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Impact of Lead-Contaminated Milk on Cytokine Pathways (IL25, IL33, and TSLP) and Filaggrin Gene Polymorphism in the Development of Atopic Dermatitis

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ABSTRACT: This study aims to evaluate the immunogical effect of atopic dermatitis patients from consuming different milk (cow, goat, and buffalo) potential contamination with some heavy metals (lead) and genetically susceptible individuals carrying FLG gene (R501X) polymorphisms in development of atopic dermatitis disease. Three samples of each milk were collected in the city of Baghdad. The pH values of the milk samples were recorded at the mean±SD for cow, buffalo and goat were (6.44 ± 0.003, 6.712 ± 0.002 and 6.551 ± 0.0015) respectively. In general, the pH values of the analyzed milk samples were within the normal physiological range for fresh milk (6.4–6.9). Also, quantitative analysis of lead was performed by using graphite furnace atomic absorption spectrometry. The result lead mean±SD values for cow milk were (1.0671±0.00081ppm), buffalo milk were (1.1277±0.00029 ppm)and goat milk were (1.1928±0.0002 ppm). In addition to, the influence of consuming different types contamined milk on plasma cytokine levels in atopic dermatitis patients than healthy individual were indicated. The result mean±SD interleukin25 (IL25) levels were highest in patients consuming goat milk were (446.71±78.65 pg/ml), followed by buffalo milk were (453.14±94.55 pg/ml) and cow milk were (320.04±113.26 pg/ml) than controls were (191.69±73.26 pg/ml). The mean±SD IL33 levels were significantly higher in patients consuming goat milk were (439.80±89.37 pg/ml), cow milk were (413.37±111.91 pg/ml)and buffalo milk were(340.04±120.40 pg/ml) compared to controls were (171.69±50.17 pg/ml). Similarly, the mean±SD of TSLP that highest levels were observed in goat milk consumers were (366.47±131.49 pg/ml), cow milk were (273.37±112.50 pg/ml) and buffalo milk were (246.71±90.57 pg/ml) than in controls were (105.02 ±73.71 pg/ml). Results showed that all milk significantly increased pro-inflammatory cytokines, these findings that lead-contaminated milk types affect immune modulation and inflammatory status. Also, the blood samples atopic dermatitis patients were analyzed by DNA sequencing for FLG gene(R501X) SNP. The results genetic sequencing showed the filaggrin gene there were highly significant difference between patients and healthy groups and with three genotypes (AA, AT, and TT). In another hand, the effect of these SNP on development of AD is depend on status of mutant TT.

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INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease characterized by pruritus, xerosis, and recurrent eczematous lesions[1]. It is considered a multifactorial condition resulting from a complex interplay between genetic predisposition, immune dysregulation, environmental exposures, and skin barrier dysfunction[2]. The disease is strongly associated with type 2 immune responses, particularly the overproduction of cytokines such as IL4, IL5, and IL13, which promote IgE synthesis and allergic inflammation[3]. In recent years, growing attention has been directed toward environmental pollutants, such as heavy metals, as potential triggers and exacerbating factors for AD[4].Milk is a widely consumed dietary source, and contamination with heavy metals such as lead (Pb) poses significant health concerns. Lead exposure through contaminated milk can influence immune regulation by promoting oxidative stress, impairing regulatory T-cell function, and altering cytokine signaling pathways[5]. Studies have demonstrated that elevated lead levels stimulate the release of pro-allergic cytokines, thereby enhancing Th2-dominant immune responses[6]. This dysregulated immune activation may contribute to the onset and progression of atopic diseases, including atopic dermatitis, especially in genetically susceptible individuals[7].Epithelial-derived cytokines, including IL-25, IL33, and thymic stromal lymphopoietin (TSLP), play a central role in initiating and amplifying type 2 immune responses. These cytokines act as "alarmins," released by stressed or damaged epithelial cells[8]. Lead exposure in milk has been reported to upregulate the expression of these cytokines, thereby activating dendritic cells and group 2 innate lymphoid cells (ILC2s). IL25 and IL33 promote the differentiation and activation of Th2 cells, while TSLP enhances dendritic cell maturation and drives Th2 polarization. Together, these pathways amplify IL4, IL5, and IL13 production, leading to increased IgE synthesis, eosinophilic infiltration, and chronic allergic inflammation[9]. Mutations in the *FLG* gene, which is produced by upper- layer epidermal keratinocytes, have been identified as one of the genetic factors that promote skin barrier dysfunction [10]. This gene affects the development of AD by promoting the production of natural moisturizing factors and the matrix of lipid which functions as mortar to hold keratinocytes in the horny layer together [11]. Dry skin is caused by a loss-of-function mutation in *FLG*, which disrupts the development of the skin barrier, and increases transepidermal water loss [12].The aim of this study is to investigate the potential role of lead contamination in milk as an environmental risk factor and understanding this relationship may provide new insights into the immunopathological mechanisms linking environmental pollutants and genetic risks contributing to development of atopic dermatitis (AD).

