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Effects of Prenatal Arsenic Exposure Via Maternal Blood on Placental GLI3 Expression and Neonatal Outcomes

Somya Singh[1], Jayashree Adhikary[2], Sukanya Biswas[3], Subhash Chandra Biswas[4*]

[1,4]Institute of Post Graduate Medical Education and Research, Kolkata, India
[2]Presidency University, Kolkata, India
[3]North City Diagnostic and Research Centre, Kolkata, India

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***Corresponding author**:
Dr. Subhash Chandra Biswas
(Email: scbiswas59sskm@gmail.com)

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ABSTRACT

Background: Prenatal arsenic exposure can harm both the mother and foetus by changing gene expression and impacting foetal growth. The study aimed to assess the impact of arsenic exposure on GLI3 expression in feto-placental tissue via maternal blood and record neonatal outcomes.

Material and Methods: The study used 54 mother-infant pairs. Atomic absorption spectrophotometer used for measuring arsenic in maternal blood. Based on the median value of arsenic content in maternal blood, samples were split into two groups: the arsenic-low and the arsenic-high group. Maternal age, gestational age, arsenic exposure history, neonatal data were compared between two groups. Using qRT-PCR fold change in GLI3 expression was determined.

Results: Many participants resided in arsenic-contaminated regions and consumed underground water. The arsenic high group had a considerably higher mean arsenic content. The arsenic high group had a considerably lower gestational age than the low group. Neonatal birth weight significantly reduced in arsenic high group. Neonatal birth length increased in arsenic high group in comparison to arsenic-low group. Exposure to arsenic significantly decreased the relative expression of GLI3.

Conclusion: Prenatal arsenic exposure via maternal blood reduces GLI3 expression and affects neonatal anthropometry.

Key words: Arsenic, Pregnancy, Maternal Health, GLI3, Fetus, Neonatal Outcome

INTRODUCTION

Geographically distributed arsenic-rich geologic strata are a natural source of arsenic (As), a harmful environmental element.[1] A threat to human life has arisen from the long-term exposure to As-contaminated ground water, which is caused by using the tainted water for irrigation, cooking, and drinking. There has been a lot of research showing a link between exposure to arsenic and several cancers, including those of the liver, lung,

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prostate, skin, and bladder.[2] As a result of prenatal exposure to arsenic (iAs), epidemiological studies have recently reported negative effects on the developing fetus, including low birth weight, birth defects, and infant mortality. [3-6]

According to Ramsey et al. (2013),[7] arsenic is easily transmitted from the placenta to the cord blood and may have a negative impact on a fetus's development. Since arsenic has the potency to cause mutagenic effect,[8] it may alter a growing fetus's gene expression through both genetic and epigenetic changes. Chronic exposure to arsenic in fetal tissue during pregnancy may alter numerous essential genes' normal expression patterns, which are crucial for normal foetal development.[9,10] This could result in serious developmental defects that could have short- or long-term repercussions.[11] Numerous biological systems play a part in the highly regulated process of embryonic development, which is largely governed by three primary developmental signaling pathways: NOTCH, WNT, and HEDGEHOG (HH).[12,13] Human developmental problems are caused by mutations in components of the HH, NOTCH, or WNT pathways,[12,13] which is consistent with their functions in cellular differentiation and embryogenesis. Though slight alterations in these pathway activities, such those caused by exposure to the environmental toxic elements, can also interfere with fetal development and affect an individual's long-term health.[14,15]Substantial disruptions of these developmental pathways in humans are likely to result in spontaneous miscarriage, stillbirth, or unique birth abnormalities. Because the placenta is a mixture of maternal and fetal tissue, we can use the feto placental tissue to identify the gene that is differentially expressed and link it to birth defects that were noted at the time of delivery. Therefore, our objectives were to ascertain whether the one of the critical developmental gene in HEDGEHOG (HH) pathway namely, GLI3 was expressed by the feto-placental tissue exposed to arsenic and to record neonatal body weight and length in the corresponding sample set.

The study was conducted with objectives to assess arsenic content in maternal blood to find the potential source of arsenic exposure during pregnancy; to study mRNA expression analysis of GLI3 gene in the feto-placental tissue exposed to arsenic; and to study neonatal body weight and length of mothers exposed to arsenic during pregnancy.

