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Transplacental exposure to arsenic disrupts offspring metabolism through sex specific pathological changes in liver and adipose tissue

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Abstract

Background: Suboptimal fetal environment may cause pathological and functional changes in the adulthood and may impact health and metabolism of the offspring. Transplacental exposure to environmentally relevant doses of arsenic may program offspring metabolism through alterations in the development of major metabolic organs such as kidneys, liver and adipose tissue. Alterations particularly in the liver and adipose tissue may cause altered glucose homeostasis and insulin sensitivity in the offspring.

Methods: Female Balb/c mice were exposed to environmentally relevant doses of arsenic (0, 0.04 and 0.4 mg/kg) from 15 days prior to conception until delivery. The offspring were divided according to sex and monitored for metabolic alterations, particularly glucose homeostasis was monitored by periodically checking for fasting glucose levels and metabolic tolerance tests. Pathological changes in major metabolic organs i.e., liver, inguinal white adipose tissue and kidneys were observed by standard pathological staining process.

Results: Adult female offspring showed marked increase in the weight gain pattern and relative adiposity owing to fat deposition in the inguinal fat depot was also found to be increased. Transplacental exposure to arsenic correlated with a marked decrease in insulin sensitivity and glucose tolerance. As the liver and adipose tissue dictates whole body insulin resistance, we checked any pathological alterations in these organs. Adipocyte hypertrophy marked by a substantial increase in cellular volume was seen in the treated groups. the liver showed micro and macro vesicular fatty changes with necrotic foci which were apparent in the female offspring. The kidneys showed minor glomerular swelling and mesangial cell infiltration showing inflammatory changes in the organ.

Conclusion: Transplacental exposure to arsenic may alter metabolic phenotype in the adulthood leading to dysfunction and pathological outcomes in major metabolic organs.

Keywords: Arsenic, in utero exposure, metabolic disorder, glucose tolerance, insulin resistance, histopathology

1. Introduction

Arsenic is a major drinking water pollutant affecting >300 million people across the globe. Chronic exposure to arsenic has been a major risk factor for the development of skin and urinary bladder cancer. In recent years, various groups have demonstrated several non-cancer endpoints in the chronic arsenic exposed population. The World Health Organization (WHO) has laid down the acceptable limit of arsenic in drinking water at 10µg/l (0.01 ppm). But prolonged exposure to inorganic arsenic in drinking water far exceeding the MCL set by WHO is widely found in the drinking water of the affected countries. Especially in India and Bangladesh where the MCL of arsenic in

drinking water is allowed at 50 ppb [1,2]. Chronic exposure to arsenic has been implicated in the development of several types of cancerous [3–6] and non-cancerous endpoints including metabolic disorders like type II diabetes and obesity [7–10]. Strong correlation has been set up between the development of metabolic deregulation and arsenic exposure in arsenic endemic areas with population studies have revealed the [8,11–14]. Inorganic arsenic in drinking water readily crosses the blood placenta barrier and therefore, it is considered as a risk factor for developmental toxicity. During the fetal development, the epigenomic marks that shape and dictate gene expression landscape of an organism is erased and established in a time-dependent fashion. Therefore, these periods of epigenomic plasticity are particularly vulnerable to environmental toxicants and/or stress. Recently, much attention of developmental toxicity research has been focused on the evaluation of non-overtly toxic exposures to environmental chemicals. And newer evidence points out that, as a developmental carcinogen, arsenic may affect the development and functioning of major metabolic organs such as liver, adipose tissue and kidneys [15–23]. These subtle changes in gene expression or epigenetic alterations may contribute towards the development of diseases in adult life. In a ground-breaking study, Waalkes et.al. pointed out that, prenatal exposure to 42.5ppm and 85ppm sodium arsenite increased the incidence of hepatic, adrenal and ovarian tumors in female mice [24,25]. Since then, many reports point out that prenatal exposure to arsenic may increase predisposition towards the development of cancers in the offspring [26–30] while, the development of non-cancerous endpoint disorders such as type 2 diabetes mellitus and metabolic syndrome have been neglected as a research topic. The metabolic alterations that can be brought about by prenatal exposure to arsenic may contribute to a person's predisposition towards the development of the said disorders. It is suggested in earlier studies that inorganic arsenic exposure could alter lipid metabolism, glucogenesis and insulin secretion in normal individuals leading to pre-diabetic effects and can worsen the effects in diabetic individuals [31–35]. The role of prenatal exposure on the progeny needs to be evaluated at length because arsenic is a potential poison to the evolving fetus and can easily cross the blood placental barrier [36–41].

