High-fat Diet Mice Following Lead Exposure. Biol Trace Elem Res 199(6):2247–2258
17. Ochoa-Martínez ÁC, Varela-Silva JA, Orta-García ST, Carrizales-Yáñez L, Pérez-Maldonado IN (2021) Lead (Pb) exposure is associated with changes in the expression levels of circulating miRNAS (miR-155, miR-126) in Mexican women. Environ Toxicol Pharmacol 83:103598
18. Wallace DR, Taalab YM, Heinze S, Tariba Lovaković B, Pizent A, Renieri E et al (2020) Toxic-Metal-Induced Alteration in miRNA Expression Profile as a Proposed Mechanism for Disease Development. Cells 9(4):E901
19. Ayers D, Baron B, Hunter T (2015) miRNA Influences in NRF2 Pathway Interactions within Cancer Models. J Nucleic Acids 2015:143636
20. Kaundal RK, Datusalia AK, Sharma SS (2022) Posttranscriptional regulation of Nrf2 through miRNAs and their role in Alzheimer's disease. Pharmacol Res 175:106018
21. Bus P, Siersema PD, Verbeek RE, van Baal JWPM (2014) Upregulation of miRNA-143, -145, -192, and – 194 in esophageal epithelial cells upon acidic bile salt stimulation. Dis Esophagus 27(6):591–600

22. Li F, Deng Y, Zhang S, Zhu B, Wang J, Wang J et al (2022) Human hepatocyte-enriched miRNA-192-3p promotes HBV replication through inhibiting Akt/mTOR signalling by targeting ZNF143 in hepatic cell lines. Emerg Microbes Infections 11(1):616–628
23. Masalha M, Sidi Y, Avni D (2018) The contribution of feedback loops between miRNAs, cytokines and growth factors to the pathogenesis of psoriasis. Exp Dermatol 27(6):603–610
24. Yan S, Xu Z, Lou F, Zhang L, Ke F, Bai J et al (2015) NF-κB-induced microRNA-31 promotes epidermal hyperplasia by repressing protein phosphatase 6 in psoriasis. Nat Commun 6. 10.1038/ncomms8652
25. Chu Q, Bi D, Zheng W, Xu T (2021) MicroRNA negatively regulates NF-κB-mediated immune responses by targeting NOD1 in the teleost fish Miichthys miiuy. Sci China Life Sci 64(5):803–815
26. Nejad C, Stunden HJ, Gantier MP (2018) A guide to miRNAs in inflammation and innate immune responses. FEBS J 285(20):3695–3716
27. Chen J, Zhang S, Tong J, Teng X, Zhang Z, Li S et al (2020) Whole transcriptome-based miRNA-mRNA network analysis revealed the mechanism of inflammation-immunosuppressive damage caused by cadmium in common carp spleens. Sci Total Environ 717:137081
28. Uribesalgo I, Ballaré C, Di Croce L (2012) Polycomb regulates NF-κB signaling in cancer through miRNA. Cancer Cell 21(1):5–7
29. Saikiran G, Mitra P, Sharma S, Kumar PK, Sharma P (2022) Selenium, oxidative stress and inflammatory markers in handicraft workers occupationally exposed to lead. Arch Environ Occup Health 77(7):561–567
30. Goyal T, Mitra P, Singh P, Sharma P, Sharma S (2021) Evaluation of oxidative stress and pro-inflammatory cytokines in occupationally cadmium exposed workers. Work 69(1):67–73
31. Goyal T, Mitra P, Singh P, Ghosh R, Lingeswaran M, Sharma S et al (2021) Alterations in Th17 and Treg Lymphocyte Subset in Workers Occupationally Exposed to Lead. Biol Trace Elem Res 199(5):1693–1700
32. Challenges in diagnosing lead poisoning: A review of occupationally and nonoccupationally exposed cases reported in India - PubMed [Internet]. [cited 2023 Jan 24]. Available from: https://pubmed.ncbi.nlm.nih.gov/32496147/
33. Batra J, Thakur A, Meena SK, Singh L, Kumar J, Juyal D (2020) Blood lead levels among the occupationally exposed workers and its effect on calcium and vitamin D metabolism: A case-control study. J Family Med Prim Care 9(5):2388–2393
34. Himani, Kumar R, Ansari JA, Mahdi AA, Sharma D, Karunanand B et al (2020) Blood Lead Levels in Occupationally Exposed Workers Involved in Battery Factories of Delhi-NCR Region: Effect on Vitamin D and Calcium Metabolism. Indian J Clin Biochem 35(1):80–87
35. Yang L, Li X, Jiang A, Li X, Chang W, Chen J et al (2020) Metformin alleviates lead-induced mitochondrial fragmentation via AMPK/Nrf2 activation in SH-SY5Y cells. Redox Biol 36:101626
36. Lu J, Jiang H, Liu B, Baiyun R, Li S, Lv Y et al (2018) Grape seed procyanidin extract protects against Pb-induced lung toxicity by activating the AMPK/Nrf2/p62 signaling axis. Food Chem Toxicol