MATERIAL AND METHODS

Samples milk collection

3 samples of raw milk selected during morning milking (cow, goat, and buffalo). The ages were each of them was a cow(13 years old) , a goat(12 years old) and buffalo(15 years old) a They were collected in plastic bottles, each sample was labelled to identify the source, site, and date of sampling, than milk pasteurization is a heat treatment process used to kill harmful bacteria and pathogens in raw milk, while preserving its nutritional quality, milk is quickly heated to 72°C, held for 15 seconds, then rapidly cooled to below 4°C and preserve in freezing till analyzed[13].

Determination of physicochemical parameters of milk samples pH-value

The pH of each sample was measured using a Digital pH-meter (BP3001/ Malaysia) equipped with combined glass electrode. The pH values of the liquid milk samples were measured directly by immersing glass electrode in the supernatant solution and pH values were recorded for each sample when the meter has stabilized [14].

Determination of Ash content

For liquid milk 2 mL of sample was blended with 10 mL 3:2 HNO_3 (65%, v/v) (BDH company): $HClO_4$ (69%, v/v) (BDH company). Then, it was digested at 90 °C on an electric hot plate and this mixture's temperature was gradually raised to 120 ^{0}C while being stirred every 30 minutes to allow improved digestion. The final volume of each sample was raised to 50mL after filtering the digests, and it was then kept in plastic bottles until analysis[15].

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Determination concentration of lead in milk

The concentration lead of presented within ash samples were determined by Flame atomic absorption spectroscopy (FAAS) (ASC-7000/USA) after wet digestion process[16].The flames of air-acetylene were single elements were used to determine metal concentrations in the range of mg/kg (ppm). All instrumental settings were performed under the terms set out in the table (1).

Table (1):The operation conditions of FAAS

Operation conditions	Pb
Flame	Air-Acetylene
Wavelength (nm)	283.3
Width(nm)	0.7
Lamp(mA)	10

Lead standard solutions (1000mg/L) were purchased from BDH company. These standards were used to prepare calibration solutions for each metal.

Subjects

This study was approved by the Ethics Committee in the College of Science, University of Baghdad (Ref.: CSEC/1025/0132). Sixty subjects were selected for this study, which included patients (N=15 each) consuming: cow, goat, and buffalo milk aged from 18 to 40years who suffered from atopic dermatitis. Also, the diagnosis, depended on the clinical signs and laboratory diagnosis by consultant physician, in addition to healthy random subjects as control group (N=15). This study was conducted in Baghdad Teaching and Al-Yarmouk Teaching Hospitals.

Blood samples

Blood samples were taken from two groups: Patients with a atopic dermatitis consuming: cow, goat, and buffalo milk samples and healthy individuals. Take 3 ml of the blood is distributed into the gel tubes, which was then left at room temperature for 30 minutes in order to initiate the clotting process, then the serum was separated by centrifugation at 3000 rpm for 10 minutes and stored sample at -20 ºC until the time of immunologic assays (IL25, IL33, and TSLP cytokines). Two ml of the blood from peripheral venous were dispensed in a sterile EDTA tube for DNA extraction and detection of SNP *FLG* genes are associated with atopic dermatitis.