In the following study, we saw the effects of prenatal arsenic exposure at an environment relevant level on the metabolic tolerance of adult Balb/c mice. Due to lifelong or multilevel exposure to arsenic in human populations, it is difficult to assess the effect of gestational arsenic exposure in isolation. We tried to end this lacuna by restricting the exposure window to only the prenatal stage and observing the offspring for any changes in metabolic profile and pathologic alterations in the major metabolic organs i.e., liver, visceral white adipose tissue and kidney. The study will reveal various physiological and histopathological alterations that contribute to the increased risk of metabolic deregulation in the individuals from arsenic endemic areas.

2. Material and Methods

Experimental protocol

6 weeks male and female Balb/c mice were procured from CSIR- Indian Institute of Toxicology Research, India. Females were randomly divided into three groups of control, 0.04 and 0.4 mg/kg arsenic. All the animals were housed in polypropylene cages and were fed with standard pellet diet and water *ad libitum*. Temperature and relative humidity were maintained at 25±5˚C and 50±15% with dark light cycle of 12-h:12-h. Freshly prepared arsenic dose in water was administered via oral gavage to females from 15 days prior to mating i.e., GD-15 till delivery of pups i.e., GD 21. The pups in the F1 generation were separated according to sex after weaning and were allowed to grow to

adulthood. Institutional Animal Ethical Committee (IAEC) approved animal experiment protocols were employed.

Physiological analysis

At termination (40 weeks), body weights were recorded and liver, visceral epididymal adipose and kidney were isolated and fixed in 4% paraformaldehyde for histopathological analysis. Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were performed on 6 hours fasted animals at 25 weeks. After fasting, 2.0 g/kg body weight D-glucose was given orally in OGTT and blood glucose levels were measured at various intervals while, in ITT, 1 U/kg body weight insulin was administered intraperitonially and blood glucose levels were measured. Using a portable glucometer (AccuChek Active, India), blood glucose levels were recorded at 0, 10, 20, 40, 60, 90 and 120 minutes for both OGTT and ITT.

Histopathological analysis

Liver and kidney tissues fixed in 4% paraformaldehyde were processed in various gradations of alcohol, xylene and paraffin wax and then paraffin embedded blocks were prepared. Whereas for visceral epididymal adipose tissue, processing was performed in various gradations of histochoice, xylene and paraffin wax and then paraffin embedded blocks were prepared. Slides were prepared by cutting the blocks in 5 µm thick sections and were stained with Hematoxylin and Eosin (H&E). The sections stained with H&E were visualized under light microscope and the images were procured via using Leica application suite (version 4.10).

Statistical analysis

All the data represented in the figures are illustrated as mean ± SE normalized to control values. The three groups were analysed comparatively by using one way ANOVA (p <0.05) using GraphPad software (GraphPad Software, v. 6.0; San Diego, CA, and Microsoft-Excel). The histological studies represent data from 6 animals per group.

3. Results

Prenatal exposure to arsenic leads to increased body weight and adipose tissue hypertrophy:

Prenatal exposure to arsenic led to a statistically significant increase in bodyweight of the exposed mice at 40 weeks of age (fig. 1A). The animals showed approximately 24% and 52% increase in body weight compared to control animals with no prenatal exposure.

To examine the metabolic tolerance profile of the prenatally exposed animals, we performed a 120-minute oral glucose tolerance test after 6 hours of fasting in the photophase of the light/dark cycle. Although there was no significant difference in the fasting blood glucose levels, the prenatally exposed groups showed an elevated area under the curve (AUC) indicative of delayed absorption of glucose from the bloodstream (fig. 1B-C), showing impaired insulin tolerance. To test insulin tolerance of the animals, we further performed a 120-minute intraperitoneal insulin tolerance test. The IP-ITT results confirmed dampened insulin sensitivity in the exposed animals as a significant increase in AUC measurements were observed (fig 1D-E).

