Association between lead exposure and DNA damage (genotoxicity): systematic review and meta-analysis

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Abstract

Introduction: Studies suggest that chronic lead (Pb) exposure may induce Deoxyribonucleic acid (DNA) damage. However, there is no synthesised evidence in this regard. We systematically reviewed existing literature and synthesised evidence on the association between chronic Pb exposure and markers of genotoxicity. **Methods**: Observational studies reporting biomarkers of DNA damage among occupationally Pb-exposed and unexposed controls were systematically searched from PubMed, Scopus and Embase databases from inception to January 2022. The markers included were micronucleus frequency (MN), chromosomal aberrations, comet assay, and 8-hydroxy-deoxyguanosine. During the execution review, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Mean differences in the biological markers of DNA damage between Pb-exposed and control groups were pooled using the random-effects model. The heterogeneity was assessed using the Cochran- Q test and I^2 statistic. **Results**: The review included forty-five studies comparing markers of DNA damage between Pb-exposed and unexposed. The primary studies utilised buccal and/or peripheral leukocytes for evaluating the DNA damage. The pooled quantitative results revealed a significantly higher DNA damage characterised by increased levels of MN and SCE frequency, chromosomal aberrations, and oxidative DNA damage (comet assay and 8-OHdG) among Pb-exposed than the unexposed. However, studies included in the review exhibited high levels of heterogeneity between the studies. **Conclusion**: Chronic Pb exposure is associated with DNA damage. However, high-quality, multicentered studies are required to strengthen present observations and further understand the Pb's role in inducing DNA damage.

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Short Title: Lead exposure & DNA damage SRMA

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Conclusion : Chronic Pb exposure is associated with DNA damage. However, high-quality, multicentered studies are required to strengthen present observations and further understand the Pb's role in inducing DNA damage.

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Highlights

1. The synthesized evidence indicates that chronic Pb exposure is associated with DNA damage.
2. The DNA damage markers with Pb exposure include higher levels of micro-nuclei (with nuclear buds and nucleoplasmic bridges), sister chromatid exchange frequency, chromosomal aberrations, and oxidative DNA damage.
3. **Introduction**

Lead (Pb), heavy metal with multiple desirable properties, viz. easy moulding, relatively inert and many others, has been extensively used in various industries, including automobiles, paint, ceramics, batteries, etc. . Therefore, workers in these industries are potentially exposed to high levels of Pb. Any levels of Pb in the biological samples (including blood) may be deemed potentially harmful. The Center for Disease Control (CDC) released reference blood Pb levels acceptable for community adults (i.e. without obvious occupational Pb exposure) is 3.5 µg/dL.

Chronic Pb exposure is associated with abnormalities in neurological , nephrological , cardiovascular , haematological , immunological and reproductive functions. The mechanistic studies have shown that Pb exposure is associated with impairment in antioxidant status of cells, i.e. depletion of reduced glutathione pools and increase in reactive oxygen species formation leading to organ toxicity. Literature on mechanistic as well as epidemiological studies suggests the carcinogenetic risk of Pb . International Agency for Research on Cancer (IARC) classified inorganic Pb in group 2A, and organic Pb in group 3 as Pb is related to excess risk of stomach, lungs, kidney, balder and brain cancers . Data from Steenland et al. 2019 and others conclude a robust positive correlation between blood lead levels (BLL) and the incidence of cancers in the lungs, brain and other organs The genotoxicity induced by Pb may cause a carcinogenic mechanism and lead to cancer incidence [14,15].

Studies used comet assay (tail length, tail moment, tail DNA), chromosomal aberrations and micronuclei frequency (MN) to screen workers' genotoxicity. The outcomes from such studies are contradictory, as many studies exhibited no or mild change [16–20], while others showed higher levels of DNA damage and chromosomal aberrations in exposed individuals . By considering the impact of Pb on genetic material, the pooled results would provide the best estimates of the effects of Pb exposure on induction of DNA damage and genotoxicity mediated various disorders. This would provide evidence for policy implications. Therefore, the present systematic review and meta-analysis synthesised the association between BLL with different biomarkers of genotoxicity among workers. The present study observations would provide insights and comprehension of the pieces of evidence on Pb-induced genotoxicity.

