EUROX 22 (2024) 100300

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology: X

journal homepage: www.journals.elsevier.com/european-journal-of-obstetrics-and-gynecology-and-reproductive-biology

DNA damage and ALAD polymorphism in high blood lead (Pb) levels of pregnant women attending a tertiary care teaching hospital

Mahesh Chandra Sahu [a,b,*,1], Kuldip Upadhyay [b], Shweta Gupta [b], Kabita Chanania [c], Sanghamitra Pati [a]

[a] ICMR-Regional Medical Research Centre, Chandrasekharpur, Bhubaneswar 751023, Odisha, India
[b] ICMR-National Institute of Occupational Health, Meghaninagar, Ahmedabad 380016, Gujarat, India
[c] Department of Gynaecology and Obstetrics, IMS and SUM hospital, Siksha 'O' Anusandhan University, Kalinga Nagar, Bhubaneswar 751003, Odisha, India

ARTICLE INFO

Keywords:
Lead (Pb)
Pregnancy
Blood lead levels (BLL)
ALAD
DNA damage
Comet assay

ABSTRACTS

Background: Pregnant women are particularly vulnerable to lead toxicity due to increased absorption and decreased elimination of lead from their bodies. The δ-aminolevulinic acid dehydratase (ALAD) gene plays a crucial role in lead metabolism, and its polymorphisms have been implicated in modifying the susceptibility to lead toxicity.

Methods: A cross-sectional study was conducted involving 90 pregnant women and blood samples were collected to measure blood lead levels (BLL) and assessed DNA damage using the comet assay. ALAD polymorphisms were genotyped using PCR-RFLP analysis with MspI restriction enzyme. Statistical analysis, including chi-square tests, logistic regression, and correlation analysis, was performed to determine associations between ALAD polymorphisms, BLL, and DNA damage.

Results: From 90 pregnant women the participants, 16 had high BLL (≥5 μg/dL), while the remaining 74 had normal levels (<5 μg/dL). The ALAD 1–2 genotype was found to be significantly associated with high BLL ($p < 0.001$). Pregnant women with the ALAD 1–2 genotype exhibited higher levels of DNA damage compared to those with other genotypes ($p < 0.001$). Furthermore, a positive correlation was observed between the transfer of lead concentration from mother to infant and DNA damage severity ($r = 0.511$, $p < 0.001$).

Conclusions: The combination of comet assay and polymorphism analysis offers a comprehensive approach to understanding the impact of lead exposure during pregnancy. These findings underscore the urgent need for effective regulatory measures to reduce lead exposure in the environment and mitigate its adverse effects of lead on maternal and child health.

Introduction

Lead exposure is a significant public health concern, particularly during pregnancy, due to its potential adverse effects on maternal and fetal health [1]. Lead is a toxic heavy metal commonly found in various environmental sources, including old paint, contaminated soil, water, and certain occupations [2]. Exposure to lead during pregnancy has been associated with a wide range of adverse outcomes, including increased risk of pregnancy complications, developmental delays, cognitive impairments, and behavioral problems in children. Exposure to lead during pregnancy can result in adverse outcomes, including preterm birth, low birth weight, developmental abnormalities, and long-term neurocognitive impairments in the offspring [3]. Understanding the implications of lead toxicity during pregnancy with δ-aminolevulinate dehydratase (ALAD) gene and different ALAD genotypes, including ALAD 1–1, ALAD 1–2, and ALAD 2–2 polymorphisms have been suggested to modulate individual susceptibility to lead toxicity [3–7].

The most significant levels of ALAD activity are observed in erythrocytes and in the liver. Specifically, prenatal lead exposure is inversely associated with genomic DNA methylation in cord blood [8]. DNA damage is a well-established consequence of lead exposure. Lead-induced oxidative stress can generate reactive oxygen species (ROS), causing DNA damage. DNA damage in pregnant women has been

* Correspondence to: Scientist 'C', ICMR-RMRC, Bhubaneswar, India.
E-mail address: mchsahu@gmail.com (M.C. Sahu).
[1] Formerly worked at ICMR-National Institute of Occupational Health, Ahmedabad.

https://doi.org/10.1016/j.eurox.2024.100300
Received 13 August 2023; Received in revised form 10 March 2024; Accepted 18 March 2024
Available online 27 March 2024

associated with adverse pregnancy outcomes, developmental abnormalities, and long-term health consequences in offspring. Therefore, understanding the relationship between ALAD polymorphisms, high blood lead levels, and DNA damage in pregnant women is crucial for identifying individuals at higher risk of lead toxicity and developing targeted intervention strategies [9].

The main cause of lead toxicity is because of high production of reactive oxygen species (ROS)for example, superoxide, hydroxyl radical, and hydrogen peroxide and inhibiting the production of antioxidants. Lead binds to GSH's sulfhydryl group, which in turn does not activate glutathione, which causes the reversal of GSH inhibition. The present reliable range for adequate blood lead level is under 5 μg/dl for healthy kids and under 25 μg/dl for grown-ups [10]. Elimination of half-life for lead is evaluated at 30–45 days in blood and as long as 22 years or more in bone. Lead is excreted primarily in the urine [11,12].

The placenta, while vital for nutrient and oxygen exchange between the mother and fetus, does not possess a selective barrier against lead. Lead can passively diffuse through the placenta due to its small size and ability to cross cell membranes. This allows lead present in the maternal bloodstream to enter the fetal circulation, exposing the developing fetus to the toxic effects of lead. [13]. Once lead enters the fetal circulation, it can accumulate in various fetal tissues, including the brain, bones, liver, and kidneys. The developing fetus is particularly vulnerable to the harmful effects of lead due to its rapid growth and development. Lead exposure during critical periods of organogenesis can disrupt normal development and potentially lead to long-term health consequences [14].

It has been examined that intrauterine exposure to lead may disturb endocrine balance during pregnancy, and lead to irregularities of renal structure and function, abnormalities of the reproductive system, and neuro- developmental toxicity in offspring [15]. It is proposed that raised lead levels may induce cognitive decline and neurobehavioral issues. Likewise, lead exposure in early life (postnatal exposure) can cause lasting brain damage. Perhaps the most dangerous conditions influencing the nervous system of children is cerebral palsy (CP). The most commonly observed cause for motor impairment in kids is CP, with a prevalence of 2 to 2.5 kids for every 1000 births around the world [16].

A heavy neurotoxic and protoxic metal lead, impersonates calcium in several subcutaneous and physiology processes. In pregnancy and lactation, increment in bone turnover can cause bone lead leakage into blood even in populations that have not been exposed to lead occupationally. Nourishment strongly affects levels of lead in pregnant women. Women with less calcium in the physiological system and in their eating, routine might be at greater danger for increased blood lead levels [17]. Further, Pb toxicity is regularly connected with anemia and increased Pb assimilation has been reported during iron deficiency anemia [18]. The levels of blood lead increase approximately 22% all through pregnancy, also in women with low levels of blood lead.The body burden of Pb among ordinary individuals generally relies upon the dietary consumption of Pb[19]. Treatment of lead toxicity has depended principally on the utilization of chelating agents. Chelating agents such as calcium disodium ethylenediamine tetra acetic acid (CaNa2 EDTA), d-penicillamine and British anti-lewisite (BAL) are most commonly utilized. CaNa2EDTA is one of the best encouraging medication for treating lead intoxication [20].

This original research article aims to investigate the association between DNA damage, ALAD polymorphisms, and high blood lead levels in pregnant women attending a tertiary care teaching hospital. By assessing DNA damage using the comet assay and genotyping ALAD, we aim to elucidate the potential role of genetic factors in modulating individual susceptibility to lead-induced DNA damage during pregnancy.This study aimed to investigate the association between ALAD polymorphisms, high blood lead levels, and DNA damage in pregnant women attending a tertiary care teaching hospital.

Materials and methods

Study design

This was a cross-sectional study conducted at a tertiary care teaching hospital. The study was designed to investigate the blood lead levels in pregnant women, who were not exposed in any lead shop or lead associated industry. Then, evaluated the association between DNA damage, ALAD polymorphisms, and high blood lead levels in pregnant women. It is important to note that the Centers for Disease Control and Prevention (CDC) defines a reference blood lead level of 5 micrograms per deciliter (μg/dL) as the action level for lead exposure in children, but there is no established safe threshold for pregnant women. Therefore, any detectable level of lead in the blood should be a cause for concern during pregnancy.