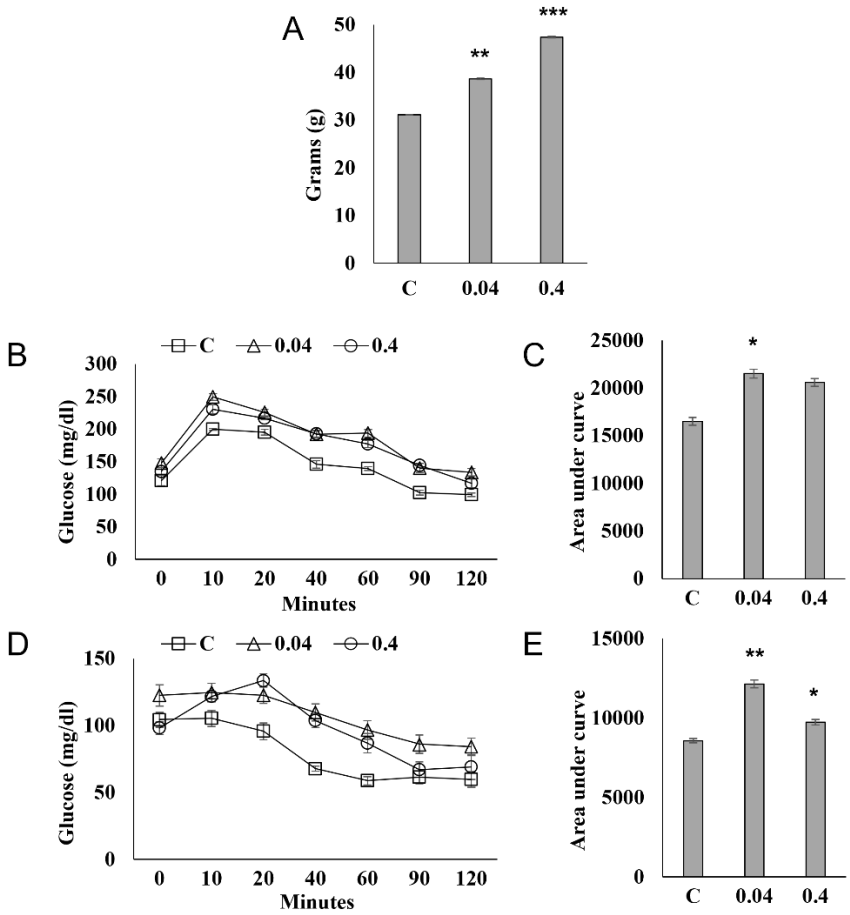
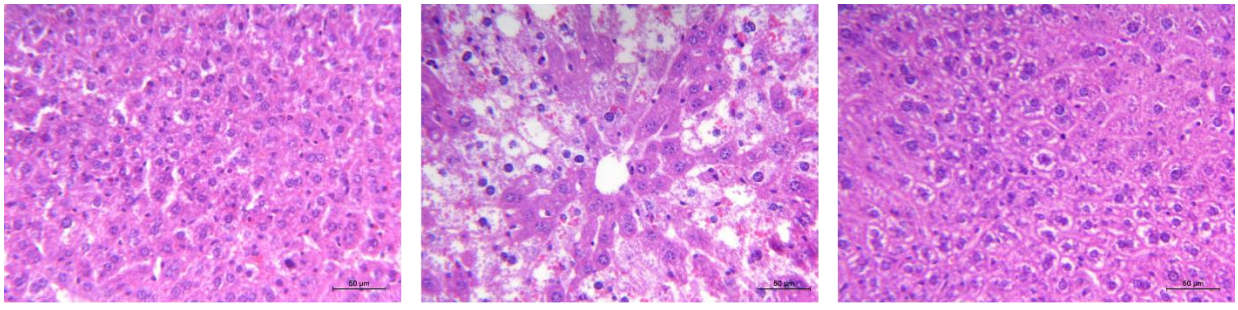


Figure 1: Prenatal exposure to arsenic disrupts glucose homeostasis with contemporaneous distortion of overall adiposity in adult animals. A) Increase in body mass of animals at termination (40 weeks). B & C) Oral glucose tolerance test (OGTT), and D & E shows Insulin tolerance test (ITT) of 20 weeks animals. All data represent the mean ±SD, n = 6 (number of experimental sets). *p < 0.05, **p < 0.01 and ***p<0.001.