Determination concentration of interleukins by ELISA

Enzyme-linked immunosorbent assay (ELISA) kits were used to quantify plasma levels of Interleukin25 (IL25), Interleukin33 (IL33) and TSLP cytokines. All assays were performed in duplicate according to manufacturer instructions(YLbiont./ China).

DNA extraction

Isolation of genomic DNA from refrigerated peripheral blood samples were collected from the healthy control and atopic dermatitis patients in EDTA tubes using Wizard® Genomic DNA Purification Kit (Promega, USA) following the manufacturer's guidelines which was followed by purity and concentration assessment. The extracted DNA was then stored at -20°C until analysis for genotyping.

PCR amplification and genotyping of *FLG* SNPs (R501X)

The fragment 400 bp containing the (R501X) SNPs placed on chromosome 1q21 in exon3 of *FLG* gene, were selected and amplified for investigation of association with atopic dermatitis using the primers The specific primers were: Fwd: 5'-AGGATGCTGTTCTTGGGTGG3'; and Rev: 5'- CTGAGGCTGCCAAGAGTGGA-3' [17]. Then 100 pmole of stock solution forward and reverse primers was prepared by dissolving lyophilized products in nuclease free water which were then used to prepare working solution. The primers, supplied by Alpha DNA Corporation, Canada, and designed using NCBI Primer-BLAST, were used in the PCR reaction. The PCR reaction was performed in a volume of 25 µl, comprising 1 µl each of forward (F) and reverse (R) primers for *FLG* SNPs (R501X) at a concentration of 10 pmol/µL. The reaction mixture also

contained 12.5 µl of master ready mix (Go Taq® Green Master Mix 2X: A reaction mixture containing DNA (2 µl, 100 ng),nuclease-free water (8.5 µl), and reagents from Promega Corporation, USA, was prepared. The mixed compound tube was processed using the Exispin system. The DNA amplification was then carried out using the thermal cycler (Multigene TM Gradient Thermal Cycler, Labnet International, USA). It was programmed as follows: First denaturationat 94°C for 5 minutes, followed by 35 cycles programmed as follows: First denaturation of denaturation at 94°C for 40 seconds, annealing for 60 seconds at 60°C (R501X), extension at 72°C for 30 seconds and followed by one cycle of a final extension 72°C for 8 minutes. Then separated products of PCR on 2% agarose gel electrophoresis at 5 v/cm ^{2}for 1 hour to confirm amplification in the existence of DNA ladder marker (100 bp) (Promega, USA) with 1 × Tris-borate-EDTA buffer and in the UV light was visualized subsequent to staining by ethidium bromide [18]. Rest of the PCR products were also sent for sanger DNA sequencing (Macrogen Corporation; South Korea) to have an automated sequencing by Genetic Analyzer system ABI-310 which gave the identity of the genotype and allele compared with the original genes in Gene Bank in NCBI.

Statistical Analysis

Parametric variables were given as mean ± standard deviation (SD) using the IBM SPSS computer software version 24. A P value of ≥0.05 was regarded as statistically significant. Allele and genotype frequencies, as well as differences, were assessed by Pearson's Chi-square test. The association between *FLG* single nucleotide polymorphism and atopic dermatitis was determined as odds ratio (OR) with the confidence of interval (CI estimate at 95%).

RESULT AND DISCUSSION

Physicochemical properties of milk samples

The pH values of all types of milk samples were illustrated in table (2). In this study, the pH values of the milk samples were recorded at the mean±SD for, Cow, Goat, and buffalo milk samples were (6.44 ± 0.003, 6.551 ± 0.0015, and6.712 ± 0.002) respectively. In general, the pH values of the analyzed milk samples were within the normal physiological range for fresh milk (6.4–6.9), when values higher than 6.9 indicate mastitis, and values less than6.4 indicate bacterial deterioration[19]. Cow milk presented the lowest pH, indicating relatively higher acidity compared to the other types, this can be attributed to its lower buffering capacity, mainly influenced by its protein and mineral content[20]. In contrast, buffalo milk exhibited the highest pH, reflecting a mild alkalinity, this finding is consistent with earlier reports that buffalo milk contains higher concentrations of protein (particularly casein), calcium, and phosphate, all of which increase the buffering capacity and elevate pH values[21]. Goat milk showed an intermediate value, closer to cow milk but slightly higher, reflecting its moderate buffering components[22]. The variations in milk pH among species may also result from genetic factors, dietary composition, metabolic activity, and the presence of bioactive compounds[23]. From a nutritional and technological perspective, the relatively higher pH of buffalo milk may contribute to greater stability and slower acidification during storage, while the slightly more acidic nature of cow milk could influence its fermentability and suitability for certain dairy products[24]. Although all three types of milk fall within the acceptable range for fresh milk, the species-specific variations in pH may affect their processing characteristics, nutritional properties, and shelf life[25]. The pH value of the buffalo milk sample is 6.71, which corresponds to the pH value of buffalo milk is 6.70 reported in a [26]. While the pH value of the cow's milk sample is 6.44, which is slightly lower than the pH value of cow sample is 6.60 due to the higher value of lactose which reported in a [27].