MATERIALS AND METHODS

Study design: The study comprises a small cohort of 54 expectant patients who saw the outpatient department (OPD) at the Institute of Post Graduate Medical Education and Research (IPGME&R), Kolkata, during an 18-month period. The study included pregnant women of different age groups who gave their informed consent, were having their first child, had no history of diabetes, inflammatory diseases, or congenital birth defects. Patients with pre-existing diabetes, acute or chronic inflammatory diseases, or unwillingness to participate were not accepted. Forms of questionnaires and delivery records were used to gather information on the mother's age, address, gestational age in weeks, neonatal birth outcome, and other topics. The work in question received ethical clearance from the IPGME&R Research Oversight Committee in Kolkata (IPGME&R/IEC/2020/340).

Collection of samples: During parturition, a sample of placental tissue and 3 to 5 milliliters of maternal blood were taken from the subjects. Both clinical information, such as gestational age, and maternal personal information, such as age, address, and drinking water source (surface or underground), were documented through individual questionnaires. Additionally, information on neonatal birth outcomes, such as birth weight and length, was gathered from the hospital's record department.

Arsenic measurement: Using an Atomic Absorption Spectrophotometer Duo (Agilent Technologies) outfitted with a vapour generation assembly (Agilent VGA 77), total arsenic (As) in maternal blood samples of patients was evaluated. Standard As standards were created for calibration purposes using a 1,000 ppm commercially available as solution (Merck, Cat no. 119773). Instrument calibration and the as standards pre-reduction experiment was conducted in accordance with the standard protocol.[16] 189 nm wavelength was used for detection after unknown samples have been diluted with varied concentrations. Every sample was examined three times, and the average of the three readings was determined in each instance.

Gene Expression analysis: Following parturition, placental tissue was taken out from the placenta's fetal side. Using quantitative real-time PCR, the expression of GLI3 in placental tissues was examined. TRIzol reagent (Invitrogen, USA) was initially used to isolate total RNA from samples in accordance with the manufacturer's instructions. cDNA synthesis utilizing Superscript III (Invitrogen, USA) reverse transcriptase was carried out followed by Semiquantitative RT-PCR analysis. Human GAPDH was used as an endogenous control in the semiquantitative RT-PCR analysis, and specific primers for the gene of interest (GLI3) was used.[17] In summary, 2µl of cDNA was amplified in a 15µl reaction solution comprising 7.5µl SYBR-green master mix (Bio-Rad Laboratories, U.S.A.) and 1µl of each primer for the purpose of real-time quantification of mRNA expression. Bio-Rad CFX DUET was used to load each sample in triplicate and run it through 40 cycles. To verify the amplification of transcripts, melting curves were produced following each run. The comparative threshold cycle (ddCT) method was used to estimate the relative level of gene expression once the expression level of the GLI3 gene has been normalized against GAPDH. The formula 2-ddCt was utilized to determine the relative expression of each gene.[18]

Statistical analysis: The study employed descriptive statistics for analyzing participants' personal information, including their demographics. Using the chi square test, the association between blood arsenic and groundwater arsenic status was established. The t-test was used for comparing the means of the neonatal body weights of the respective mother who were exposed to varying levels of arsenic. For neonatal body length, a similar procedure was used. All statistical tests are 2-sided and every two-sided statistical test is deemed significant when the probability value is less than 0.05, or p<0.05. GraphPad Prism was used for the analysis.

RESULTS

Features of the Study Group: 54 mother-child pairs that were enrolled for an 18-month period at the Institute of Post Graduate Medical Education and Research (IPGME&R), Kolkata, made up the study cohort. The mean arsenic concentration in the mother's blood was 38.57 parts per billion (ppb). In the investigated sample, the median maternal blood arsenic concentration was 28.54 ppb. The sample set is then split into two groups: the arsenic-low group and the arsenic-high group, based on the median value of the maternal blood arsenic content. There are 27 samples in each group; values below the median are categorised as belonging to the arsenic-low group, while values above the median are categorised as belonging to the arsenic-high group. In the arsenic-low group, the mean maternal blood arsenic concentration was 22.73±0.6589 ppb, while in the arsenic-high group, it was54.42±4.156 as shown in Table 1.