Effects on target organs

Liver:

Histological examination of 5um thin, H&E-stained sections of liver revealed significant hepatocyte ballooning and macro vesicular fatty changes in the animals exposed to 0.04 mg/kg arsenic. Cytoplasmic accumulation of glycogen was apparent in hepatocytes with rarefaction in cytoplasm with centrally located nuclei in both the treatment groups. some centrilobular hepatocellular hypertrophy was also seen (fig. 2).



Control | 0.04 mg/kg | 0.4 mg/kg

Figure 2: Effects of prenatal exposure of arsenic on pathological changes in the liver tissue of 40 weeks old mice. From left to right control, 0.04 mg/kg and 0.4 mg/kg exposed offspring. Representative images of each treatment group (n=6) are shown. Stained haematoxylin and eosin liver showed focal micro vesicular fatty changes and intracellular glycogen deposition in the liver of the prenatally exposed animals. Magnification 160x of original.

Visceral adipose tissue:

Along with the increased fat mass in the visceral adipose tissue, the prenatally exposed animals showed significant changes in adipocyte morphology. Significant hypertrophic changes were clear in both the treatment groups. Some inflammatory cell infiltration was also seen in the interstitial spaces (fig. 3).

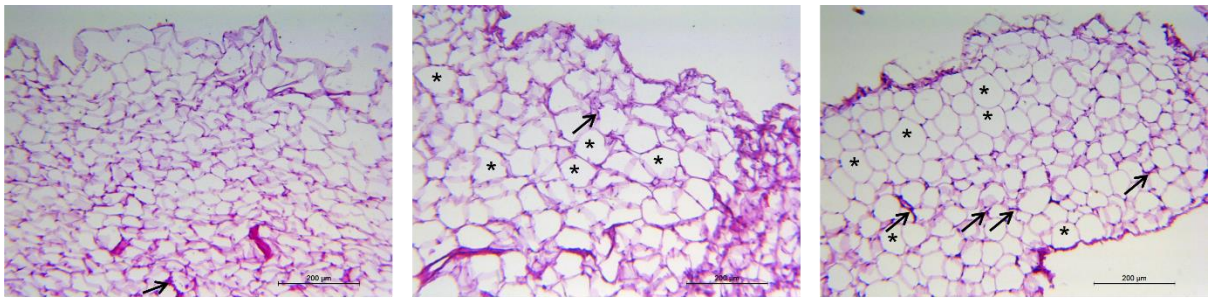


Figure 3: Effects of prenatal exposure of arsenic on pathological changes in the visceral epididymal adipose tissue of 40 weeks old mice. From left to right control, 0.04 mg/kg and 0.4 mg/kg exposed offspring. Representative images of each treatment group (n=6) are shown. Stained haematoxylin and eosin epididymal fat pad showed hypertrophic adipocytes (asterisks) with inflammatory cells infiltration (black arrows). Magnification 160x of original.

Kidneys:

Histological examination of the kidneys revealed glomerular swelling and a clear increase in the number of mesangial cells with depreciated urinary space and mesangial expansion. No damage to the proximal and distal convoluted tubules were seen in any of the groups, although some animals showed significant inflammatory cell infiltration in the peritubular/interstitial spaces (fig. 4).