116:59–69

37. Ye F, Li X, Li L, Lyu L, Yuan J, Chen J (2015) The role of Nrf2 in protection against Pb-induced oxidative stress and apoptosis in SH-SY5Y cells. Food Chem Toxicol 86:191–201
38. Egea J, González-Rodríguez Á, Gómez-Guerrero C, Moreno JA (2019) Editorial: Role of Nrf2 in Disease: Novel Molecular Mechanisms and Therapeutic Approaches. Front Pharmacol 10:1149
39. Babiker F, Al-Kouh A, Kilarkaje N (2019) Lead exposure induces oxidative stress, apoptosis, and attenuates protection of cardiac myocytes against ischemia-reperfusion injury. Drug Chem Toxicol 42(2):147–156
40. Vacchi-Suzzi C, Viens L, Harrington JM, Levine K, Karimi R, Meliker JR (2018) Low levels of lead and glutathione markers of redox status in human blood. Environ Geochem Health 40(4):1175–1185
41. C AFPAA, A SMC, C P (2012) Serum oxidative stress-induced repression of Nrf2 and GSH depletion: a mechanism potentially involved in endothelial dysfunction of young smokers. PLoS ONE 7(1). 10.1371/journal.pone.0030291
42. Mozzini C, Garbin U, Stranieri C, Pasini A, Solani E, Tinelli IA et al (2015) Endoplasmic reticulum stress and Nrf2 repression in circulating cells of type 2 diabetic patients without the recommended glycemic goals. Free Radic Res 49(3):244–252
43. Mozzini C, Fratta Pasini A, Garbin U, Stranieri C, Pasini A, Vallerio P et al (2014) Increased endoplasmic reticulum stress and Nrf2 repression in peripheral blood mononuclear cells of patients with stable coronary artery disease. Free Radic Biol Med 68:178–185
44. Roles for NF-κB in nerve cell survival, plasticity, and disease | Cell Death & Differentiation [Internet]. [cited 2023 Feb 3]. Available from: https://www.nature.com/articles/4401837
45. Hoseinrad H, Shahrestanaki JK, Moosazadeh Moghaddam M, Mousazadeh A, Yadegari P, Afsharzadeh N (2021) Protective Effect of Vitamin D3 Against Pb-Induced Neurotoxicity by Regulating the Nrf2 and NF-κB Pathways. Neurotox Res 39(3):687–696
46. Liu C-M, Sun Y-Z, Sun J-M, Ma J-Q, Cheng C (2012) Protective role of quercetin against lead-induced inflammatory response in rat kidney through the ROS-mediated MAPKs and NF-κB pathway. Biochim Biophys Acta 1820(10):1693–1703
47. Almutairi MM, Nadeem A, Ansari MA, Bakheet SA, Attia SM, Albekairi TH et al (2022) Lead (Pb) exposure exacerbates behavioral and immune abnormalities by upregulating Th17 and NF-κB-related signaling in BTBR T+ Itpr3tf/J autistic mouse model. Neurotoxicology 91:340–348
48. Attafı IM, Bakheet SA, Ahmad SF, Belali OM, Alanazi FE, Aljarboa SA et al (2022) Lead Nitrate Induces Inflammation and Apoptosis in Rat Lungs Through the Activation of NF-κB and AhR Signaling Pathways. Environ Sci Pollut Res Int 29(43):64959–64970
49. Metryka E, Chibowska K, Gutowska I, Falkowska A, Kupnicka P, Barczak K et al (2018) Lead (Pb) Exposure Enhances Expression of Factors Associated with Inflammation. Int J Mol Sci 19(6):1813
50. Churg A, Xie C, Wang X, Vincent R, Wang RD (2005) Air pollution particles activate NF-kappaB on contact with airway epithelial cell surfaces. Toxicol Appl Pharmacol 208(1):37–45