Methods

The systematic review was registered at Prospero (Reg No PROSPERO 2022CRD42022286810) and executed as per Preferred Reporting Items of Systematic reviews and Meta-Analysis (PRISMA) . Observational studies reporting the comparison of genotoxicity and /or DNA damage between occupationally Pb-exposed and healthy controls were systematically searched. The search was performed in PubMed- Medline, Scopus, and Embase online repositories. The last search was performed on 10^{th} January 2022. The search terms and strategies adopted in the current review are tabulated in Supplementary Table 1. The search parameters were constructed by using the conventional PICO approach. The "Participant" (individuals occupationally exposed to Pb), "Intervention" (i.e., exposure to Pb), "Comparator" (individuals without occupational exposure to Pb) "Outcome" (DNA damage and genotoxicity parameters) were used. The search on "Outcome" measures included parameters on DNA damage (comet assay), i.e. the percentage of tail DNA, tail intensity, tail length, tail moment and Olive tail moment (OTM), sister chromatid exchange (SCE) frequency,

Micronuclei frequency (MN) (micro-nucleated, Bi-nucleated) and other nuclear abnormalities like Pycnosis, Condensed chromatin, Karyorrhexis, Nuclear buds, Nucleoplasmatic bridges and Mitotic index and oxidative DNA damage markers (8-hydroxy-2-deoxyguanosine) respectively. The studies which included parameters on chromosomal analysis, i.e. Gaps, chromatid aberrations (chromatid breaks, chromatid deletions, chromatid rings, dicentrics, acentric fragments, gaps) and chromosomal aberrations (chromosomal breaks, chromosomal deletions, chromosomal rings, dicentrics, acentric fragments, gaps) were also considered. A sensitivity and precision maximizing strategy was adopted to identify relevant studies, and additional keywords identified during the search were included in the systematic search.

2.1 Screening and reviewing of studies : The authors independently screened the titles and abstracts of all citations, resulting from the systemic search of various electronic databases for their potential inclusion (NR and KRB). Authors (NR and KRB) independently reviewed the full text of articles scrutinized during screening. The final list of studies meeting the inclusion and exclusion criteria was prepared after removing duplicates based on the authors' mutual consensus (NR, KRB, and BSB) (Fig. 1). The studies meeting the following inclusion criteria were selected: (1) Published in English; (2) Occupational investigation; (3) article must contain an exposure group and control group; (4) the two groups were comparable in terms of age and health status; and (5) availability of outcome variables in mean ± standard deviation (SD) or can be convertible into mean ± S.D form. Studies were excluded according to the following criteria; (1) Studies involving participants with only <18 years of age; (2) Case reports, reviews, letters to editors, editorials, and methodological papers; (3) duplicated data, and incomplete information studies; and (4) animal experiments and basic research. Lateral search for potential studies using the studies identified during the full-text review was additionally attempted. The Rayyan online platform was used for screening and selection of studies .

2.2 Data extraction, analysis, and management : The Microsoft excel ver. 2016 was used to extract relevant details from studies to achieve planned objectives. The details of publications (author(s), title, journal, and year of publication), participant details (study location, age, gender, and clinical details), Pb exposure details (source(s), duration and Pb levels in biological samples) and outcome measures (DNA damage and genotoxicity markers) were extracted from the primary studies and recorded. The corresponding authors of the primary studies were contacted by email when relevant data was unavailable. The authors were contacted and reminded on at least two occasions with an interval of two weeks between the reminders before excluding the study / declaring "non-availability of data". However, these studies were part of the critical evaluation for generating necessary evidence.

Data on central tendency (mean/median) and dispersion [(SD) / Standard error (SE) / Interquartile range / 95% confidence interval (CI)] for available parameters were independently extracted from the included studies (NR and KRB) and verified for consistency before further analysis (KRB). The outcome variables reported in units other than the conventional units/standard units were converted to standard / conventional units [Blood Pb as µg/dL, SCE frequency and chromosomal aberrations as per cell, MN frequency, Tail DNA, tail intensity as a percentage, tail length in µM and 8-hydroxy-2-deoxyguanosine (8-OHdG) in ng/ml etc.] using standard conversion factors. The measures of central tendency & data dispersion, when provided alternate to mean (e.g., median and mode) and SD (e.g., 95 % CI, Interquartile range, standard error of the mean) appropriate conversions were adopted to pool the results . Mean differences of the outcome variables (where available) were pooled between Pb- exposure and unexposed/control groups when [?] 3 studies were available for quantitative assessment. For studies reporting more than one group in the control and exposed group , the grand mean and SD were calculated . Key details recorded in the data extraction sheet are summarized in Supplementary Table 2.