Table (2) : The pH Mean±SD in the various milk samples

Milk Sample	pH Mean±SD
Cow Milk	6.44±0.003
Buffalo Milk	6.712±0.002
Goat Milk	6.551±0.0015

Determination of lead concentration levels by FAAS

Linearity (Calibration Curve)

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The linearity with respect to Pb is depicted in Figure (1). As shown from the figure the calibration curve prepared from aqueous standards was ranged from 0.4-0.8 µg/ml (R^2 : 0.9993).

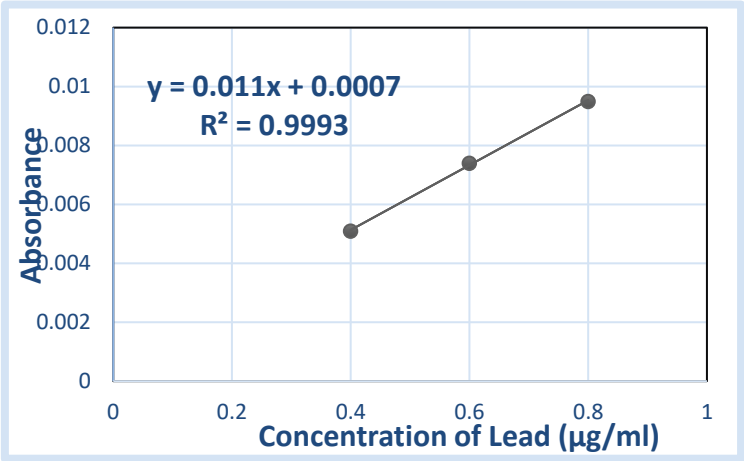


Figure (1): Calibration curve of Pb ppm

In this study, estimated the concentration of lead levels in different milk samples most commonly used by FAAS. The results were summarized in table (3) and figure(2). All milk samples show lead concentrations far exceeding the WHO safety limit (0.02–0.03 parts per million (ppm)).Goat milk has the highest level mean±SD (1.1926±0.0002)ppm, followed by buffalo milk (1.1276±0.00028) ppm and then cow milk(1.067±0.0008) ppm. All three milk types contain amounts of the contaminant (lead), Cow Milk: Despite having the lowest concentration, cow milk contains detectable lead levels that may combined with lower pH (6.44) to increase metal solubility and absorption in the gastrointestinal tract and trigger cytokine release[28]. Also buffalo milk lead level is significantly higher than the WHO safety limit. Although slightly lower than goat milk, it is still extremely above the safe threshold and poses a serious health risk for consumers[29]. Compared to cow milk, buffalo milk shows higher lead content, indicating that buffalo milk may be more prone to accumulating environmental contaminants. This could be due to factors such as: Feeding habits and grazing areas, Environmental exposure (water, soil),Physiological differences in mineral absorption[30]. Goat Milk: Highest concentration indicates greater exposure potential. pH is 6.551, slightly lower than buffalo milk, which may enhance metal bioavailability[31]. Individuals consuming goat milk with genetic susceptibility may experience stronger Th2-skewed immune responses leading to more severe atopic dermatitis[32]. Risk of lead absorption is not solely dependent on concentration, but also on pH (solubility) and genetic susceptibility[33]. The results [34; 35] demonstrate the mean±SD of lead in was (4.3± 0.2) ppm of goat milk , (3.1± 0.2) ppm of cow milk and was (2.8± 0.2) ppm of buffalo milk in Pakistan. But the mean±SD of lead was for cow and buffalo milk were (0.343 ± 0.039) (0.485±0.050) ppm respectively in Egyptian[36].