51. Lidén J, Ek A, Palmberg L, Okret S, Larsson K (2003) Organic dust activates NF-kappaB in lung epithelial cells. Respir Med 97(8):882–892
52. Koo JW, Russo SJ, Ferguson D, Nestler EJ, Duman RS (2010) Nuclear factor-κB is a critical mediator of stress-impaired neurogenesis and depressive behavior. Proceedings of the National Academy of Sciences. ;107(6):2669–74
53. Ramesh V, Thatte HS, McCarley RW, Basheer R (2007) Adenosine and sleep deprivation promote NF-kappaB nuclear translocation in cholinergic basal forebrain. J Neurochem 100(5):1351–1363
54. García-García VA, Alameda JP, Page A, Casanova ML (2021) Role of NF-κB in Ageing and Age-Related Diseases: Lessons from Genetically Modified Mouse Models. Cells 10(8):1906
55. Carlsen H, Haugen F, Zadelaar S, Kleemann R, Kooistra T, Drevon CA et al (2009) Diet-induced obesity increases NF-κB signaling in reporter mice. Genes Nutr 4(3):215–222
56. Nowak AJ, Relja B (2020) The Impact of Acute or Chronic Alcohol Intake on the NF-κB Signaling Pathway in Alcohol-Related Liver Disease. Int J Mol Sci 21(24):9407
57. Cigarette smoke condensate activates nuclear transcription factor-κB through phosphorylation and degradation of IκBα: correlation with induction of cyclooxygenase-2 | Carcinogenesis | Oxford Academic [Internet]. [cited 2022 Dec 25]. Available from: https://academic.oup.com/carcin/article/23/9/1511/2608286
58. Perkins ND, Gilmore TD (2006) Good cop, bad cop: the different faces of NF-kappaB. Cell Death Differ 13(5):759–772
59. McGrath J, Kane LE, Maher SG (2022) The Influence of MicroRNA-31 on Oxidative Stress and Radiosensitivity in Pancreatic Ductal Adenocarcinoma. Cells 11(15):2294
60. Chuang AY, Chuang JC, Zhai Z, Wu F, Kwon JH (2014) NOD2 Expression is Regulated by microRNAs in Colonic Epithelial HCT116 Cells. Inflamm Bowel Dis 20(1):126–135
61. Kotsyfakis M, Patelarou E (2019) MicroRNAs as biomarkers of harmful environmental and occupational exposures: a systematic review. Biomarkers 24(7):623–630
62. Tumolo MR, Panico A, De Donno A, Mincarone P, Leo CG, Guarino R et al (2022) The expression of microRNAs and exposure to environmental contaminants related to human health: a review. Int J Environ Health Res 32(2):332–354
63. Qiao M, Yang H, Liu L, Yu T, Wang H, Chen X et al (2024) Chronic Lead Exposure in Adult Mice: Associations with miR-671/CDR1as Regulation, NF-κB Signaling, and Alzheimer's Disease-like Pathology. Toxics 12(6):410
64. Mir FA, Lark ARS, Nehs CJ (2025) Unraveling the interplay between sleep, redox metabolism, and aging: implications for brain health and longevity. Front Aging 6:1605070
65. Nennig SE, Schank JR (2017) The Role of NFkB in Drug Addiction: Beyond Inflammation. Alcohol Alcohol 52(2):172–179