Arsenic exposure and maternal parameters: In the arsenic-low group, the mean maternal age was 25.44 ± 0.9627 years; in the arsenic-high group, the mean maternal age was 26.96 ± 0.8103 years. The p-value obtained from an unpaired t-test comparing the mean age of the two groups was 0.233. The group with low arsenic levels had a mean gestational age of 38.67 ± 0.4203 weeks, whereas the group with high arsenic levels had a mean gestational age of 35.52 ± 0.4634 weeks. The mean gestational age was found to have a significant p-value (p <0.0001) between the groups. There was a significant difference (p <0.0001) in the mean maternal blood arsenic concentration between the arsenic-low group (22.73±0.6589 ppb) and the arsenic-high group (54.42±4.156 ppb). After noting the individual's address and checking the area's ground water arsenic level, [19] it was discovered that, of the 27 participants in the arsenic-low group, 14 lived in an arsenic-safe area and 13 in a moderately to severely affected area. However, of the 27 participants in the arsenic-high group, 4 resided in an arsenic-safe area and 23 in an arsenic-moderately to arsenically afflicted area. The number of individuals coming from the safe and moderately affected areas to the affected area in the arsenic-low group against the arsenic-high group showed a statistically significant p value of 0.0084 upon chi-square analysis. The participants' sources of drinking water were documented; of those in the arsenic-low group, 13 drank water from the surface reservoirs and 14 drank water from underground sources. Out of the 27 participants in the arsenic-high group, 24 drank water from underground sources, and just 3 drank water from the surface water sources. A significant p value of 0.0063 was found in the chi-square analysis comparing the number of people in the two groups who consumed surface and underground water. (Table 1)

Table 1: Maternal parameter of the study participants

Maternal Parameters	Arsenic Low Group (AsL) (n=27) (%)	Arsenic High Group (AsH) (n=27) (%)	P value
Maternal blood arsenic concentration (Mean±Std. Deviation):	22.73 ± 0.6589	54.42 ± 4.156	<0.0001*
Arsenic exposure history			
Safe area	14 (51.8)	4 (14.8)	0.0084*
Exposed area	13 (48.2)	23 (85.2)	
Drinking water source			
Surface water	13 (48.2)	3 (11.1)	0.0063*
Underground water	14 (51.8)	24 (88.9)	
Age (in years) (Mean ± Std. Deviation):	25.44 ± 0.9627	26.96 ± 0.8103	0.233
Gestational Age (in weeks) (Mean ± Std. Deviation):	38.67 ± 0.4203	35.52 ± 0.4634	<0.0001*

*All statistical tests are two-sided, and when the probability value is less than 0.05, or p<0.05, the test is considered significant. P value less than or equal to 0.0001 is considered as highly significant.

Table 2: Neonatal anthropometry measurement

Neonatal Parameter	Arsenic Low Group (AsL) (n=27)	Arsenic High Group (AsH) (n=27)	P value
Body weight at birth (in gm) (Mean ± Std. Deviation):	2574 ± 75.55	2217 ± 41.57	0.0001*
Body length at birth (in cm) (Mean ± Std. Deviation):	50.68 ± 0.2442	53.91 ± 0.5485	<0.0001*

*All statistical tests are two-sided, and when the probability value is less than 0.05, or p<0.05, the test is considered significant. P value less than or equal to 0.0001 is considered as highly significant.

Arsenic exposure and neonatal parameters: Following delivery, mothers included in the study group's neonates' birth weight and length were noted. According to the previous categorisation based on the median value of maternal blood arsenic concentration in Table 1, the mothers who were in the arsenic-low group were categorised together with their respective neonates in the arsenic low group. Similarly, the mothers who were categorised in the arsenic-high group their respective neonates were categorized in arsenic-high group. Neonates in the arsenic-low group weighed 2574 ± 75.55 grams on average at delivery. Conversely, newborns in the arsenic-high group had a mean birth weight of 2217 ± 41.57 grams. Neonates in the low and high arsenic groups had significantly different birth weights on average (p=0.0001). In the arsenic-low group, the mean birth length of neonates was 50.68 ± 0.2442 centimetres, but in the arsenic-high group, it was 53.91 ± 0.5485 centimeters. P value (p<0.0001) was found to be the significant between the groups after comparing the means using an unpaired t-test. (Table 2)