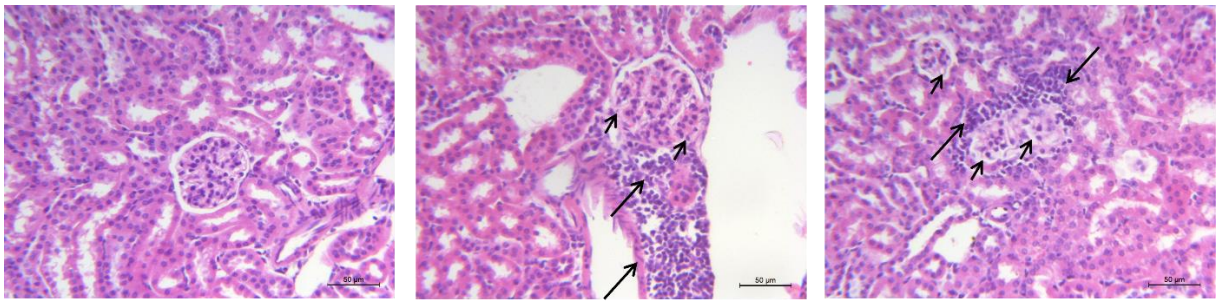


Figure 4: Effects of prenatal exposure of arsenic on pathological changes in the Kidney tissue of 40 weeks old mice. From left to right control, 0.04 mg/kg and 0.4 mg/kg exposed offspring. Representative images of each treatment group (n=6) are shown. Stained haematoxylin and eosin kidney showed focal inflammatory cell infiltration (long arrow) and glomerular damage (short arrow) in the kidneys of prenatally exposed animals. Magnification 160x of original.

4. Discussion

Exposure to trivalent, inorganic arsenic during the gestational period has been shown to cause adverse birth outcomes in humans. Inorganic arsenic readily crosses the blood-placenta barrier and

may deleteriously affect the developing fetus. During the gestational period, several important developmental events unfold, which shapes the gene expression profile of the offspring during the adulthood. Any toxicant, environmental factor such as maternal nutrition, substance abuse and stress which hampers this process may leave a lasting mark on the epigenome of the developing organism. These marks are further translated into disease states and susceptibility to various diseases in adult life. The developmental origin of health and disease hypothesis supports this view of adult life diseases to be partly rooted in the fetal and/or maternal environment. During the maturation phase, several other environmental factors may come into play and aggravate the disease state of the organism. However, a straightforward cause-effect understanding in the subject is still developing. Also, in human populations, the effects of maternal environment on the health and well-being of the offspring are difficult to assess. This is especially true in cases of prenatal toxicant exposure such as, arsenic in drinking water, because often the exposure is lifelong (i.e., starting at conception till the disease development) in the endemic areas. Thus, to assess the role of maternal exposure to arsenic and its impacts on fetal epigenome and later, health and disease we must employ animal models of only gestational exposure. Emerging research in the chronic arsenic toxicity area suggest that, even in low-moderate exposure levels arsenic is associated with the development of type 2 diabetes. A cross sectional study among American Indian adults (median age 36 years) revealed arsenic exposure was associated with incident diabetes among non-prediabetic individuals. The authors also reflected that, low monomethyl arsenate percentage in the urine (an indicator of As3MT activity and arsenic metabolism) was correlated with a higher HOMA2-IR [42]. Previously, in cross sectional studies in arsenic endemic areas of Mexico, it was revealed that, higher levels of exposure to arsenic are directly associated with an elevation in fasting blood glucose and later, the development of diabetes [43]. However, a mechanistic understanding on this subject is still at its infancy. The toxicity of arsenic is influenced by its metabolism, primarily in the liver and other tissues [44]. Ater its absorption from the gastrointestinal tract, arsenic is metabolised in its mono and di -methylated forms which are progressively less toxic and are excreted in the urine [45,46]. Lower methylation ability and any defect in the methylation of the inorganic arsenic may lead to significantly higher accumulation of arsenic across tissues and as a result, higher level of toxicity. Indeed, this phenomena of individual differences between arsenic metabolism significantly increases the risk of skin lesions, cardiovascular disease, bladder cancer and skin cancer [47]. In cases of prenatal exposure to arsenic, the metabolism and clearance ability of the mother may thus influence degrees of toxicity in the developing fetus. Indeed, a study conducted among pregnant women from an arsenic endemic population in Mexico, a positive correlation is proved among birth outcomes and individual capacity of arsenic metabolism and clearance [48]. In laboratory animals, the individual differences between arsenic metabolism and its clearance are minimised. We thus looked to replicate the arsenic exposure induced toxicity in a model of prenatal exposure.