Figures

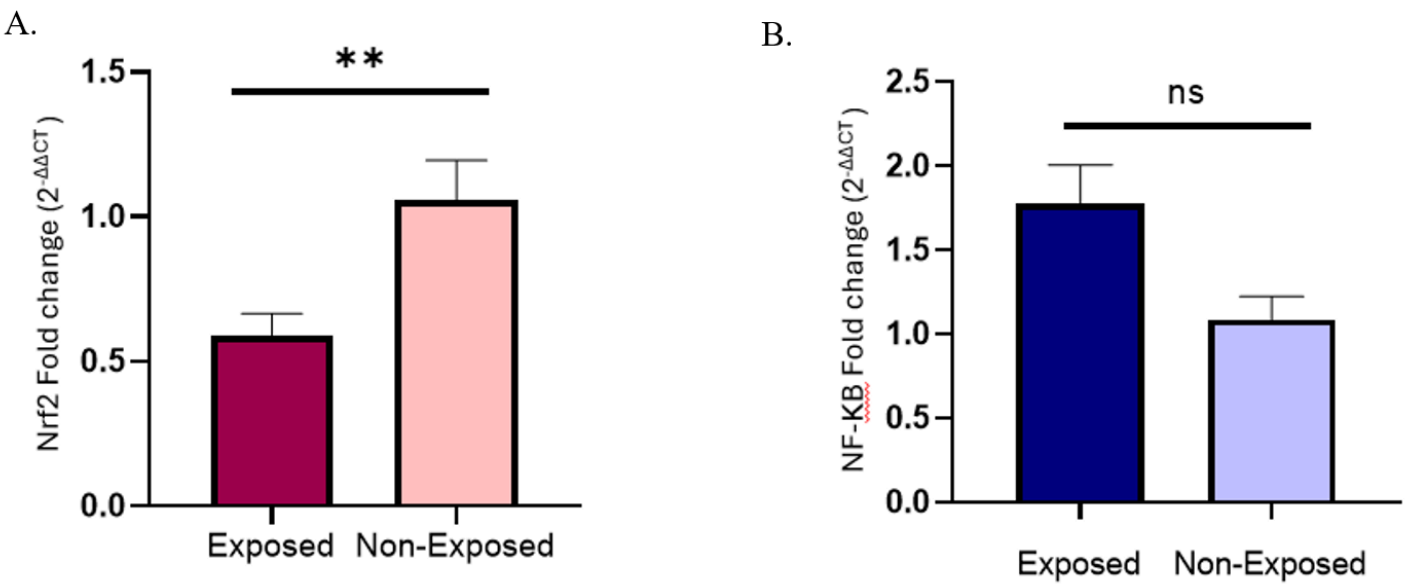


Figure 1

Relative mRNA expression of redox-sensitive transcription factors Nrf2 and NF- κB in the study group

(A)Relative fold change of Nrf2 mRNA expression showing a significant downregulation in lead-exposed individuals compared to controls (p = 0.006) (B). Relative fold change of NF- κB mRNA expression; although an increasing trend (fold change = 1.76) was observed in the exposed group, the difference was not statistically significant (ns). Error bars represent SEM, and significance was determined via the Mann-Whitney U test.