Arsenic induced differential GLI3 gene expression in placenta: Next, we investigated the expression of our candidate gene, GLI3, which was connected to the length and weight of the fetus at birth. We considered about studying the feto-placental tissue as the fetal side of the placenta and the fetus have comparable genetic makeup. To figure out if the normal expression of GLI3 in feto-placental tissue is affected by maternal arsenic exposure. We isolated RNA from 50 placental samples, 25 samples from each group. The control group consisted of samples from the arsenic-low group, while the case group consisted of samples from the arsenic-high group. The comparative threshold cycle (ddCT) method was used to calculate the relative expression of the GLI3 gene. GAPDH, the endogenous control of the corresponding sample, was used to normalize the ct values of the GLI3 gene in the arsenic-low group (n=25) and the arsenic-high group (n=25). The GLI3 gene's Dct values were compared between the case group (arsenic-high) and the control group (arsenic-low) to determine the relative change in gene expression (i.e., 2^-ΔΔctGLI3). In the control group, the expression of 25 samples was combined and interpreted as 1±0 (mean±SE). In the arsenic-high group (case group), the mean fold change in GLI3 gene expression was 0.295151±0.011222 (mean±SE). Showing a decrease in the expression of the GLI3 gene in placentas exposed to high levels of arsenic in comparison to low levels. It was determined that there was a statistically significant difference in the GLI3 gene expression between the two groups, with a p value of p<0.0001. (Figure 1)

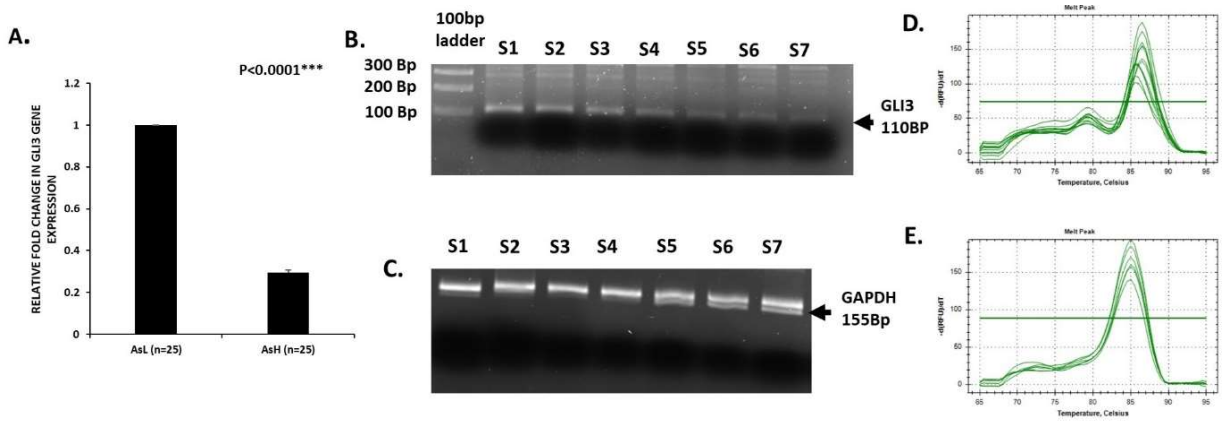

Figure 1: (A) Arsenic induced differential GLI3 gene expression. The comparative threshold cycle (ddCT) method was utilized to calculate the relative expression of the GLI3 gene. For both the arsenic low group (n=25) and the arsenic high group (n=25), the ct values of the GLI3 gene were normalized using the endogenous control GAPDH of the corresponding sample. The relative change in gene expression (i.e., 2^-ΔΔctGLI3) was calculated using the Dct values of the GLI3 gene from the case group (arsenic-high group) vs the control group (arsenic-low group). The error bar displays the 95% confidence intervals, with a significant p-value of p<0.0001. (B-C) Representative Agarose Gel-electrophoresis image (2% agarose) of real-time qPCR amplified products: (B) An image displaying the GLI3's differential gene expression, at 110 bp. (C) 155 bp amplicon size of the endogenous control GAPDH. Samples (S1-S3) from the case group (Arsenic-high) and Samples (S4-S7) from the control group (Arsenic-low) are shown in both panels (B and C). (D-E) Melt curve generated from the real-time qPCR. (D) Melt curve for GLI3. (E) Melt curve for GAPDH.