Participants

A total of 90 3rd trimester pregnant women attending the antenatal clinic at the tertiary care teaching hospital and their newborn baby were recruited for the study. Participants were selected based on the following criteria: (a) gestational age between> 24 weeks, (b) age range of 18 to 40 years, and (c) no history of chronic diseases or previous exposure to lead associated industry or battery shops.

Sample collection

In this study,peripheralblood sample from pregnant women and cord blood for babies were collected in a sterile sodium heparin BD vacutainer for lead toxicity study and patient history were noted down in a self-explained questioner form.

Measurement of blood lead levels

Blood lead levels were determined with previous method [21] using atomic absorption spectrometry (AAS) (Analyst 800, Make Perkin Elmer, USA) and its detection limit 0.16 microgram/dL and quantification limit 0.51 microgram/dL[22]. The analysis was performed in the ICMR-NIOH, Ahmedabad and GBRC, Gandhinagar, which follows standard quality control and calibration procedures.

Designing and validation of primers

To perform the Polymerase Chain Reaction (PCR) of the extracted DNA samples, primers specific to ALAD gene are synthesized using Primer 3 software. A nucleotide sequence of ALAD gene (Homo sapiens) is obtained from NCBI and copied the FASTA sequence of the gene and pasted in Primer 3 software, then primers are picked with default parameters. In-silico PCR is run for the primers using UCSC genome browser to check the length of PCR products. All the nucleotide sequence of the primers were checked using primer stat tool (Sequence Manipulation Suite, SMS) online browser to avoid loop formation and primer dimer accumulation. The sequence of forward and reverse primers (5−7) is then sent to company (IDT- Integrated DNA Technology) for synthesis. After receiving of primers from company (100 mM), required amount of nuclease free water was added to individual primer vials according to instruction of the company. Then, primers were diluted to 10 mM for PCR experiment.

Genotyping of ALAD polymorphisms

Genomic DNA was extracted from the blood samples using a commercially available DNA extraction kit. The ALAD gene polymorphisms were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis (Table S1 and S2). Primers specific to the ALAD gene region containing the polymorphisms

of interest were used in the PCR reactions. The PCR products were then digested with appropriate restriction enzymes to distinguish between different ALAD genotypes.

DNA Damage assessment

DNA damage was assessed using the comet assay. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples using density gradient centrifugation. The comet assay was performed following the standard protocol. Briefly, PBMCs were embedded in low-melting-point agarose on a microscope slide, lysed to remove cellular proteins, and subjected to electrophoresis. After staining with a fluorescent DNA-binding dye Ethidium bromide (EtBr), the comet images were captured using fluorescence microscopy and analyzed using CASP software and the comet's DNA damage score was calculated. The comet tail length, tail intensity, and tail moment were used as quantitative measures of DNA damage.

Statistical analysis

Statistical analysis was performed using SPSS 2020 software. Descriptive statistics, such as mean, standard deviation, and frequency, were used to summarize the demographic characteristics of the participants. The chi-square test was employed to determine the association between ALAD polymorphisms and high blood lead levels. Logistic regression analysis was used to assess the independent effect of ALAD genotypes on high blood lead levels and DNA damage. Additionally, correlation analysis was conducted to explore the relationship between blood lead levels and DNA damage severity.

Ethical considerations

This study was approved by scientific advisor committee and institutional ethical committee of ICMR-NIOH (Ahmedabad). Informed consent was obtained from all participants before their enrollment in the study. Confidentiality of participants' data was ensured throughout the study.

Limitations

The study's limitations include the cross-sectional design, which does not establish a cause-and-effect relationship, and the relatively small sample size, which may limit the generalizability of the findings. Future studies with larger and more diverse populations will be to validate the results.

Results

In this cross sectional study, all reproductive age groups were included. The demographic data and clinical history of all participants were documented (Table 1). The participants had a mean age of 27.5 years (standard deviation [SD] = 4.6). It is observed from medical history that in all age groups, unexplained pregnancy loss was delivered. Similarly, in all age group, husbands were taking gutkha, masala, and cigarette. Hemoglobin were normal in all the age group of the participants (Table 1).

Blood lead levels were determined using atomic absorption spectrometry (AAS) (Analyst 800, Make Perkin Elmer, USA) (its detection limit 0.16 microgram/dL and quantification limit 0.51 microgram/dL) in all 90 pregnant women and it was revealed that 16 (17.77%) had high blood lead levels (>5 mg/dl). Similarly, in cord blood from infants only 5 (5.55%) had high blood lead levels (>5 mg/dl). Among the participants, 16 had high blood lead levels (≥5 μg/dL), while the remaining 74 had normal blood lead levels (<5 μg/dL). From regression analysis, it was found that a significant amount of lead transferred from mother to infants (Table 2, Fig. 1). It was revealed that 3 infants were died and 3

Table 1
The demographic data of pregnant women evaluated for blood lead concentration.

Pregnant women's data	16-20 years (n = 9)	21-25 years (n = 44)	26-30 years (n = 24)	31-35 years (n = 8)	36-40 years (n = 5)
Working	10%	48.88%	26.66%	8.88%	5.55%
Mix diet	50%	42.59%	65.38%	62.5%	38%
BMI	21.19 ± 0.64	21.00 ± 0.72	25.24 ± 1.14	27.68 ± 4.45	23 ± 42
Unexplained pregnancy loss	44.44%	27.27%	33.33%	75%	80%
Hb	11.67 ± 0.36	11.18 ± 0.57	11.18 ± 0.54	12.2 ± 1.90875	11.34 ± 0.62
Husband's habit (pan masala)	31%	33%	34%	12%	42%
Habit of smoking	12.5	10.25%	7.14%	62%	58%
Medical history of Spontaneous abortion	11.11%	2.27%	4.16%	12.5%	0%

Table 2
Lead concentration from 3rdtrimester of pregnant women and cord blood of infants.

Sl. No	Mother Lead BLL	Baby Lead BLL	Sl. No	Mother Lead BLL	Baby Lead BLL
1	11.7	5.669	2	3.596	0.686
3	1.752	0.81	4	1.532	0.89
5	1.52	1.163	6	2.893	2.334
7	0.822	0.57	8	2.456	1.664
9	1.436	0.62	10	2.965	1.013
11	1.312	0.6794	12	3.428	1.506
13	1.068	0.723	14	1.047	1.783
15	0.842	1.417	16	2.865	1.932
17	2.337	1.527	18	4.714	3.268
19	2.539	0.4522	20	1.736	3.258
21	0.874	0.31	22	5.067	0.357
23	0.556	0.466	24	0.883	1.084
25	0.879	0.951	26	2.8	1.867
27	0.92	1.416	28	5.5	3.467
29	5.197	1.827	30	2.429	1.142
31	1.874	1.353	32	1.7	1.165
33	2.645	1.005	34	3.57	1.169
35	0.05	0.01	36	4.711	2.717
37	1.98	0.473	38	3.136	1.134
39	0.917	1.175	40	2.14	0.865
41	26.89	9.322	42	3.987	3.216
43	2.87	2.869	44	3.59	0.598
45	2.627	2.332	46	4.187	3.374
47	1.541	3.285	48	2.322	0.25
49	4.147	3.34	50	2.816	1.228
51	3.905	1.466	52	2.448	0.187
53	1.257	1.055	54	1.531	1.865
55	3.621	0.794	56	6.12	2.535
57	1.45	0.567	58	4.709	0.987
59	6.775	4.215	60	2.86	2.371
61	13.151	5.326	62	5.112	0.861
63	2.449	11.95	64	6.524	5.01
65	1.695	3.035	66	6.49	1.621
67	2.092	1.79	68	1.458	0.779
69	1.434	1.486	70	2.66	1.472
71	1.332	1.466	72	1.607	0.711
73	1.405	1.471	74	5.089	4.09
75	1.942	1.785	76	1.768	1.32
77	5.21	3.26	78	1.76	1.23
79	1.224	0.729	80	4.254	3.157
81	0.769	1.123	82	1.834	4.407
83	1.638	0.87	84	12.323	3.286
85	1.466	0.3	86	7.271	4.399
87	2.4	0.9	88	4.56	2.22
89	0.562	3.099	90	8.32	5.758

Note: 16 pregnant women having more than 5 mg/dl of blood lead level (BLL)