Table (3) : The results Mean±SD of Pb in different milk samples

Milk Sample	Mean±SD ppm
Cow Milk	1.067±0.0008
Buffalo Milk	1.1276±0.00028
Goat Milk	1.1926±0.0002

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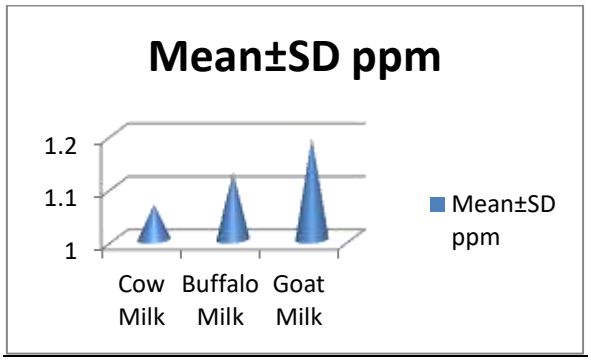


Figure (2): Lead concentration in different milk samples (ppm).

Determination of interleukin25,IL33 and TSLP by ELISA

The mean±SD interleukin25 (IL25) levels were highest in patients consuming goat milk (446.71±78.65 pg/ml), followed by buffalo milk (453.14±94.55 pg/ml) and cow milk (320.04±113.26 pg/ml) than controls (191.69±73.26 pg/ml) (Table 4).This indicates that milks intake stimulate IL25 production. IL25 is a Th2-promoting cytokine that drives IgE production and activates mast cells and eosinophils, thus contributing to allergic diseases such as asthma and eczema[37]. Also, The mean±SD IL33 levels were significantly higher in patients consuming goat milk (439.80±89.37 pg/ml), cow milk (413.37±111.91 pg/ml)and buffalo milk (340.04±120.40 pg/ml) compared to controls (171.69±50.17 pg/ml) (Table 4). IL33 is considered an "alarmin" cytokine released by epithelial cells upon stress or damage. It amplifies Th2 responses and enhances tissue inflammation. Its elevated levels suggest a strong link with chronic immune stimulation, possibly triggered by contaminants(lead)[38]. In addition to, the mean±SD of TSLP that highest levels were observed in goat milk consumers (366.47±131.49 pg/ml), cow milk (273.37±112.50 pg/ml) and buffalo milk (246.71±90.57 pg/ml) than in controls (105.02 ±73.71 pg/ml) (Table 4).TSLP plays a key role in priming dendritic cells (DCs) to direct the immune system towards a Th2 profile, leading to increased IL4, IL13, and IgE production [39]. Elevated TSLP along with IL25 and IL33 forms the epithelial cytokine triad due to environmental contaminants (such as heavy metals like lead), considered the initiating signal in allergic immune responses[40].

Table (4): The mean ± standard deviation of IL25,IL33 and TSLP levels in atopic dermatitis patients

Parameter Pg/ml	Patients Cow milk consuming N=15	Patients Buffalo milk consuming N=15	Patients Goat milk consuming N=15	Control N=15
IL25 Mean ±SD	320.04±113.26 ^{A}	453.14±94.55 ^{A}	466.71±78.65 ^{A}	191.69±73.26^{B}
IL33 Mean ±SD	413.37±111.91^{A}	340.04±120.40^{A}	439.80±89.37^{A}	171.69±50.17^{B}
TSLP Mean ±SD	273.37±112.50 ^{A}	246.71±90.57 ^{A}	366.47±131.49 ^{A}	105.02±73.71 ^{B}
The different letters referred to significant variation (P > 0.05)				

Genotype distribution and allele frequencies of the *FLG* SNP (R501X)

The results of amplified *FLG* (R501X) SNP PCR products was visible in agarose gel electrophoresis as shown in Figure (3), demonstrated that a yield of 400 bp single band for the desired result for atopic dermatitis patients.