2.3 Heterogeneity, sensitivity, subgroup, and risk of Bias assessment: The heterogeneity among included studies was assessed using visual inspection of forest plots, the Cochran-Q test, and$I$ -squared (I^2) statistics. Either$I^2 > 25\%$ or Cochrane-Q <0.1 was regarded as evidence for the presence of heterogeneity among the included studies. The random-effects model of Der Simonian and Laird was used after confirming heterogeneity . Further, the sources of heterogeneity were explored by fitting the co-variables such as age and duration of exposure in the meta-regression model depending on the availability of data minimum of

ten studies are essential for meta-regression analysis . The variable responsible for reducing I^2 by 50% in the meta-regression model was regarded as the potential source of heterogeneity; subsequently, a bubble plot was used to explore the covariate's influence. Asymmetrical funnel plot or significant Egger's test ($p < 0.05$) of the effect measures were regarded as evidence for potential publication bias . However, the funnel plot and Egger's test of effect measures were valuable when adequate (i.e. > 10) primary studies were available for pooling the particular outcome variable. Contour-enhanced funnel plot was additionally explored to investigate the sources of biases. Lastly, the influence of the type of sample used (i.e. buccal cells *vs* peripheral leukocytes) was explored by subgroup analysis. However, in view of fewer studies, the proposed sensitivity & subgroup analyses (i.e. < 3 studies under each subgroup) were not executed for the genotoxicity parameters. Data was recorded using a Microsoft Excel sheet and analyzed using Stata version 16 (2019) . Two-sided $p<0.05$ was considered statistically significant.

2.4 Assessment of the risk of bias : New Castle Ottawa scale (NOS) was used to evaluate the risk of bias for each of the included studies . The details of NOS followed in this study are described in our previous publication .

Results:

The search in databases retrieved 4,050 studies. Subsequently, with duplicate removal and screening, Forty-five studies were selected for the review and data synthesis. The study selection details at various screening steps are shown in the PRISMA selection flow chart (Figure 1). The particulars of extracted data related to assessments, primary study participants, and their occupation-related details from the selected studies are listed in Supplementary Table 2. Included studies' participants were occupationally exposed to Pb as smelters, welders, Pb battery manufacturing & recycling workers, automobile workers, petrol station attendants, E-waste workers, and workers involved in the foundry, painting work and chemical plants with occupational Pb usage.

Among 45 studies included in the meta-analysis, seven biomarkers of DNA damage (MN, 8-OHdG, chromosomal aberrations, the comets assay, cell apoptosis, telomere length, and necrosis rate) were reported in biological materials like buckle cells and peripheral leukocytes/ lymphocytes. The MN frequency (n=20) is the most commonly monitored biomarker. In contrast, telomere length , necrosis and apoptosis rate were investigated independently by single individual studies and hence synthesis of quantitative information using meta-analysis was not executed for these parameters. The risk of bias assessment results using the NOS is reported in Supplementary Table 3.

3.1 BLL : Forty studies reported BLL comparison between Pb-exposed and controls. The majority of Pb-exposed had a significantly higher BLL among the exposed group than the control group. The included studies were classified based on BLL as per the guidelines of new york state for health workers few studies observed BLL >10 µg/dL (elevated BLL) among the controls . The Pb-exposed in the included studies exhibited wide range of BLL, majority of the studies observed elevated Pb levels in blood (i.e. BLL between of 25–40 µg/dL) , while few observed seriously elevated levels (i.e. 40–80 µg/dL and one study observed very high levels of BLLs (> 80 µg/dL) . The effect measure of mean difference in BLL from studies demonstrates that the study participants with occupational Pb exposure (n = 2,570) had significantly higher BLL than age-matched controls (n = 1,981). The pooled mean difference in BLL is 23.55 (95 %CI, 19.96 to 27.15) µg/dL, and there was high heterogeneity between the studies ($I^2 = 99.72$) (Supplementary Fig.1). The subgroup, sensitivity, and meta-regression analyses did not aid in exploring and identifying the factors attributing to heterogeneity. There is publication bias, as suggested by the asymmetry in the funnel plot (p = 0.271); the contour-enhanced funnel plot indicates the possible existence of other biases. (Supplementary Fig. 2).

3.2 Micronuclei frequency: MN frequency is an extensively studied biomarker to investigate chromosomal damage induced by cytotoxic agents (/genotoxicity) . In this review, twenty studies reported the MN frequency in mono nucleated cells (MNC) among Pb-exposed individuals. The few studies utilized buccal cells as a source of sample, while others used peripheral leukocytes .

Nineteen out of twenty studies observed considerably higher MN frequency among the Pb-exposed than

the controls, with most studies reporting statistical significance. Consistent with the observations from primary studies, the pooled results revealed significantly higher MN frequency in the Pb-exposed with a mean difference of 1.50 (95%CI 1.17 to 1.84)% than the controls, with high heterogeneity between studies ($I^2 = 99.74$ %) (Fig.2A). The asymmetric funnel plot is suggestive of potential publication bias ($p = 0.011$) and plausible other biases as suggested by the contour-enhanced funnel plot (Supplementary Fig.3). The subgroup analysis with the type of sample (i.e. buccal cells vs. peripheral leukocytes) used for assessing the MN frequency did not indicate the source of heterogeneity or the direction of the results. The subgroup pooled mean difference in MN involving buccal cells and peripheral leukocytes was respectively 1.78 (95 %CI 0.00 to 3.57)% and 1.37 (95 %CI 1.05 to 1.69) %withI^2 99.93% and 98.98% (Fig.2A).