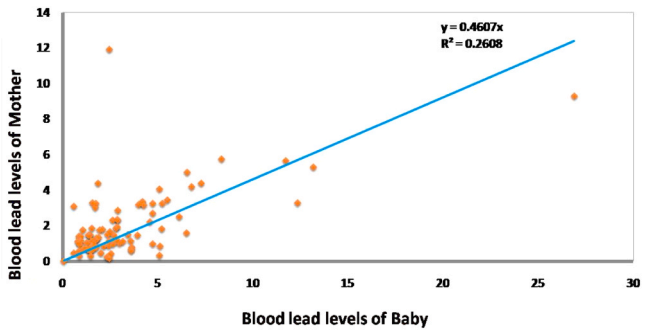

Fig. 1. Regression analysis showing a significant amount of lead transferred from mother to infants.

were admitted in NICU from the 16 high blood lead level pregnant women. In addition to 8 premature deliveries and low birth weight baby were revealed (Table 3). All studied pregnant women including the 16 high blood lead level pregnant women were studied for genotoxicity of DNA damage with comet assay. It was revealed that in high blood lead levels had more DNA damage as compared to the low blood lead levels (Table 4, Fig. 2a-d). DNA damage was assessed using the comet assay, with parameters such as tail length, tail intensity, and tail moment serving as quantitative measures. The mean tail length was significantly higher in pregnant women with high blood lead levels compared to those with normal levels ($p < 0.001$). Pregnant women with the ALAD 1–2 genotype exhibited significantly longer tail lengths (mean difference = 20.4 μm, $p < 0.001$), higher tail intensities (mean difference = 15.2 arbitrary units, $p < 0.001$), and increased tail moments (mean difference = 3.8, $p < 0.001$) compared to those with other ALAD genotypes. Furthermore, a positive correlation was observed between blood lead levels and the severity of DNA damage. Pregnant women with higher blood lead levels had significantly longer tail lengths ($r = 0.472$, $p < 0.001$), higher tail intensities ($r = 0.389$, $p < 0.001$), and increased tail moments ($r = 0.421$, $p < 0.001$).

For ALAD polymorphism, DNA was extracted from blood and quantified with 0.8% agarose gel with taking uncut known concentrate lambda phase DNA as control (Fig. 3a-d). It was revealed that a good quantity of DNA was extracted from all samples. Primers for ALAD gene were designed with primer tool 3 browser and synthesized by outsourcing (Table S3). All validated primers were used for PCR to amplify the ALAD gene and revealed good amplicons with sequences (Fig. 4a-c, Table S4). For polymorphism study, all PCR product of ALAD gene were digested with MspI restriction enzyme and polymorphism bands were documented (Fig. 5). A total of 16 blood samples from mother and 5 blood having high blood levels were used for ALAD poly morphism study with taking control from lower blood lead samples. The primers ALAD 4, forward primer (5′AGGAGCCTTCCACAGCCGAAT3′) and reverse primer (5′CC TTCCTTTTTCTGTTTGTATTGGAGAC3′) and ALAD 5 forward primer (5′CCTGAGTGAA AACGGAGCAT3′) and reverse primer (5′GGCTTGACTTAGCTGCATCC3′) revealed good amplification of ALAD gene. Genotyping of ALAD polymorphisms revealed that 3 participants had the ALAD 1–1 genotype, 11 participants had the ALAD 1–2 genotype, and 2 participants had the ALAD 2–2 genotype (Table 5). The distribution of ALAD genotypes was consistent with Hardy-Weinberg equilibrium ($p > 0.05$).

Overall, our results demonstrate a significant association between ALAD polymorphism, high blood lead levels, and DNA damage in pregnant women attending the tertiary care teaching hospital. Pregnant women carrying the ALAD 1–2 genotype showed increased susceptibility to lead toxicity, as evidenced by higher blood lead levels and more severe DNA damage. These findings emphasize the importance of considering genetic factors, such as ALAD polymorphisms, in assessing individual vulnerability to lead-induced genotoxicity during pregnancy.

Discussion

The study was carried out by taking blood samples from pregnant women at BJ Medical, Ahmedabad and only unexposed lead associated industry / shop pregnant women were included. Exposure to air pollution, particularly high levels of lead in the air, has been associated with adverse outcomes during pregnancy. Lead is a toxic metal that can have harmful effects on the developing fetus. It's important to note that lead exposure can come from various sources, including air pollution from industrial activities, lead-based paint, contaminated soil, and certain types of water pipes. Efforts to reduce air pollution, regulate industrial emissions, and address lead exposure sources can contribute to improving maternal and fetal health outcomes. Pregnant women are often advised to be cautious about potential lead exposure and take preventive measures to minimize risks during pregnancy. Regular prenatal care and consulting with healthcare professionals are crucial for monitoring and managing potential environmental risks.

Lead exposure is a significant public health concern, particularly during pregnancy, as it poses serious risks to both maternal and fetal health. High blood lead levels in pregnant women have been associated with adverse outcomes such as preterm birth, low birth weight, developmental delays, and neurobehavioral abnormalities in children [23, 24]. While environmental factors play a crucial role in lead exposure, recent research has shed light on the genetic component influencing individual susceptibility to elevated blood lead levels [25]. In this study, Blood samples were collected during the 3rd trimester, and the comet

Table 3
Pregnancy outcome and Status of newborn baby of high lead concentrated (>5 μg/dl) pregnant women.

Sl. No.	Sample ID	BLL in Mother (vein blood)	BLL in infants (cord blood)	Weight of infants (KG)	Maturity	APGAR	Status of baby
1	M1	11.7	5.669	2.7	FT	4/5/6	NICU admission
2	M22	5.067	0.357	1.6	28 W	5/7/8	Death
3	M28	5.5	3.467	1.4	29 W	6/7/8	NICU admission
4	M29	5.197	1.827	2.8	FT	7/8/9	Live
5	M41	26.89	9.322	2.7	FT	7/8/9	Live
6	M56	6.12	2.535	0.8	26 W	5/6/7	NICU admission
7	M59	6.775	4.215	2.6	FT	7/8/9	Live
8	M61	13.151	5.326	3	FT	7/8/9	Live
9	M62	5.112	0.861	1.3	27 W	4/5/6	Death
10	M64	6.524	5.01	1.8	28 W	6/7/8	Live
11	M66	6.49	1.621	2.1	FT	7/8/9	Live
12	M74	5.089	4.09	2.8	FT	7/8/9	Live
13	M77	5.21	3.26	1.9	32 W	0/1/3	Death
14	M84	12.323	3.286	1.2	31 W	7/8/9	Live
15	M86	7.271	4.399	1.9	32 W	7/8/9	Live
16	M90	8.32	5.758	3.1	FT	8/8/9	Live

Note: M: represents for mother, BLL: represents Blood lead levels

Table 4
Percentage of DNA damage in comet assay with respect to blood lead levels (BLL)of pregnant women.