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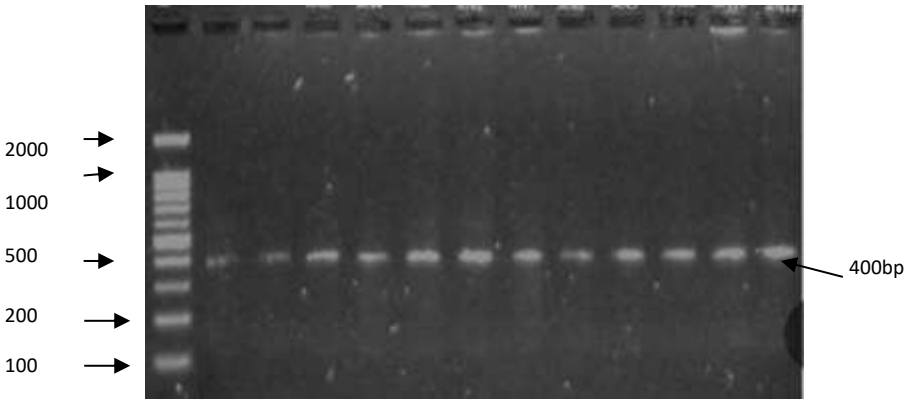


Figure (3): Electrophoresis of *FLG* (R501X) SNP PCR products on 2% agarose at 5 v/cm2 for 1 h. Lane L: 100bp DNA ladder; Lanes 1-12: atopic dermatitis patients

Two alleles were detected at *FLG* (R501X) SNP (A and T), which were corresponded to three genotypes (AA, AT, and TT). The result *FLG* A/T polymorphism genotype distributions and frequencies of allele in the atopic dermatitis consuming cow milk and controls are indicated in Table 5. AA genotype was prevalent in the consuming cow milk patients and controls (13% vs 87%); OR = 1) but appeared with decreased frequency in patients. The AT genotype (20% vs 13%); OR = 1.625; pc= 0.073) had no significant differences in the frequency between the consuming cow milk patients and controls (p>0.05), and the TT genotypes also showed significantly increased occurrence in patients than controls(67% vs 0%); OR = 59.2; pc= 0.001). A alleles had a corrected significantly decreased frequency in consuming cow milk patients compared to controls (20vs. 87%; OR = 0.038; pc = 0.001). The T allele showed a corrected significantly increased frequency in patients (80 vs. 13%; OR = 26; pc=0.001). Logistic regression analysis demonstrated that the *FLG* gene SNP was significantly associated with the atopic dermatitis patients consuming cow milk.

Table 5: Genotype and allele frequencies of *FLG* (R501X) gene polymorphism in healthy control subjects and atopic dermatitis patients consuming cow milk.

Allele or Genotype		**Patients cow milk consuming N=15 (%)**	**Controls n= 15 %**	**Odds ratio (OR)**	**95% C.I.**	**P value**	**PC-Value**
FLG	**A**	6 (20 %)	26(87%)	0.038	0.16-0.009	0.001>	0.001> S
	T	24(80%)	4(13 %)	26	103.5-6.53	0.001>	0.001>S
	AA	2 (13 %)	13(87%)	1.0	-	-	-
	AT	3 (20 %)	2(13%)	1.625	11.0-0.24	0.073	0.073NS
	TT	10(67%)	0 (0 %)	59.2	1070-3.3	0.001>	0.001>S

C.I.: Confidence interval; p: Fisher's exact probability; pc: Corrected p; NS: p > 0.05

The *FLG* (R501X) gene polymorphism genotypes distributions and frequencies allele in the buffalo milk consuming patients and controls are revealed in Table 6. The AA genotype was prevalent in the consuming cow milk patients and controls (10% vs 87%); OR = 1), and the TT genotypes also showed significantly increased occurrence in patients than controls(60% vs 0%); OR = 58.9; pc= 0.001). A alleles had a corrected significantly decreased frequency in consuming buffalo milk patients compared to controls (25vs. 87%; OR = 0.06; pc = 0.001). The T allele showed a corrected significantly increased frequency in patients (75 vs. 13%; OR = 16.25; pc=0.001). Logistic regression analysis demonstrated that the *FLG* gene SNP was significantly associated with the atopic dermatitis patients consuming buffalo milk.