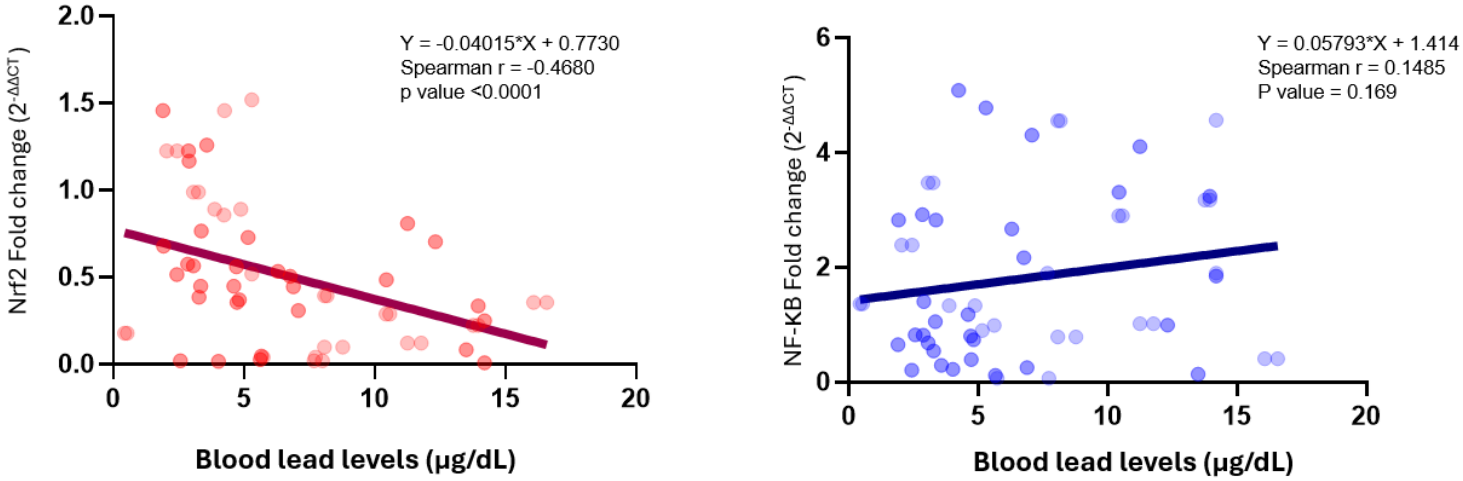


Figure 2

Correlation Analysis of Nrf2 and NF- κB expression with Blood Lead Levels (BLL) in the exposed population

(A) Spearman correlation plot demonstrating a significant negative association between BLL and Nrf2 gene expression. (B) Spearman correlation of of NF- κB mRNA expression plot showing a weak positive association between BLL and gene expression.