Sl. No	BLL	Head Area	Tail Area	Head DNA	Tail DNA	Head DNA%	Tail DNA%	Head Radius	Tail Length	Comet Length	Head MeanX	Tail MeanX	Tail Moment	Olive Tail Moment
1	11.70	24614	24167	900.39	375.33	70.58	29.42	87	180	355	166.596	278.186	52.9579	32.831
2	3.59	7537	2084	1438.61	97.14	93.67	6.33	50	13	114	142.072	165.919	0.822275	1.50838
3	1.75	3780	545	804.57	23.75	97.13	2.87	34	9	78	160.032	188.412	0.258068	0.813792
4	1.53	3346	27	199.96	0.36	99.82	0.18	32	5	70	289.995	322.917	0.00895533	0.0589649
5	1.52	2510	86	102.24	0.97	99.06	0.94	28	10	67	172.592	200.994	0.0938312	0.2665
6	2.89	4929	39	158.14	0.29	99.82	0.18	39	5	84	215.345	256.305	0.00921536	0.0754923
7	0.82	3158	321	79.20	2.48	96.96	3.04	31	14	77	143.135	172.192	0.425875	0.883922
8	2.46	4478	448	136.87	3.53	97.49	2.51	37	8	83	121.254	145.821	0.200989	0.617234
9	1.44	8289	13	311.09	0.11	99.96	0.04	52	3	108	145.937	199.282	0.00108495	0.0192921
10	2.97	6083	652	245.24	6.31	97.49	2.51	43	24	111	202.399	238.06	0.601898	0.894341
11	1.31	7620	215	1526.06	6.63	99.57	0.43	49	4	103	169.868	215.241	0.0172999	0.196237
12	3.43	3999	360	163.22	3.87	97.69	2.31	35	4	75	161.767	180.278	0.0925683	0.428375
13	1.07	4448	174	100.42	1.22	98.80	1.20	37	13	88	168.614	205.668	0.156065	0.444834
14	1.05	5175	78	163.95	0.53	99.68	0.32	40	13	94	179.464	224.132	0.0421066	0.144676
15	0.84	7830	2	1266.07	0.03	100.00	0.00	50	3	104	120.275	172.456	6.39E-05	0.00111083
16	2.87	4822	29	181.08	0.25	99.86	0.14	39	3	82	184.422	224.063	0.00417633	0.055184
17	2.34	4231	38	173.13	0.36	99.79	0.21	36	5	78	193.497	230.952	0.0102898	0.0770796
18	4.71	5245	716	270.67	14.62	94.87	5.13	40	12	93	152.736	185.217	0.615038	1.66477
19	2.54	3757	33	319.46	0.67	99.79	0.21	34	3	72	138.399	172.8	0.00624857	0.0716514
20	1.74	7848	2	1169.60	0.02	100.00	0.00	50	3	104	208.116	260.454	6.38E-05	0.00111271
21	0.87	6864	3	267.86	0.02	99.99	0.01	46	4	97	160.676	211.222	0.0002642	0.0033386
22	5.07	7287	7330	721.36	189.00	79.24	20.76	49	78	177	148.319	197.837	16.1938	10.2806
23	0.56	7580	20	1403.31	0.55	99.96	0.04	49	3	102	188.561	238.742	0.00118369	0.0197996
24	0.88	8290	2	724.50	0.00	100.00	0.00	52	3	108	205.764	265.02	1.38E-05	0.00027322
25	0.88	11975	2	1800.67	0.03	100.00	0.00	61	3	126	197.279	261.456	4.49E-05	0.000960656
26	2.80	7427	107	1246.61	2.92	99.77	0.23	48	4	101	205.309	253.049	0.00936131	0.111727
27	0.92	12639	22	580.80	0.22	99.96	0.04	63	6	133	169.224	235.831	0.00223634	0.0248258
28	5.50	6613	2673	638.62	91.47	87.47	12.53	48	21	118	175.706	186.707	2.63102	1.37834
29	5.20	9030	1891	1600.24	79.36	95.28	4.72	53	18	125	226.234	267.325	0.850486	1.94153
30	2.43	7512	555	446.46	7.80	98.28	1.72	48	17	114	145.398	194.516	0.291773	0.843013
31	1.87	9276	96	2145.06	3.16	99.85	0.15	54	3	112	169.189	221.105	0.00440671	0.0762595
32	1.70	8001	50	1857.85	1.40	99.92	0.08	50	3	104	139.559	189.801	0.00225892	0.037831
33	2.65	9743	38	1577.24	1.02	99.94	0.06	55	3	114	153.569	209.496	0.00194312	0.0362244
34	3.57	7373	1060	1379.99	33.59	97.62	2.38	49	8	107	135.152	165.219	0.190121	0.714532
35	0.05	9670	2	1182.90	0.00	100.00	0.00	55	3	114	139.079	197.5	9.41E-06	0.000183325
36	4.71	6706	1384	1292.21	48.14	96.41	3.59	46	21	114	99.6192	138.182	0.754272	1.38509
37	1.98	8483	32	1158.34	0.73	99.94	0.06	51	6	109	159.753	212.797	0.00380174	0.0336096
38	3.14	4455	199	104.35	1.64	98.46	1.54	37	13	88	146.704	186.43	0.200655	0.613172
39	0.92	7158	8	742.38	0.13	99.98	0.02	48	3	100	148.04	196.268	0.00053457	0.00859381
40	2.14	4837	13	93.44	0.06	99.94	0.06	39	5	84	333.641	375.491	0.00310729	0.0260081
41	26.89	22823	22228	799.76	451.13	63.94	36.06	87	131	306	117.255	196.085	47.2449	28.4298
42	3.99	8674	909	1981.36	35.45	98.24	1.76	52	7	112	206.224	241.095	0.123039	0.612923
43	2.87	39811	799	965.47	6.53	99.33	0.67	112	36	261	210.32	338.811	0.241777	0.862943
44	3.59	30366	1785	777.16	14.35	98.19	1.81	97	17	212	326.728	393.477	0.308126	1.20982
45	2.63	24569	581	644.18	6.26	99.04	0.96	87	17	192	448.901	540.104	0.163542	0.877381
46	4.19	10947	610	269.73	6.89	97.51	2.49	59	29	148	121.549	192.505	0.722494	1.76777
47	1.54	6519	4	121.46	0.02	99.99	0.01	45	3	94	165.369	222.338	0.00040041	0.00760373
48	2.32	21984	398	982.53	5.17	99.48	0.52	83	10	177	152.929	236.868	0.0523801	0.439673
49	4.15	21621	2187	642.11	22.97	96.55	3.45	82	24	189	159.865	230.893	0.828733	2.45262
50	2.82	35205	538	2183.35	10.05	99.54	0.46	110	25	246	199.469	328.611	0.114549	0.591722
51	3.91	41311	2	990.87	0.00	100.00	0.00	114	3	232	248.908	371.267	4.02E-07	1.64E-05
52	2.45	37939	595	810.21	4.49	99.45	0.55	110	21	242	303.336	424.514	0.115776	0.668073
53	1.26	34687	19	515.54	0.11	99.98	0.02	104	6	215	466.387	578.994	0.00123229	0.0231273
54	1.53	5929	5	725.87	0.11	99.99	0.01	43	4	91	214.287	259.84	0.00058979	0.00671659
55	3.62	7216	16	716.72	0.32	99.96	0.04	47	3	98	144.716	192.415	0.00134401	0.0213695
56	6.12	9833	2383	297.59	28.47	91.27	8.73	55	61	172	180.34	248.191	5.32688	5.92516
57	1.45	5292	8	161.13	0.06	99.97	0.03	40	5	86	224.898	270.621	0.0017279	0.0158008
58	4.71	8612	1320	226.81	14.45	94.01	5.99	51	35	138	211.417	274.843	2.0969	3.7999
59	6.78	6288	3068	304.38	39.38	88.54	11.46	45	18	109	245.922	230.287	2.06222	1.79131
60	2.86	44038	9	710.19	0.04	99.99	0.01	118	3	240	231.128	368.204	0.00018629	0.00851188
61	13.15	3565	4768	72.44	39.12	64.93	35.07	33	98	165	150.279	203.821	34.3659	18.7755
62	5.11	5242	3218	169.13	33.15	83.61	16.39	40	27	108	245.532	255.183	4.42504	1.58159
63	2.45	7066	2	177.05	0.00	100.00	0.00	48	3	100	131.696	187.642	6.74E-05	0.00125675
64	6.52	17573	12231	674.60	175.74	79.33	20.67	74	85	234	187.842	261.588	17.5673	15.2413
65	1.70	50716	19	801.48	0.09	99.99	0.01	126	3	256	229.861	372.817	0.00035193	0.0167703
66	6.49	16220	10503	493.14	133.51	78.69	21.31	72	59	204	159.265	226.1	12.5702	14.2397
67	2.09	35481	4858	617.22	36.64	94.40	5.60	105	61	272	459.463	576.512	3.41862	6.55978
68	1.46	43705	23	891.23	0.13	99.99	0.01	115	11	242	300.05	426.798	0.00158105	0.0182177
69	1.43	52306	11	751.46	0.07	99.99	0.01	127	5	260	292.666	444.696	0.00043396	0.0131951
70	2.66	11942	2146	1397.58	76.39	94.82	5.18	64	22	151	182.701	234.293	1.14024	2.67396
71	1.33	8388	5	1232.18	0.08	99.99	0.01	51	3	106	175.156	226.922	0.00020033	0.00345674
72	1.61	4246	7	112.24	0.04	99.97	0.03	36	3	76	278.421	315.534	0.00096358	0.0119204
73	1.41	1698	8	87.97	0.06	99.93	0.07	23	3	50	191.392	216.075	0.00195697	0.0161009

(*continued on next page*)

Table 4 (*continued*)