Table 6: Genotype and allele frequencies of *FLG* (R501X) gene polymorphism in healthy control subjects and atopic dermatitis patients consuming buffalo milk.

Allele or Genotype		Patients buffalo consuming N=15(%)	Controls n= 15 %	Odds ratio (OR)	95% C.I.	P value	PC-Value
FLG	**A**	10(25%)	26(87%)	0.06	0.20-0.02	0.001	0.001 S
	T	20 (75%)	4(13 %)	16.25	57.5-4.6	0.001	0.001 S
	AA	2 (10%)	13(87%)	1.0	-	-	-
	AT	4 (30%)	2(13%)	13.0	105.2-1.6	0.012	0.036 S
	TT	9 (60%)	0 (0 %)	58.9	1018-3.4	0.001	0.001 S

C.I.: Confidence interval; p: Fisher's exact probability; pc: Corrected p; NS: p > 0.05.

The *FLG* (R501X) gene polymorphism genotypes distributions and frequencies allele in the goat milk consuming patients and controls are revealed in Table 7. The AA genotype was prevalent in the consuming cow milk patients and controls (6.7% vs 87%); OR = 1) but appeared with decreased frequency in patients. The AT genotype (26.7% vs 13%); OR = 12.3; pc= 0.093) had significant differences in the frequency between the consuming goat milk patients and controls (p>0.05), and the TT genotypes also showed significantly increased occurrence in patients than controls(66.6% vs 0%); OR = 59.2; pc= 0.001). A alleles had a corrected significantly decreased frequency in consuming goat milk patients compared to controls (27vs. 87%; OR = 0.08; pc = 0.003). The T allele showed a corrected significantly increased frequency in patients (73 vs. 13%; OR = 12.1; pc=0.003). Logistic regression analysis demonstrated that the *FLG* gene SNP was significantly associated with the atopic dermatitis patients consuming goat milk.

Table 7: Genotype and allele frequencies of *FLG* (R501X) gene polymorphism in healthy control subjects and atopic dermatitis patients consuming goat milk.

Allele or Genotype		Patients Goat milk consuming N=15 %	Controls n=15 %	Odds ratio (OR)	95% C.I.	P value	PC-Value
FLG	**A**	8 (27%)	26(87%)	0.08	0.28-0.02	0.001	0.003 S
	T	22 (73%)	4(13 %)	12.1	43.5-3.4	0.001	0.003 S
	AA	1 (6.7 %)	13(87%)	1	-	-	-
	AT	4 (26.7 %)	2(13%)	12.3	128.7-1.2	0.031	0.093 S
	TT	10(66.6%)	0 (0 %)	59.2	1018-3.4	0.001	0.001 S

C.I.: Confidence interval; p: Fisher's exact probability; pc: Corrected p; NS: p > 0.05.

The TT genotype of *FLG* is strongly associated with increased disease risk and likely plays a causative role. The AA genotype is protective against the disease. The AT genotype may have an intermediate or non-significant effect. This pattern indicates that the T allele acts as the risk allele, while the A allele is protective.The *FLG* gene plays a crucial role in mediating IL33 signaling pathways, regulating Th2 immune responses[41]. Mutations or polymorphisms in *FLG* can predispose individuals to heightened Th2 activity and AD susceptibility[42]. Filaggrin abnormalities are intimately linked to transepidermal water loss

(TEWL) and dry skin in people with atopic dermatitis [43]. Skin barrier disorders have been linked to a truncated mutation in filaggrin, which alters phase-separation dynamics [44].

CONCLUSION

Lead contamination in milk may contribute to the development of atopic dermatitis by inducing epithelial cytokines (IL25, IL33, TSLP) and enhancing Th2 immune responses. Reducing exposure to contaminated milk could help prevent or mitigate AD in susceptible individuals. There was highly significant difference in genetic sequencing of the *FLG* gene SNP was observed between the patient consuming lead contamination in milk and control groups with three genotypes (AA, AT, and TT) that increase development of AD disease.

Conflicts of interest

There are no conflicts of interest.

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