In addition, ten studies reported MN frequency among binucleated (BNC) in buccal cells , and peripheral leukocytes . All studies consistently observed higher MN frequency in BNC in the Pb-exposed than in the controls. We noticed contradictory results among studies with low and high BNC in the exposed population vs the control group, however, two of these studies observed a trend, while the remaining studies reported a significant difference between the duo. The pooled difference was 1.97 (95 %CI 1.19 to 2.74) with high heterogeneity ($I^2 = 99.42\%$) (Fig. 2B). Asymmetric funnel plots and contour-enhanced funnel plots suggest possible publication bias (p = 0.048) and other biases (Supplementary Fig. 4). The subgroup mean-difference in BNC between Pb-exposed and controls with buccal cells and peripheral leukocytes was respectively 3.03 (95%CI 1.56 to 4.51,I^2=99.50) % and 1.23 (95 %CI 0.60 to 1.85,I^2=96.93%)% (Fig. 2B).

3.3 Other Biomarkers of Cytogenic alteration : The other biomarkers of cytogenic alteration such as condensed chromatin (CC), lobed nucleus (LN), nuclear buds (NB), mitotic index (NDI), nucleoplasmatic bridges (NPB), pycnosis (PYC) and karyorrhexis (KARY) were available in addition to MN frequency. The CC was quantified by two studies in buccal cells, and both studies reported a higher percentage of CC among Pb exposed than controls. Alabi et al. (2020) was the only study to report a lobed nucleus (the nucleus is segmented into two or more connected lobes), wherein the Pb exposed group exhibited a significantly higher percentage of the lobed nucleus than controls . The NB frequency was reported by five of the included studies; four of these studies used peripheral leukocytes , and one study with buccal cells . Aksu et al.(2019) observed considerably more frequent NB in buccal cells of the Pb-exposed than in the controls, while the other studies showed no significant difference. The pooled difference between Pb-exposed and controls was 0.04 (95 %CI -0.28 to 0.36) % with high between-study heterogeneity ($I^2 = 99.62\%$) (Supplementary Fig. 5).

The NDI frequency was reported among eight of the included studies. Palus et al. was not part of the quantitative analysis in view of the non-availability of data dispersion details. All studies reported NDI frequency using peripheral leukocytes , where Kasuba et al. (2020) and Minozzo et al. (2004) observed statistical significance. The pooled mean difference between the duo was -0.0003 (95 %CI -0.00 to 0.00) % with low between-study heterogeneity ($I^2 = 1.75\%$) (Supplementary Fig. 6).The frequency of NPB was reported in three of the included studies , and two of these observed significant differences. The pooled mean difference between the Pb-exposed and controls was 0.08 (95 %CI 0.02 to 0.13) % with no obvious heterogeneity ($I^2 = 0.00\%$) (Supplementary Fig.7).

The frequency of PYC and KARY was reported using the buccal cells of the participants. The PYC and KARY frequency was considerably higher among Pb-exposed workers compared to the controls. The pooled mean KARY difference between the Pb-exposed and controls was 1.67(95 %CI -0.12 to 3.46)% (Supplementary Fig. 8), with high (99.95%) between-study heterogeneity. Funnel plots and contour-enhanced funnel plots were not analyzed due to fewer available studies (n<10). The results from included studies demonstrated a strong and consistent association between Pb exposure and high rates of MN frequency in buccal cells and lymphocytes of occupationally Pb exposed than controls.

3.4 Sister chromatid exchange (SCE) : Ten of the included studies reported the SCE frequency among lymphocytes . Majority of the studies observed considerably ($p > 0.05$) higher SCE levels among exposed group than the controls . Consistent with the results from primary studies, the pooled mean difference between the duo was 1.35 (95 %CI 0.87 to 1.82) per cell, with high heterogeneity ($I^2 = 93.21\%$) (Fig.3A).

There was the presence of publication and other biases as suggested by the asymmetric funnel plot (p = 0.036) and contour-enhanced funnel plot (Supplementary Fig. 9).

3.5 Chromosomal aberrations : Six of the included studies reported chromosomal aberrations in lymphocytes, with consistent evidences of chromosomal aberrations among occupational Pb exposed workers. The pooled mean difference between Pb-exposed and controls was 2.25 (95 %CI 0.30 to 4.20) per cell with high between-study heterogeneity (I^2 = 99.80%) (Fig.3B). Three studies reported chromosomal breaks in lymphocytes, while in vew of non-availability of data dispersions, Carere et al., 1995 wasn't part of the quantitative analysis. All three studies observed higher chromosomal breaks among the Pb exposed than the controls. The chromosomal acentrics, dicentrics and rings were higher among Pb exposed group analyzed . However, the pooled difference was not available for these parameters due to a single study . Funnel plots and contour-enhanced funnel plots were not analyzed due to fewer available studies (n <10).