Sl. No	BLL	Head Area	Tail Area	Head DNA	Tail DNA	Head DNA%	Tail DNA%	Head Radius	Tail Length	Comet Length	Head MeanX	Tail MeanX	Tail Moment	Olive Tail Moment
74	5.09	4964	1176	137.52	14.84	90.26	9.74	39	20	99	264.449	300.516	1.94832	3.51354
75	1.94	4807	6	88.37	0.02	99.98	0.02	39	3	82	183.753	231.613	0.00057003	0.00909384
76	1.77	61007	13	1083.32	0.07	99.99	0.01	137	4	279	293.765	454.821	0.00025878	0.0104196
77	5.21	26637	9554	737.33	146.23	83.45	16.55	91	71	254	530.577	670.721	11.7505	23.1938
78	1.76	58108	6	999.68	0.03	100.00	0.00	136	5	278	316.778	486.28	0.00014042	0.00476039
79	1.22	35498	5	576.51	0.03	99.99	0.01	105	4	215	335.226	449.093	0.00021452	0.00610657
80	4.25	33451	6896	566.01	40.93	93.26	6.74	101	104	307	491.795	615.396	7.01344	8.3353
81	0.77	32539	2	554.14	0.00	100.00	0.00	100	3	204	394.77	509.5	1.97E-05	0.000754339
82	1.83	41211	5	560.34	0.02	100.00	0.00	114	3	232	307.285	443.318	8.95E-05	0.00405667
83	1.64	31903	2	395.11	0.01	100.00	0.00	100	3	204	370.907	492.538	4.44E-05	0.00179931
84	12.32	38919	27713	1714.74	778.26	68.78	31.22	112	144	369	171.096	348.88	44.9535	55.5
85	1.47	20496	6	344.05	0.03	99.99	0.01	81	4	167	244.638	354.266	0.00035828	0.00981945
86	7.27	9559	5131	430.95	122.56	77.86	22.14	56	32	145	154.807	200.135	7.08553	10.0366
87	2.40	12326	2508	764.83	48.30	94.06	5.94	62	20	145	186.837	232.238	1.18793	2.69665
88	4.56	13520	3096	404.96	32.89	92.49	7.51	64	18	147	214.317	257.443	1.35218	3.23963
89	0.56	13126	2	1130.50	0.00	100.00	0.00	65	3	134	211.221	282.673	5.21E-06	0.000123996
90	8.32	19034	8931	457.78	81.97	84.81	15.19	76	46	199	140.784	181.885	6.98583	6.2418

assay was performed to evaluate DNA damage. The results revealed a significant increase in DNA damage in the high blood lead group compared to the control group. These findings highlight the potential genotoxic effects of lead exposure during pregnancy and emphasize the importance of monitoring blood lead levels to mitigate adverse health outcomes.

Studies have shown that certain variants or polymorphisms within the ALAD gene can affect an individual's susceptibility to lead toxicity. One of the most well-studied polymorphisms is the ALAD2 allele, which results in reduced enzymatic activity compared to the more common ALAD1 allele. The ALAD2 allele has been associated with higher blood lead levels, suggesting that individuals carrying this variant may be more vulnerable to the toxic effects of lead exposure [9,11,26]. Our study reveled ALAD 1–2 genotype exhibited significantly longer tail lengths (mean difference = 20.4 μm, $p < 0.001$), higher tail intensities (mean difference = 15.2 arbitrary units, $p < 0.001$), and increased tail moments (mean difference = 3.8, $p < 0.001$). Our study correlated with the findings of Pawalas et al., they concluded that genetic changes in the link between blood lead and toxic effects have significant consequences in terms of risk assessment (Pawalas et al.2012) [27]. The ALAD gene's influence on lead toxicity becomes particularly significant during pregnancy due to the potential for maternal-fetal transfer of lead. Maternal blood lead levels directly impact fetal exposure, as lead can cross the placental barrier. Consequently, the presence of ALAD gene variants that increase maternal blood lead levels may have implications for fetal development and health[14].

Lead concentration should be monitored during pregnancy to mitigate potential adverse clinical outcomes. Lead exposure during pregnancy has been associated with various health risks for both the mother and the developing fetus. Elevated blood lead levels have been linked to increased risk of pregnancy complications, such as gestational hypertension, preeclampsia, and preterm birth. Additionally, lead exposure during pregnancy can negatively impact fetal development, leading to developmental delays, cognitive impairments, and behavioral problems in children (Behera et al.2016)[28]. According to Li et al., ALAD plays an important role in the health effects of lead poisoning. Lead exposure may alter disease susceptibility through the epigenome, which may provide a sufficient mechanism [6,29].

Shaik et al. found that the wild type ALAD 1–1 to be characterized by a 582 bp fragment and a homozygote variant with to be characterized by 511 bp fragment which is different from our result carried out with the two primers that show a band at 600–1000 bp.[8]. Yang et al., documented a genotyping technique based on the PCR reaction which is common among most investigators. He concluded that a 916 bp sequence having the ALAD1/2 polymorphic site is amplified and then further restricted with restriction enzymes [30,31]. In our study, Genotyping of ALAD polymorphisms revealed that 3 participants had the ALAD 1–1 genotype, 11 participants had the ALAD 1–2 genotype, and 2 participants had the ALAD 2–2 genotype. The distribution of ALAD genotypes was consistent with Hardy-Weinberg equilibrium ($p > 0.05$).

In a study by Ortega et al. factors related to lead toxicity in newborn babies due to breast feeding was also evaluated which shows that newly born breastfed children showed a higher blood lead level (4.6–2.5 μg/dL) if the mother has a high amount of lead in their blood. He concluded that high concentrations of lead in the milk of lactating mothers can be correlated to high level of lead in their babies (Ortega et al.2019) [14]. This study was somewhat similar to our findings of lead estimation in blood samples of pregnant women and their babies, in all the digested blood samples the amount of lead transferred from mother and their baby. According to the results by Kayaalti et al., the maternal blood lead levels are higher than cord blood and he concluded that lead moves to the fetus through the placenta which accounts for the notion that the mothers with high blood lead levels during pregnancy are responsible for the high amount of lead in their newborn babies. ALAD activity is higher in cord blood, and there is a strong link between maternal blood, cord blood, and ALAD activity [13,31]. In our study, also the blood lead concentration was more as compared to the cord blood.

Early identification of elevated blood lead levels allows for the implementation of appropriate interventions to reduce lead exposure. This may involve identifying and eliminating potential sources of lead, such as lead-based paint or contaminated water [32–34]. Pregnant women should be advised to avoid occupational or environmental settings with a high risk of lead exposure. Education on lead-safe practices, including proper hygiene, nutrition, and home maintenance, can also help minimize exposure [35,36]. In cases where pregnant women are found to have elevated blood lead levels, close monitoring of their clinical outcomes is crucial. Healthcare providers should carefully assess maternal health parameters, including blood pressure, kidney function, and liver function, to detect any signs of lead-related complications. Additionally, fetal monitoring through regular ultrasounds and other appropriate tests can help assess fetal growth and development. By implementing lead concentration monitoring during pregnancy, healthcare providers can identify and manage potential risks associated with lead exposure [33]. Collaborative efforts between healthcare professionals, public health agencies, and environmental agencies are essential in raising awareness, providing education, and implementing effective strategies to reduce lead exposure and improve clinical outcomes for pregnant women and their offspring. Regular monitoring of lead concentration in pregnant women can help identify individuals with high blood lead levels and enable timely interventions to minimize the risks associated with lead exposure[37]. Screening for lead levels can be performed through blood tests, typically using atomic absorption spectrometry or other validated methods. It is important to note that the Centers for Disease Control and Prevention (CDC) defines a reference