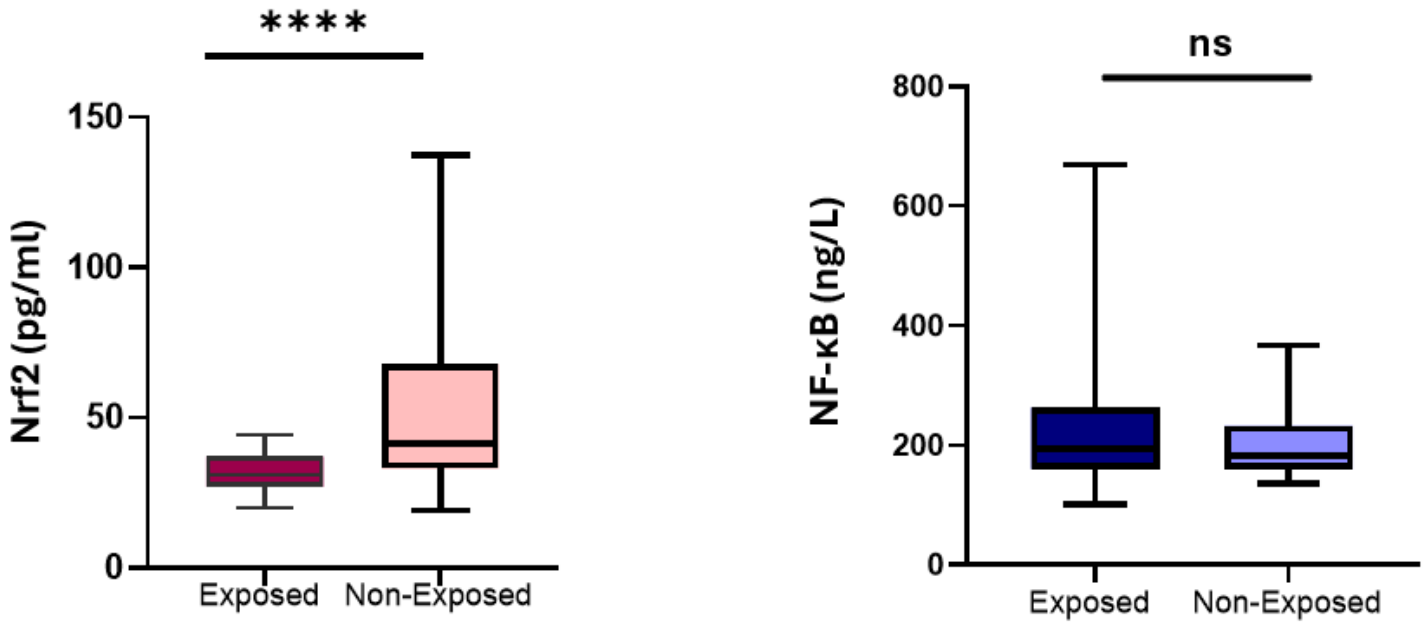


Figure 3

Serum levels of Nrf2 and NF- κB in the study group

(A). Box-and-whisker plot showing significantly lower serum Nrf2 (pg/ml) levels in occupationally exposed workers compared to non-exposed controls ($p < 0.0001$). (B). Comparison of serum NF- κB (ng/L) levels. Error bars represent SEM, and significance was determined via the Mann-Whitney U test.

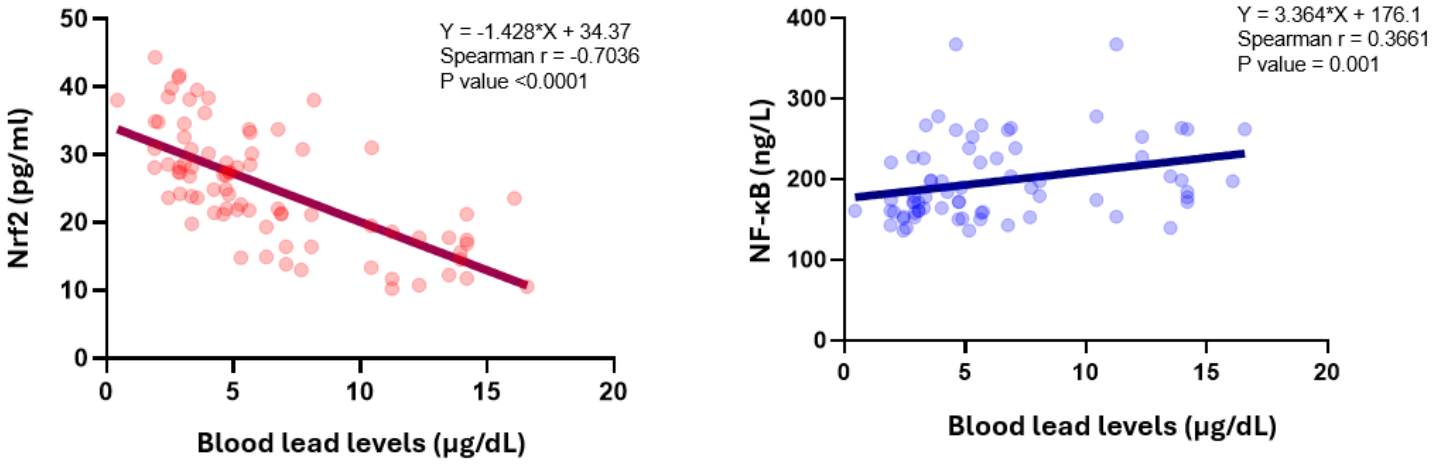


Figure 4

Correlation of Serum Proteins with Blood Lead Levels (BLL)

Association between circulating lead levels and antioxidant/inflammatory protein markers. (A) Spearman correlation analysis showing a significant negative correlation between BLL and serum Nrf2 (pg/ml) levels. (B). Spearman correlation analysis showing a significant positive association between BLL and serum NF-κB (ng/L) levels.