3.6 Chromatid aberrations : Two studies reporting chromatid aberrations observed higher chromatid aberrations among Pb exposed group compared to the control group . In case of chromatid acentrics, studies monitored aberrations in lymphocytes and found considerably higher levels among Pb-exposed than in controls. The Pb-exposed group exhibited higher chromatid breaks with pooled mean difference 0.91 (95 %CI -0.07 to 1.89, I^2 = 99.79%) per cell (Supplementary Fig.10). The dicentrics were higher among the Pb-exposed. The funnel plots and contour-enhanced funnel plots were not analyzed due to fewer available studies (n<10).

3.7 Comet assay parameter s: The studies included in the current review evaluated the parameters of the comet's tail, such as its intensity, DNA content, moment and length, to assess the DNA damage. Nine studies monitored the percentage of DNA content in the lymphocyte comet tail nterestingly, all studies reported a significantly higher percentage of DNA in the Pb-exposed group than in the controls. Consistently, the pooled mean difference in the percentage of tail length between the duo revealed the same with a statistical significance, i.e.7.92 (95 %CI 4.77 to 11.07) %, with high heterogeneity between the studies (I^2 = 99.56%) (Fig.4A). Five of the included studies reported tail intensity using peripheral leukocytes . The pooled mean difference between Pb-exposed and controls was 1.79 (95 %CI 0.28 to 3.30 % with high heterogeneity (I^2 = 86. 60%) (Fig.4B). The length of the comet's tail was reported in nine of the included studies using peripheral leukocytes . The majority of the studies observed significantly longer comet's tail among the Pb-exposed as compared to controls , The pooled mean difference in comet's tail length between the duo was 6.31 (95 %CI 2.23 to 10.38) µm with high between-study heterogeneity (I^2 = 98. 60%) (Fig.4C). The tail moment was reported by seven studies using peripheral leukocytes . The tail moment of Pb-exposed was higher as compared to the controls, with pooled tail moment difference of 10.03 (95 %CI 5.71 to 14.35) and heterogeneity (I^2 = 99.07%) (Fig.4D). The OTM was analyzed in lymphocytes , and studies found higher levels of OTM among samples of the Pb-exposed group than in the controls. The comet length was reported by Akram et al., 2019 and Palus et al., 2003 in isolated lymphocytes of the participants; similar to the other parameters of the comet assay, the comet length was considerably higher among Pb-exposed than in controls. A single study using lymphocytes reported the DNA in the head region. The observation of DNA content of the comet's head was lower among the Pb-exposed than the controls was consistent with the other comet assay parameters, suggestive of a relatively greater DNA damage among the Pb-exposed.

3.8 Oxidative DNA damage : The 8-OHdG is a product of oxidative DNA damage and is considered a biomarker of genotoxic exposure while examining genome stability. Five of the included studies reported 8-OHdG in the study participants . The results of these studies marked a higher level of blood 8-OHdG content in the exposed population than control. Similarly, the pooled mean difference between the duo was high, i.e. 32.45 (95 %CI 10.32 to 54.59, I^2 = 96.35%)ng/ml with high heterogeneity (Supplementary Fig. 11).

3.9 Others

3.9.1 Telomere length : One study examined telomere length in peripheral blood lymphocytes of control and exposed workers and demonstrated a significant decrease in telomere length among the exposed

population (1.91±0.46 vs 1.66±0.63).

4.9.2 Apoptosis and Necrosis rate : Kasuba et al., 2012found a significant increase in the apoptosis (5.5 ± 5.9% vs19.6 ± 20.8%) and necrosis rate (2.1 ± 2.9% vs 3.3 ± 5.7%) in whole blood of Pb exposed workers as compared to the control group.

Discussion

Present systematically reviewed the current literature examining the impact of chronic Pb-exposure on DNA damage and genomic instability using updated standard guidelines. Studies describing the DNA damage or genetic instability among occupationally Pb-exposed than the controls without a history of noticeable occupational exposure were primarily included. Occupational Pb-exposed workers were reported with significantly higher BLL consistent with their exposure, and exhibited relatively increased DNA damage, i.e. elevated MN frequency, SCE frequency, total chromosomal aberrations (chromosomal and chromatid aberrations), oxidative DNA damage, apoptotic and necrosis rate. The comet assay results were consistent with the above results, i.e.higher DNA content, intensity, length and moment of the tail. In contrast, telomere length was significantly decreased among Pb-exposed workers compared to the respective controls.