DISCUSSION

Worldwide variations exist in the amount of arsenic found in geological layers. It is commonly recognized that there is an abundance of arsenic-contaminated underground water in India's Indo-Gangetic belt.[20] Although the study comprises a very small cohort of just 54 individuals but it is worthy of mention that the study group's members were drawn from a variety of west Bengali districts, and our data shows that a sizable portion of them are from areas afflicted by arsenic contamination (p=0.0084*) (Table 1). Our findings clearly show

that drinking water from underground sources is one of the main ways that arsenic enters the body (p=0.0063*) (Table 1). The maternal blood arsenic content, which in the investigated sample set had a median of 28.54 ppb, indicates that the mother was exposed to arsenic. Adverse effects on fetal growth and development are also caused by exposure to environmental toxic substances during pregnancy. [21] These implications could affect early childhood and become apparent later in life. [22] Our research revealed a noteworthy drop in weeks of gestational age between the low and high arsenic groups (p<0.0001), indicating a substantial impact of arsenic throughout the pregnancy period. While examining the newborn anthropometry, we discovered a significant decrease in neonatal birth weight in thearsenic-high group compared to the arsenic-low group (p=0.0001) (Table 2). When comparing the arsenic-high group to the arsenic-low group, it was discovered that the neonatal birth length measured at the time of birth was substantially longer in the arsenic-high group (p<0.0001). This information points to the role that arsenic plays in embryonic development. A 2015 study by Emily F. Winterbottom et al. [10] identified multiple genes that expression was correlated in a fetal sex-specific way with maternal arsenic exposure. Among which, there was a negative correlation between arsenic exposure and the expression of GLI3, an element of the HEDGEHOG pathway and a positive correlation between it and the birth weight of the newborn. The GLI3 gene, which encodes a transcription factor crucial to controlling the HEDGEHOG (HH) pathway, was the subject of our investigation. Among the different signaling pathways, the HH pathway is regarded as one of the key developmental signaling pathways involved in fetal growth. We discovered that the arsenic high group in our cohort had considerably lower GLI3 gene expression than the arsenic low group (p<0.0001) (Figure 1). Thus, we can deduce that the placenta's exposure to arsenic through maternal blood is what triggered the differential gene expression. The HEDGEHOG (HH) pathway is essential for the formation of embryonic skeletal. [9] This further explains the adverse anthropometry of the neonate found in our cohort. Arsenic induced reduced expression of GLI3 gene might have influenced the HEDGEHOG (HH) pathway. Hence, we observed abnormality in the body weight and body length at birth. However, detailed molecular mechanism behind these results needs to be investigated in increased number of samples before drawing a justifiable conclusion.

STRENGTH AND LIMITATIONS

This is the first research of its sort; while other studies on arsenic exposure have been done in the past, none have looked at the relationship between arsenic exposure and the health of mothers and newborns. The current research is extremely important given the amount of arsenic in the Indo-Gangetic belt, as exposure to the metal during pregnancy may have an impact on the processes involved in a child's early embryonic development. The study's limited sample size is its main drawback; while it does reveal some significant findings, additional participants are needed to effectively understand the association between the GLI3 gene expression and negative newborn outcomes.

CONCLUSION

Our research shows that drinking water from underground sources exposes pregnant women to arsenic during their pregnancy, which affects both the mother's and the fetus' tissue. Arsenic also tampers with certain components of developmental signaling, causing the signaling pathway to alter and significant transcriptional regulators like GLI3 to express differently. This further impairs fetal development, which leads to unfavorable delivery outcomes such as low birth weight and longer birth duration. Nevertheless, further research is needed to determine the precise mechanism by which arsenic induces these changes. To comprehend the molecular process underlying it, a thorough investigation involving a larger cohort of mothers and newborns, animals, and cell lines should be conducted in the near future. Understanding whether arsenic has any effect on fetal growth was helpful. In order to prioritize the implementation of corrective actions, the data in this regard will aid in understanding the degree of the harmful effects of arsenic exposure, particularly during the gestational period. Finding the molecular marker linked to the developmental pathway would aid in the creation of innovative treatments with the goal of reversing the negative effect in the future.

Data availability

On request, the corresponding author will provide the data supporting the study findings.

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Authors' Contributions

Somya Singh: Sample collection, documentation of maternal and neonatal information in the questionnaire, literature search. Jayashree Adhikary: Experimental work, data analysis, compilation, writing of manuscript, review and editing. Sukanya Biswas: Writing of manuscript, review of literature and data interpretation. Subhash Chandra Biswas: Conceptualization, design, review and final editing. *Somya Singh and Jayashree Adhikary contributed equally as first authors.

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