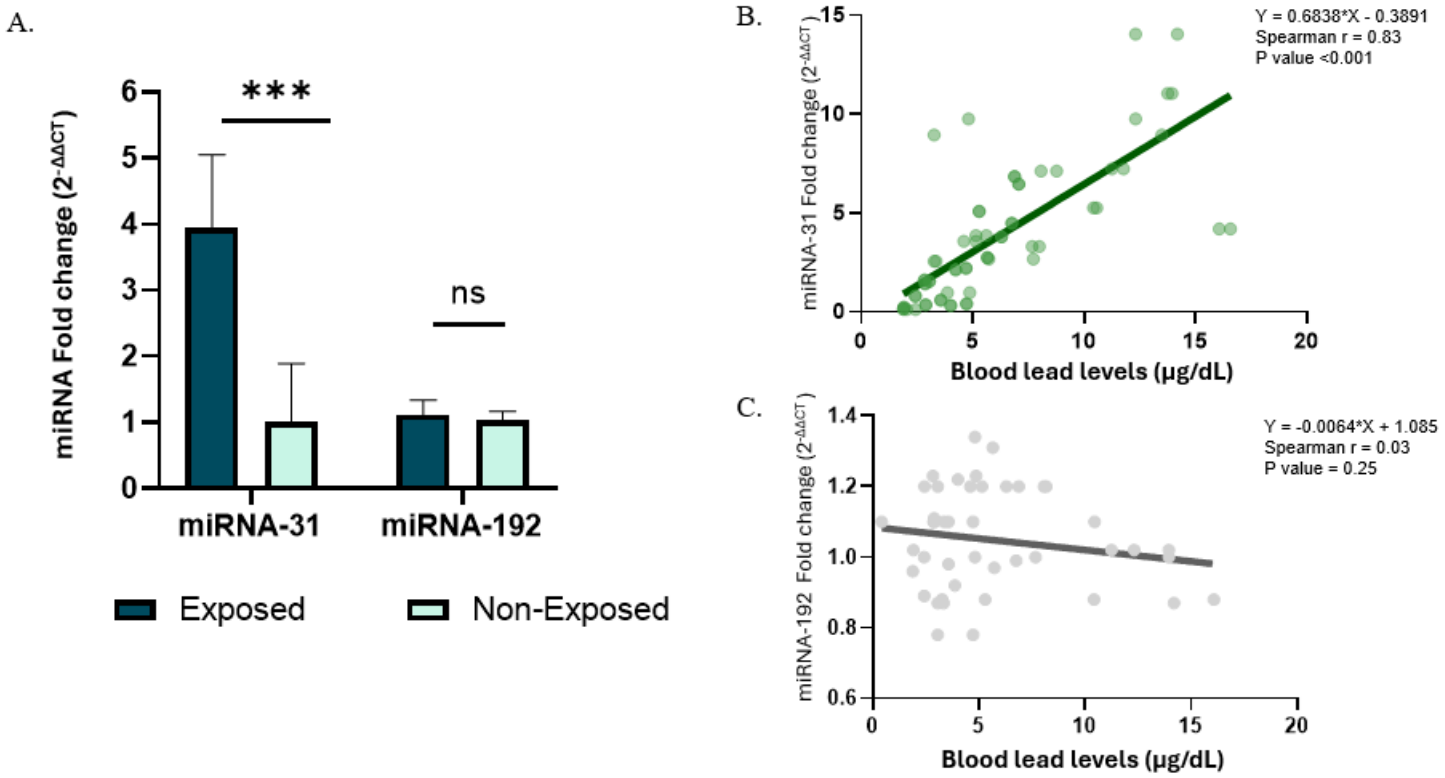


Figure 5

Expression of circulating miRNA-31 and its association with blood lead levels (BLL) in study group.

(A)Comparison of relative fold change of miRNA-31 and miRNA-192 in occupationally exposed individuals versus non-exposed controls. (B) Scatter plot and Spearman correlation analysis demonstrating the relationship between miRNA-31 expression and blood lead levels. (C) Scatter plot and Spearman correlation analysis demonstrating the relationship between miRNA-192 expression and blood lead levels.