The Pb-exposed group exhibited higher (23.55μg/dL) BLL than the control group. This observation was consistent across the included studies irrespective of the workplace's nature and exposure duration. As there is no safe level for Pb exposure, The CDC recommends investigating the potential source among the adults with BLL [?] 3.5μg/dL, which are categorized as "elevated BLL" (https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6254a4.htm). Similarly, few studies reported BLL [?] 10 μg/dL among the controls (with no obvious occupation Pb exposure) as well; however, significantly lower BLL as compared to the corresponding Pb exposed group.

The evidence from the current study noted a trend of higher MN frequency among Pb exposed than unexposed control groups. MN frequency is the common biomarker used to monitor genotoxic exposure, it is a good indicator of clastogenesis (chromosomal breaks) and aneugenic effects of xenobiotics on the genome. It is a standardized and validated method of predicting the risk of cancer . In this review, 20 studies reported higher MN frequency among Pb exposed group, consistent with the pooled mean difference. The study from Fench and Bonnasi demonstrated the influence of lifestyle factors on DNA damage (i.e.MN frequency in the peripheral leukocytes), suggesting the careful consideration of lifestyle factors while assessing the genotoxic impact of the exposure . Hamurcu et al. (2001) observed significantly higher MN frequency among tobacco smokers without obvious Pb exposure (controls) as compared to controls with non-smoking ($p = 0.048$). However, the MN frequency was significantly higher among Pb exposed (irrespective of the smoking status) as compared to control groups , suggesting the association between Pb exposure and higher MN frequency.

The MN frequency is typically scored in binucleated cells after nuclear division in telophase by blocking cytokinesis using cytochalasin-B; while recently, both mononucleosis and binucelus were considered for MN frequency analysis . The lymphocyte cytokinesis-block micronucleus test (CBMN) is OECD recommended, robust method of genotoxicity assessment . Also, CBMN is a robust predictor biomarker for various disorders . The MN frequency from bi-nucleated cells and other parameters viz. NB, NDI, pycnosis, karyorrhexis and NPB consistently showed greater DNA damage among Pb-exposed than the controls. Notably, NDI is the marker of cell proliferation. In contrast, cells with greater chromosomal damage (lower NDI) will either prematurely attain cellular death prior to cell division or fail to complete the phase of cell division. Therefore, the decreased NDI rate is suggestive of genome instability.

The MN, NPB, and NB are biomarkers of genomic instability , as they predict clastogens-induced aberrations in cell division and structural chromosomal rearrangements . Current observations of increased NB and NPB congruent with MN frequency among the Pb-exposed group suggest an elevated risk of genotoxicity among the Pb-exposed group. Further, increased cellular degeneration markers viz. pycnosis and karyorrhexis among the Pb-exposed workers are consistent with higher DNA damage associated with Pb exposure . Lastly, apoptosis and necrosis, the markers of planned/regulated cellular death either due to ageing or prematurely due to irreversible cellular injury (i.e. beyond cellular repair) were higher among the Pb-exposed.

The frequency of the peripheral lymphocyte chromosomal aberrations is considered a biomarker of genotoxicity due to carcinogens of occupational and environmental sources. Chromosomal aberrations may include the entire chromosomal or only the chromatid, wherein the former is a better indicator of cancer risk . The contrast between these indicators is that S-independent clastogens cause those chromosomal aberrations, while chromatid aberration is induced by S-dependent mutagens . Both chromosome and chromatid aberrations are sub-divided into exchanges and breaks, where breaks represent major aberration in chromatid type, while breaks and chromosome rearrangements (dicentrics) are the foremost part of chromosomal aberration . The current review observed both chromatid and chromosomal type aberrations were considerably higher among the Pb-exposed population, suggesting the potential geno and cytotoxicity associated with chronic Pb-exposure.

The SCEs are biomarkers of genetic instability conventionally used for hazard identification and risk assessment among those occupationally exposed . The SCEs are usually used to examine the cytogenic responses to carcinogen (chemical) exposure. SCEs are considered a more sensitive biomarker of genotoxicity than structural aberrations; however, they are less reliable for assessing cancer risk . Current observations of elevated SCE frequency among the Pb-exposed suggest increased cytotoxicity with Pb exposure.

Comet assay test detects cellular level DNA damage, which is simple and sensitive. The assay detects single-strand breaks, alkali labile and cross-linking sites; hence it is extensively employed in genotoxic regulatory studies. The shape, size and amount of DNA within a comet are assessed either by manual visualization or automated software applications to assess DNA damage particularly of occupational genotoxicity such as those included in this review. Interestingly, all parameters were consistently higher among the Pb-exposed workers than in the controls, suggesting a potential association between DNA damage and Pb exposure.

Enzymatic cleavage of the guanine base, 8-OHDG, is the hallmark of general oxidative DNA damage and carcinogenesis of occupational and environmental exposures . Current results of high 8-OHDG among the Pb exposed group suggest an association between oxidative stress (& possibly carcinogenesis) and chronic Pb exposure.