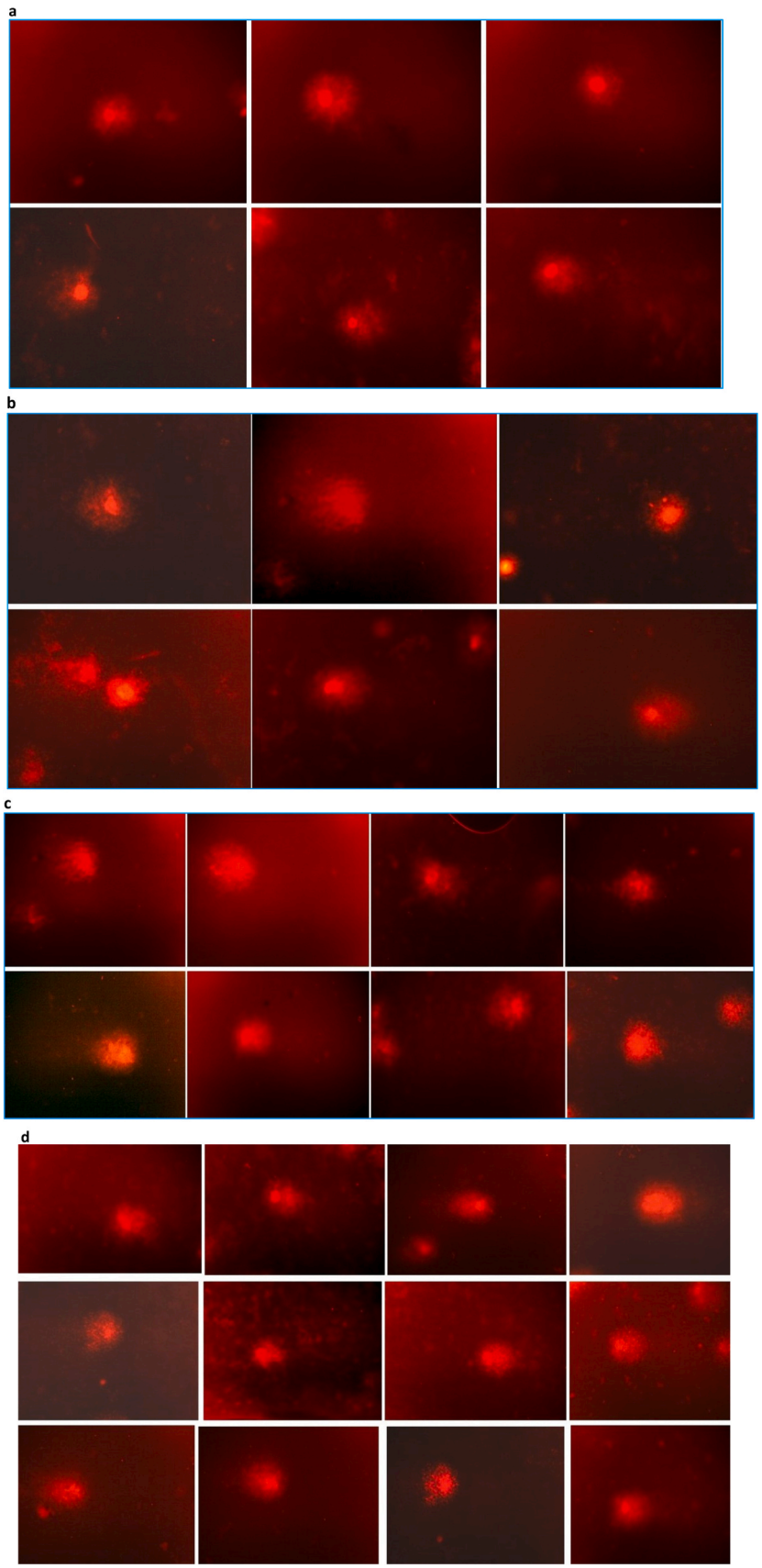

Fig. 2. a Comet assay image of mothers having lead concentration 0.1 to 2.0 mg/dl. b Comet assay image of mothers having lead concentration 2.1 to 4.0 mg/dl. c Comet assay image of mothers having lead concentration 4.1 to 5.0 mg/dl. d Comet assay image of mothers having lead concentration > 5.0 mg/dl.

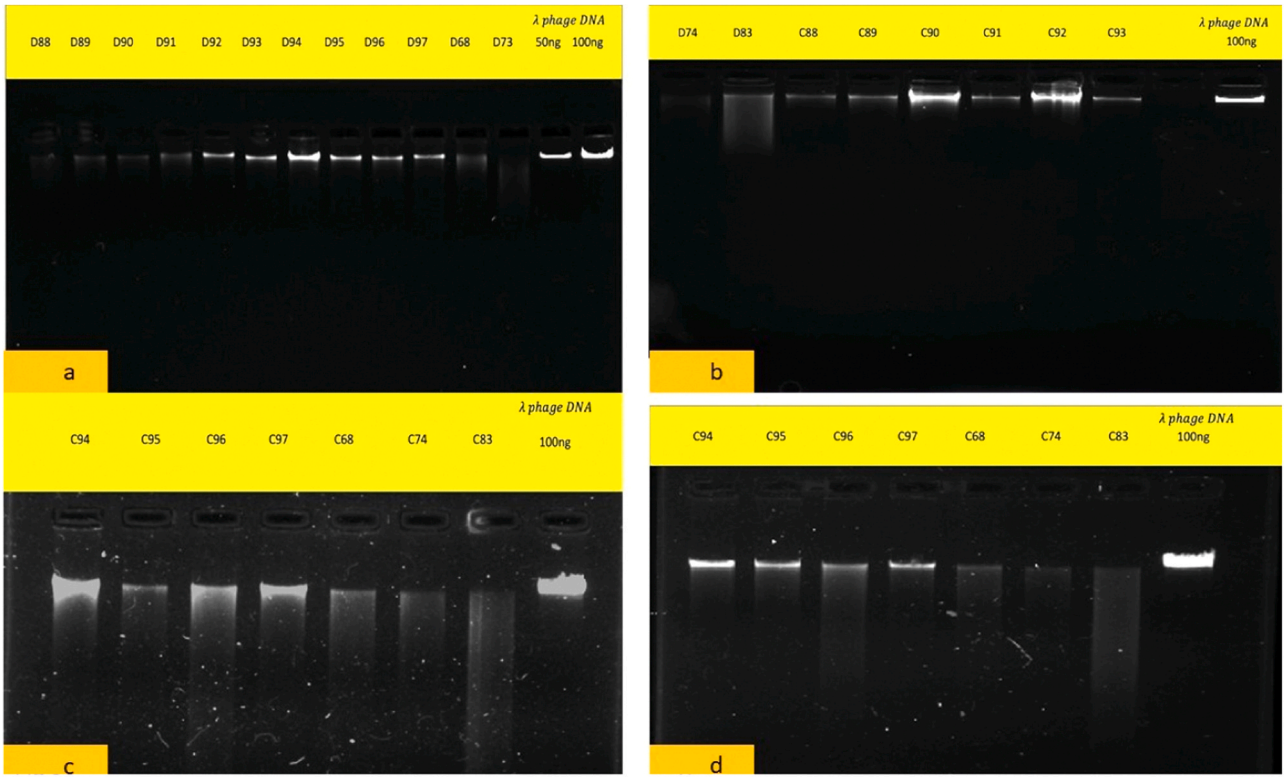

Fig. 3. a-d Quantification of DNA with λ phage DNA in 0.8% agarose gel.

blood lead level of 5 micrograms per deciliter (μg/dL) as the action level for lead exposure in children, but there is no established safe threshold for pregnant women [38]. Therefore, any detectable level of lead in the blood should be a cause for concern during pregnancy.

This study provides evidence of an association between ALAD polymorphism, high blood lead levels, and DNA damage in pregnant women. The ALAD 1–2 genotype appears to increase susceptibility to lead toxicity, as evidenced by elevated blood lead levels and greater DNA damage. These findings highlight the importance of considering genetic factors in lead exposure risk assessment during pregnancy.

Future prospective

While the association between ALAD gene variants and high blood lead levels during pregnancy is an important finding, further research is needed to elucidate other genetic factors that may influence lead toxicity. Large-scale genome-wide association studies (GWAS) and comprehensive analysis of genetic variations in pathways related to lead metabolism are ongoing to identify additional candidate genes. Such investigations will enhance our understanding of the genetic basis of lead susceptibility and facilitate the development of personalized interventions.

Traditional methods for lead detection in blood samples are time-consuming and require specialized equipment. Future work aims to investigate the feasibility of using Reverse Transcription Polymerase Chain Reaction (RT-PCR) as a rapid and sensitive method for detecting lead toxicity in pregnant women. The study will involve collecting blood samples from pregnant women exposed to lead, extracting RNA, reverse transcribing it into complementary DNA (cDNA), and amplifying target genes related to lead toxicity using PCR. The results will be compared with traditional lead detection methods to evaluate the accuracy and efficiency of RT-PCR as a diagnostic tool for lead toxicity in pregnancy.