Apart from chronic exposure studies, several population studies among arsenic-endemic populations across the globe has pointed out adverse birth outcomes such as low birth weight, decreased skull circumference and increased susceptibility to infectious diseases in the childhood [49]. Arsenic exposure in the endemic areas start from the preconception period and continues throughout gestation. Significant contribution of preconception exposure was found in one study involving preconception and gestational exposure in a genetically diverse collaborative cross mouse strain. This study confirmed dysregulated insulin response and glucose tolerance with or without the consumption of high fat diet [50]. Holistically, these studies have suggested the association of *in utero* exposure to low to moderate levels of arsenic can augment the development of type 2

diabetes (T2D) in the exposed individuals. T2D is a metabolic disorder characterized by disruption of the insulin-signaling pathway, resulting in insulin resistance, pancreatic β-cell dysfunction, impaired glucose utilization, and high fasting blood glucose [51]. Skeletal muscles, liver and adipose tissues are the major drivers of whole-body insulin resistance in T2D. Thus, accumulation of visceral fat mass because of excessive calorie intake, sedentary lifestyle and other genetic/non-genetic or environmental factors may drive the progression of T2D in susceptible individuals.

In this pilot study, we evaluated overall glucose and insulin tolerance profiles, fasting blood glucose (FBG) levels and pathological alterations in *in utero* arsenic exposed animals. Our data suggest that preconception and *in utero* exposure to moderate levels of arsenic significantly disrupts the metabolic tolerance of the animals resulting in a prediabetic phenotype when fed a balanced laboratory diet. There were significant pathological changes in the visceral at mass, indicating adipocyte hypertrophy and hyperplasia. Recent findings suggest low- and moderate-dose arsenite exposure induces lipolysis and impairs adipogenesis [19]. Mechanistic studies indicate a dose-dependent inhibition of adipocyte differentiation, altering critical pro-adipogenic programming. Increased ectopic lipid deposition in both the liver and skeletal muscle was reported as an effect of arsenic, which may possibly promote to the advancement of insulin resistance [19]. This is the first study to show that *in utero* exposure to iAs induces diet-induced obesity and type 2 diabetes in laboratory mice. Our data does not supply mechanistic approach for obesogenic effects of iAs. Nevertheless, earlier studies have established that iAs-III inhibits signal transduction mechanisms that are responsible for adipocyte differentiation [52,53]. Thus, it is plausible that, during the gestation period, exposure to arsenic creates a metabolic imprint in the fetal genome/epigenome which induces the increased adiposity and storage of fat in form of WAT in adult life.

Our findings of high glucose tolerance, insulin resistance and dysregulation in liver glycogen deposition and adipose tissue dysregulation are consistent with the initial stages of type 2 diabetes. An increase in the body weight was indicative of the prediabetic/obese phenotype of the animals. The shape of the OGTT curve also changed, showing high blood glucose levels already at 15–30 min after the glucose dose. iAs exposure may affect hepatic metabolism of glucose as this period shows postprandial uptake of glucose from portal circulation by the liver [54,55].

Various epidemiological studies have set up the link between exposure to arsenic and liver disease and kidney failure [56,57]. The most specific characteristic seen in our study in the kidney was glomerular damage and inflammatory cell infiltration. Kidney continuously excretes arsenic through urine, which may result in deposition of the residual arsenic and its further consequences leading to the architectural changes of kidney [58]. Various *in vivo* and *in vitro* studies prove that exposure to arsenic may lead towards development of glomerular sclerosis and tubular necrosis [59,60], increased oxidative stress and DNA oxidative damage [61–63] and increased inflammation (Escudero-Lourdes et al., 2010).

It can be concluded that *in utero* exposure to arsenic is responsible for reprogramming the metabolic regulators which leads to the development of various histological alterations in visceral white adipose tissue, liver and kidney. These histological changes are the outcome of the arsenic induced dysregulation in the metabolic pathway which need to be further studied for better understanding of the underlying mechanism.

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