This evidence appraisal is possibly the earliest to systematically review and document the association between chronic Pb exposure, DNA damage, and cytotoxicity. The primary studies included in the review exhibited high levels of heterogeneity, risk of bias, fewer numbers limiting the subgroup and meta-regression analyses, cross-sectional design and low powered / quality. The current review suggests the need for longitudinal studies with larger samples and better quality to investigate the association between chronic Pb exposure and investigate the pathways for efficient DNA repair. Given the potential genotoxicity and cytotoxicity of Pb, present observations suggest the need for periodic screening of individuals (workers) employed in Pb (or its associated) industries.

4.1 Conclusion: Current evidence synthesis infers that occupational Pb exposure is associated with a higher frequency of MN and SCE, chromosomal aberrations (cytotoxicity) and DNA damage (comet assay and 8-OHDG) as compared to the control group. Chronic Pb exposure is potentially genotoxic (because of higher NB and NPB rates) and induces oxidative DNA damage (elevated 8-OHDG). Current results could provide a scientific basis while considering the revision of strategies used to prevent occupational disease, particularly occupational cancers among the Pb exposed workers. Further, longitudinal & high-quality primary studies are crucial for recommending regular screening for genotoxicity among the Pb-exposed.

References:

Figure Legends

Figure 1: PRISMA flow chart

(Legends / footnotes) The flow chart illustrates the number of articles included and excluded at various steps

Figure 2: Forest plot for MN frequency among mononucleus and binucleus cells.

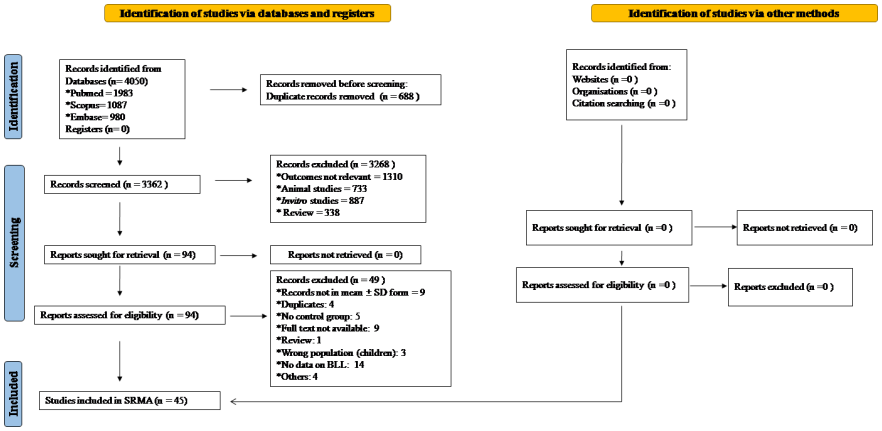
(Legends / footnotes) Group differences in MN frequency among mononucleus(A) and binucleus cells (B) between the occupationally Pb exposed and unexposed workers.

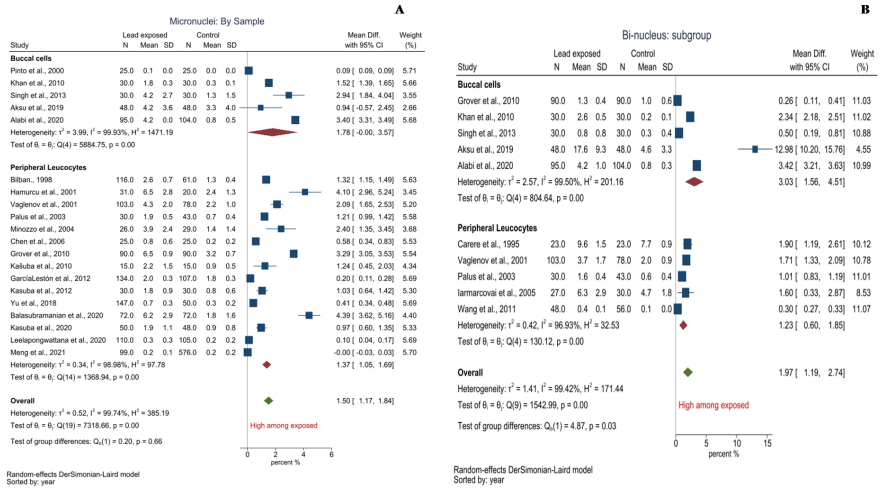
Figure 3: Forest plot for Chromosomal aberrations.

(Legends / footnotes) Group differences in Sister chromtid exchange (A) and chromosomal aberration (B) between the occupationally Pb exposed and unexposed workers.