Conclusion

The findings regarding high blood lead levels in comet assay results and polymorphism analysis underscore the significant health risks associated with lead exposure. The comet assay, which assesses DNA damage, and polymorphism analysis, which explores genetic susceptibility, provide valuable insights into the complex mechanisms through which lead toxicity manifests in the body. The presence of elevated blood lead levels in conjunction with evidence of DNA damage suggests a direct link between lead exposure and genetic instability. Furthermore, the identification of specific genetic polymorphisms associated with increased susceptibility to lead toxicity highlights the importance of individual variations in response to environmental pollutants. In conclusion, the combination of comet assay and polymorphism analysis offers a comprehensive approach to understanding the impact of lead exposure during pregnancy. These findings underscore the urgent need for effective regulatory measures to reduce lead exposure in the environment and mitigate its adverse effects of lead on maternal and child health. Additionally, targeted interventions tailored to individuals with genetic predispositions to lead toxicity may help minimize the risk of associated pregnancy complications. Ultimately, addressing lead pollution requires a multifaceted approach that combines regulatory action, public health initiatives, and personalized interventions to safeguard human health and well-being.

Funding

The study was executed by intramural ICMR-National Institute of Occupational Health funds.

CRediT authorship contribution statement

Mahesh Chandra Sahu: Conceptualization, Data curation, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. Kuldip Upadhyay: Conceptualization, Data

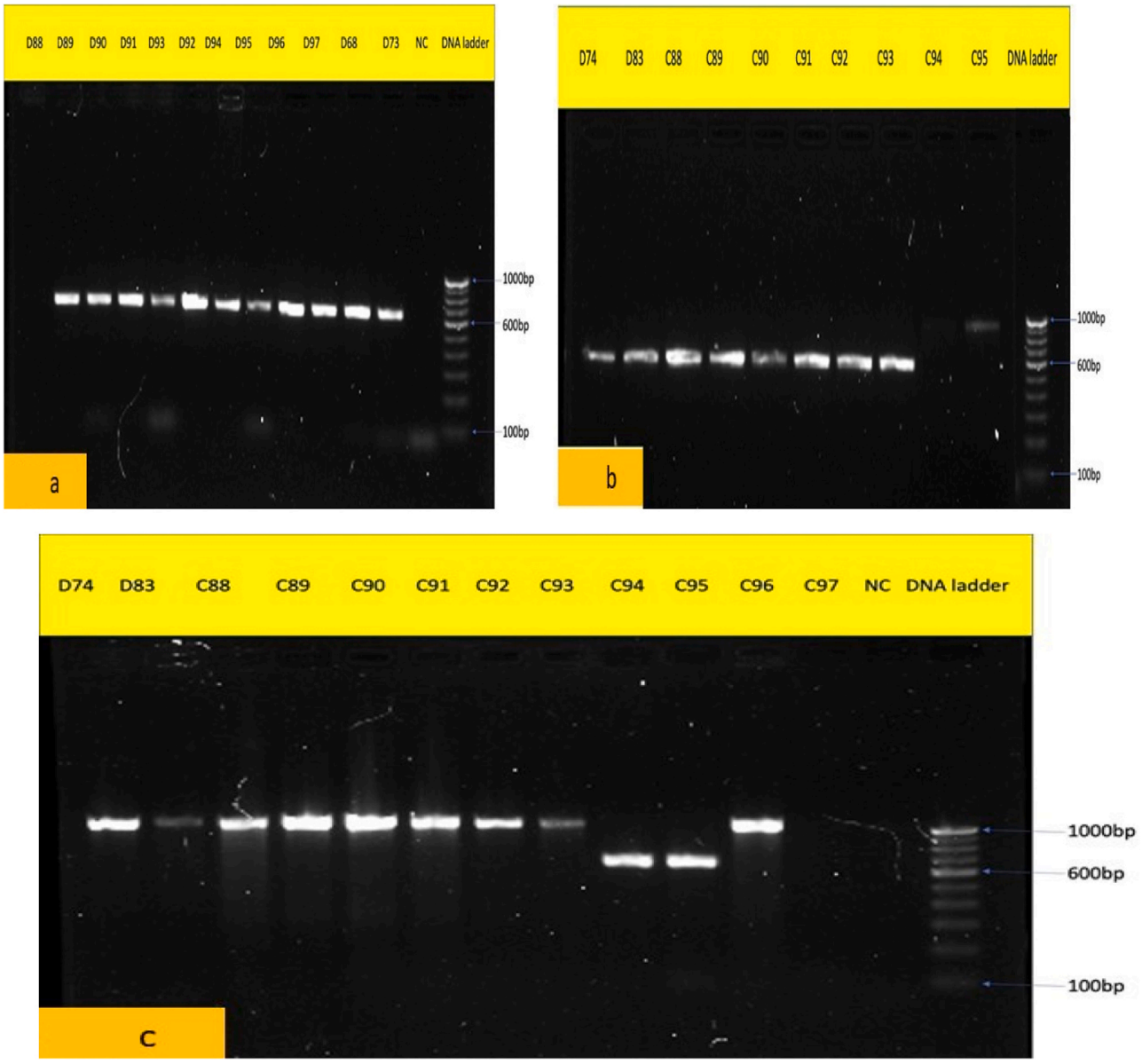

Fig. 4. a-b Amplicons of ALAD gene by PCR with different synthesized primers. a-b Amplicons of ALAD gene by PCR with different primers ALAD 5.

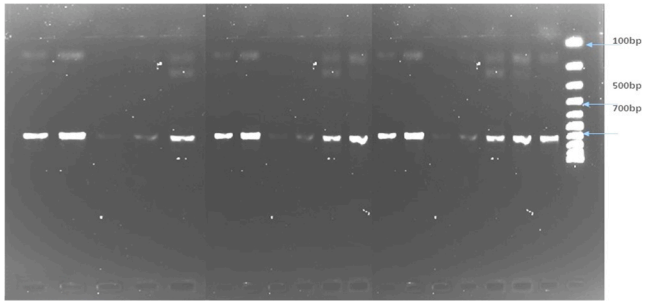

Fig. 5. The ALAD gene PCR product digested with MspI and RFLP of ALAD gene.

curation, Formal analysis, Investigation, Methodology, Software, Supervision, Visualization.Shweta Gupta: Methodology, Validation. Kabita Chanania: Data curation,Formal analysis, Writing – review & editing. Sanghamitra Pati: Visualization, Writing – review & editing

Table 5
The comet assay results of high BLL in relation to ALAD polymorphism.

ALAD Polymorphism	Number of individual	Tail DNA percentage in Comet Assay Results (Mean ± SD)
ALAD 1-1	3	16.17 ± 5.82
ALAD 1-2	11	32.74 ± 4.70
ALAD 2-2	2	18.66 ± 4.92

Note: BLL- blood lead levels

Declaration of Competing Interest

I confirm that all individuals who have made significant contributions to the article are acknowledged appropriately. Furthermore, I declare that I have obtained all necessary permissions from the scientific advisory committee and Institutional Ethics Committee to carry out this project work.

I take full responsibility for the content of the article and declare that all information provided is accurate to the best of my knowledge. I also affirm that the research presented in the article has been conducted in

compliance with applicable laws and regulations regarding human subjects, including obtaining informed consent and maintaining participant confidentiality.

Thank you for considering the submission of my article to *European Journal of Obstetrics & Gynecology and Reproductive Biology:X*. I appreciate your time and attention to this matter.

As a corresponding author, I declare that there is no conflict of interest for this article entitled "DNA damage and ALAD polymorphism in high blood lead (Pb) Levels of pregnant women attending a tertiary care teaching hospital".

Acknowledgment

The authors are grateful to Mr. Idrish Shaikh for his technical support to estimate blood lead concentration. Authors are thankful to Dr. Hina Oza and Dr. Mehta R Yogeshbhai for providing blood samples from pregnant women from BJ medical college, Ahmedabad. We are grateful to the Director, ICMR-NIOH for the extended facility to carry out this study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eurox.2024.100300.