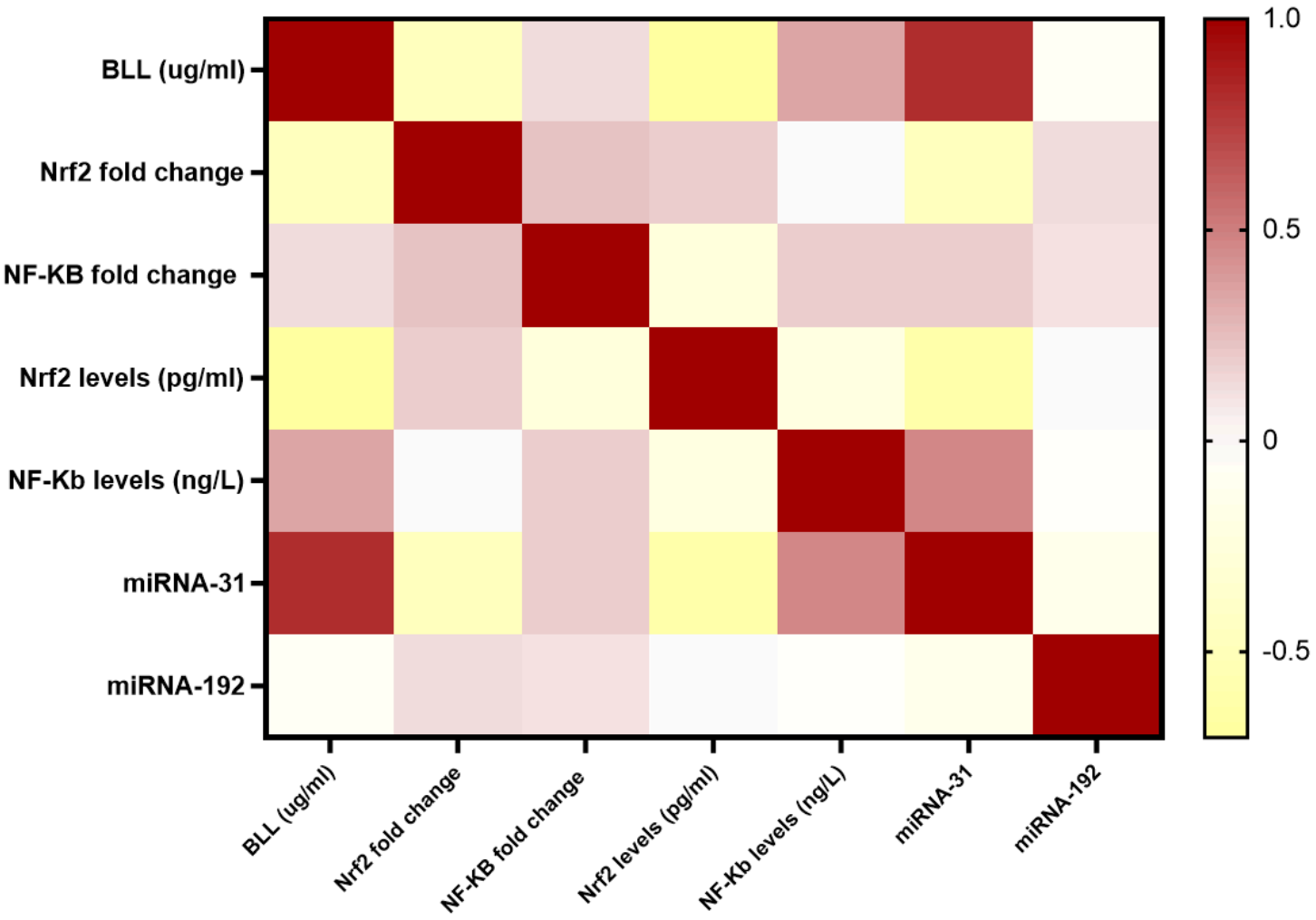


Figure 6

Correlation Matrix of Lead Exposure and Molecular Markers

Heatmap representation of Spearman correlation coefficients (r) among blood lead levels (BLL, µg/ml), Nrf2 fold change, NF-κB fold change, Nrf2 protein levels (pg/ml), and NF-κB protein levels (ng/L), miRNA-31 and miRNA-192 (fold change). Color intensity corresponds to the strength and direction of correlation, with red indicating positive correlation and yellow indicating negative correlation. The diagonal represents self-correlation (r = 1.0). Correlations were computed using two-tailed Spearman analysis.

Supplementary Files

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