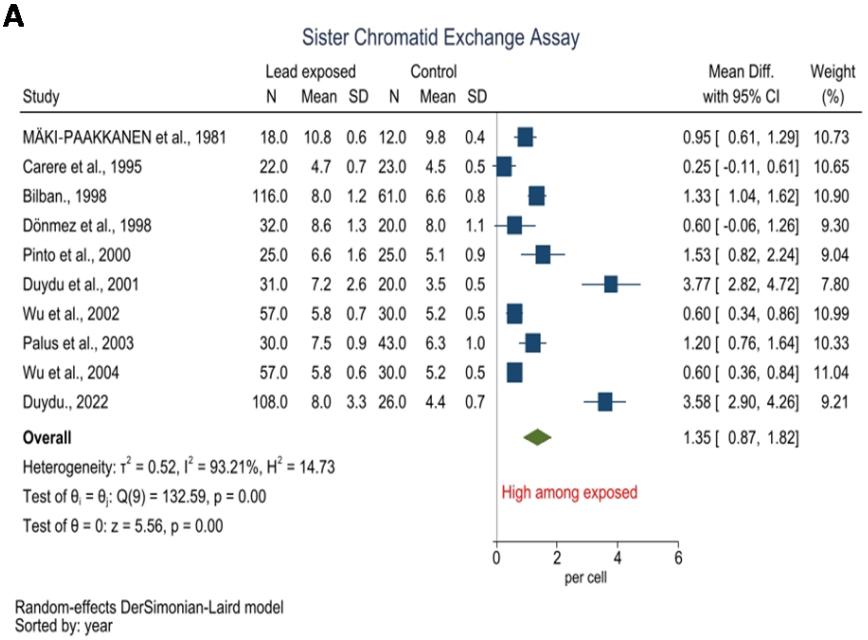
Figure 4: Forest plot for comet assay

(Legends / footnotes) Group differences in % of DNA in tail (A), tail intensity (B), tail length (C) and tail moment (D) between the occupationally Pb exposed and unexposed workers.

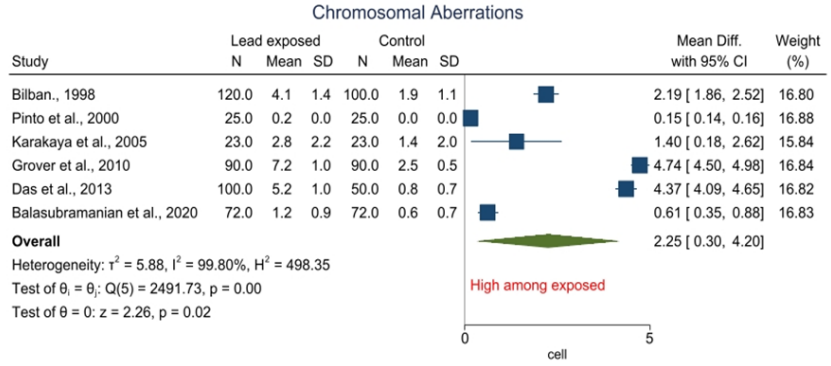


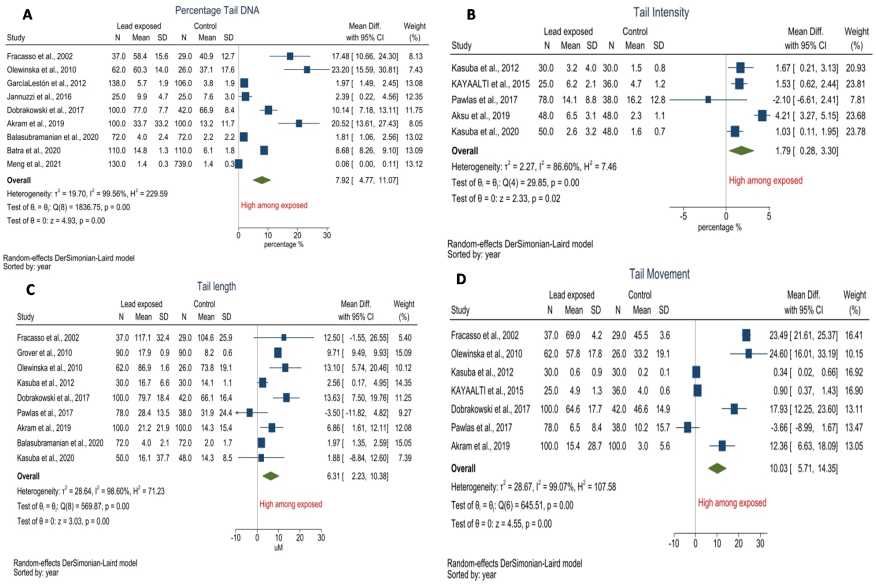


A



B





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Tables.docx available at https://authorea.com/users/495766/articles/577420-association-between-lead-exposure-and-dna-damage-genotoxicity-systematic-review-and-meta-analysis