References

[1] Niedzwiecki MM, Eggers S, Joshi A, Dolios G, Cantoral A, Lamadrid-Figueroa H, et al. Lead exposure and serum metabolite profiles in pregnant women in Mexico City. Environ Health 2021;20:1–11.
[2] Karrari P, Mehrpour O, Abdollahi M. A systematic review on status of lead pollution and toxicity in Iran; guidance for preventive measures. DARU J Pharm Sci 2012;20:1–17.
[3] Taylor CM, Golding J, Emond AM. Adverse effects of maternal lead levels on birth outcomes in the ALSPAC study: a prospective birth cohort study. BJOG: Int J Obstet Gynaecol 2015;122:322–8.
[4] Kerr BT, Ochs-Balcom HM, López P, García-Vargas GG, Rosado JL, Cebrián ME, et al. Effects of ALAD genotype on the relationship between lead exposure and anthropometry in a Cohort of Mexican children. Environ Res 2019;170:65–72.
[5] Puspitaningrum R., Adhiyanto C., Firdausi A., Nurmasari Sartono R.A., Ferania M., Hattori Y., et al. Identification of the levulinate dehydratase (ALAD) gene polymorphism and whole blood hemoglobin in the students of elementary school in Kalideres, Jakarta, Indonesia. Jakarta, Indonesia. 2017;19:897–904.
[6] Wu Y, Wang M, Zhang J, Sun N, Li C. A new model of the mechanism underlying lead poisoning: SNP in miRNA target region influence the AGT expression level. Hereditas 2019;156:1–6.
[7] Szymańska-Chabowska, Łaczmański A, Jędrychowska I, Chabowski M, Gać P, Janus A, et al. The relationship between selected VDR, HFE and ALAD gene polymorphisms and several basic toxicological parameters among persons occupationally exposed to lead. Toxicology 2015;334:12–21.
[8] Shaik A, Jamil K. A study on the ALAD gene polymorphisms associated with lead exposure. Toxicol Ind Health 2008;24:501–6.
[9] Palir N, Stajnko A, Tratnik JS, Mazej D, Briški AS, France-Štiglic A, et al. ALAD and APOE polymorphisms are associated with lead and mercury levels in Italian pregnant women and their newborns with adequate nutritional status of zinc and selenium. Environ Res 2023;220:115226.
[10] Gautam K, Pradhan S, Thuppil V, Pyakurel D, Shrestha A. Blood lead level among school children in an industrial city of Nepal. J Pathol Nepal 2017;7:1091–4.
[11] Scinicariello F, Murray HE, Moffett DB, Abadin HG, Sexton MJ, Fowler BA. Lead and δ-aminolevulinic acid dehydratase polymorphism: where does it lead? A meta-analysis. Environ Health Perspect 2007;115:35–41.
[12] Kabeer A, Mailafiya MM, Danmaigoro A, Rahim EA, bu Bakar MZA. Therapeutic potential of curcumin against lead-induced toxicity: a review. Biomed Res Ther 2019;6:3053–66.
[13] Kayaaltı Z, Sert S, Kaya-Akyüzlü D, Söylemez E, Söylemezoğlu T. Association between delta-aminolevulinic acid dehydratase polymorphism and placental lead levels. Environ Toxicol Pharmacol 2016;41:147–51.
[14] Alvarez-Ortega N, Caballero-Gallardo K, Olivero-Verbel J. Toxicological effects in children exposed to lead: a cross-sectional study at the Colombian Caribbean coast. Environ Int 2019;130:104809.
[15] Tiwari AKM, Mahdi AA, Zahra F, Sharma S, Negi MPS. Evaluation of low blood lead levels and its association with oxidative stress in pregnant anemic women: a comparative prospective study. Indian J Clin Biochem 2012;27:246–52.
[16] Abdel Hamid OI, Khayal EE-SH, Tolba SAR, Orabi EE. Maternal Δ-aminolevulinic acid dehydratase 1-2 genotype enhances fetal lead exposure and increases the susceptibility to the development of cerebral palsy. Environ Sci Pollut Res 2020;27:44709–23.
[17] Rahman A, Al-Rashidi HA, Khan A-R. Association of maternal blood lead level during pregnancy with child blood lead level and pregnancy outcome in Kuwait. Ecol Food Nutr 2012;51:40–57.
[18] Srinivasa Reddy Y, Pullakhandam R, Radha Krishna K, Uday Kumar P, Dinesh Kumar B. Lead and essential trace element levels in school children: a cross-sectional study. Ann Hum Biol 2011;38:372–7.
[19] Kim J, Lee Y, Yang M. Environmental exposure to lead (Pb) and variations in its susceptibility. J Environ Sci Health, Part C 2014;32:159–85.
[20] Mohan S, Mahonski S, Koziatek C, Cohen ET, Smith S, Su MK. Induction of labor at term for severe antenatal lead poisoning. J Med Toxicol 2023:1–4.
[21] Sahu MC, Yogeshbhai MR, Oza H, Upadhyay K, Chanania K, Pati S. Blood lead levels in pregnant women and their newborn infants at an Indian teaching hospital. J Fam Med Prim Care 2024;13(1):348–55.
[22] Upadhyay K, Viramgami A, Balachandar R, Pagdhune A, Shaikh I, Sivaperumal P. Development and validation of Graphite Furnace Atomic Absorption Spectrometry method and its application for clinical evaluation of blood lead levels among occupationally exposed lead smelting plant workers. Anal Sci 2023;39:517–26.
[23] Kadawathagedara M, Muckle G, Quenel P, Michineau L, Le Bot B, Hoen B, et al. Infant neurodevelopment and behavior in Guadeloupe after lead exposure and Zika maternal infection during pregnancy. NeuroToxicology 2023;94:135–46.
[24] Olivero-Verbel J, Duarte D, Echenique M, Guette J, Johnson-Restrepo B, Parsons PJ. Blood lead levels in children aged 5–9 years living in Cartagena, Colombia. Sci Total Environ 2007;372:707–16.
[25] Clay K., Hollingsworth A., Severnini E. The Impact of Lead Exposure on Fertility, Infant Mortality, and Infant Birth Outcomes. NBER Working Paper. 2023.
[26] Todd AC, Wetmur JG, Moline JM, Godbold JH, Levin SM, Landrigan PJ. Unraveling the chronic toxicity of lead: an essential priority for environmental health. Environ Health Perspect 1996;104:141–6.
[27] Pawlas N, Broberg K, Olewińska E, Prokopowicz A, Skerfving S, Pawlas K. Modification by the genes ALAD and VDR of lead-induced cognitive effects in children. Neurotoxicology 2012;33:37–43.
[28] Behera R, Kothekar M, Kale D, Krishnamurthi K, Sirothia A, Kalorey D, et al. Study of mutations in aminolevulinic acid dehydratase (ALAD) gene in cattle from fly ash zone in Maharashtra, India. Indian J Anim Res 2016;50:19–22.
[29] Li C, Wang M, Wang Y, Zhang J, Sun N. A new model of the mechanism underlying lead poisoning: SNPs in miRNA target region influence the δ-aminolevulinic acid dehydratase expression level. Epigenomics 2017;9:1353–61.
[30] Yang Y, Wu J, Sun P. Effects of delta-aminolevulinic acid dehydratase polymorphisms on susceptibility to lead in Han subjects from southwestern China. Int J Environ Res Public Health 2012;9:2326–38.
[31] La-Llave-León O, Méndez-Hernández EM, Castellanos-Juárez FX, Esquivel-Rodríguez E, Vázquez-Alaniz F, Sandoval-Carrillo A, et al. Association between blood lead levels and delta-aminolevulinic acid dehydratase in pregnant women. Int J Environ Res Public Health 2017;14:432.
[32] Binns HJ, Campbell C, Brown MJ. Prevention ACoCLP. Interpreting and managing blood lead levels of less than 10 μg/dL in children and reducing childhood exposure to lead: recommendations of the centers for disease control and prevention advisory committee on childhood lead poisoning prevention. Pediatrics 2007;120:e1285–98.
[33] Meyer PA, Brown MJ, Falk H. Global approach to reducing lead exposure and poisoning. Mutat Res/Rev Mutat Res 2008;659:166–75.
[34] Dignam T, Kaufmann RB, LeStourgeon L, Brown MJ. Control of lead sources in the United States, 1970-2017: public health progress and current challenges to eliminating lead exposure. J Public Health Manag Pr: JPHMP 2019;25:S13.
[35] Kumar S. Occupational and environmental exposure to lead and reproductive health impairment: an overview. Indian J Occup Environ Med 2018;22:128.
[36] ESKENAZI B, PEARSON K. Validation of a self-administered questionnaire for assessing occupational and environmental exposures of pregnant women. Am J Epidemiol 1988;128:1117–29.
[37] Centers for Disease Control (US). Preventing lead poisoning in young children: a statement. CDC; 1991.
[38] Burns MS, Gerstenberger SL. Implications of the new centers for disease control and prevention blood lead reference value. Am J Public Health 2014;104:e27–33.