Comparative Analysis of Arsenic Levels in the Blood of Arsenic Induced Female Mice and Its Correlation with Ovarian Cancer Patients

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Abstract : Arsenic is noxious metalloid that subsist everywhere in the surrounding and disturb worldwide health issue due to its carcinogenicity. Progressive increase of arsenic-related diseases and various types of cancer incidences were reported for the last few years in Bihar. Our work has focused on the toxic effects of arsenic exposure in the blood of mice and women. Female Swiss albino mice were selected as the experimental animals. In the treatment group 1.8 mg/kg body weight sodium arsenite and in control group distilled water was given orally by gavage method daily for 1 month, 2 months, 3 months, 4 months and 5 months. After the treatment of mice, blood samples were collected. Normal women and arsenic exposed ovarian cancer patients from arsenic affected districts of Bihar were selected as human model and blood samples were collected with their prior consent of the subjects. The levels of sodium arsenite of blood of all arsenic treated mice groups were significantly ($p < 0.05$ for Group A and for rest of the Groups, $p < 0.01$) higher than that of the control mice group. The arsenic levels were significantly ($p < 0.05$ for Bhagalpur district and for rest all districts $p < 0.01$) higher in ovarian cancer patients from all sample districts as compared to the normal group of women. Thus, the present work reveals the toxic effect of arsenic exposure in the blood of Swiss albino mice and women of arsenic hit districts of Bihar.

IndexTerms **– Sodium Arsenite, Swiss Albino Mice, Blood, Ovarian Cancer Patients.**

I. INTRODUCTION

According to [1,2] arsenic is a serious worldwide health concern around the earth broadly based on its carcinogenic potential after occupational or environmental exposure. Generally the presence of arsenic has been found in soil, water and air. The arsenic enters into the human body through different processes i.e., inhalation, ingestion and skin absorption. The ingestion of polluted food or water is the primary route of arsenic exposure for the most of the population. [3] reported that for the general population, inhalation or skin absorption of arsenic from contaminated sources was a less exposure route.

[4] reported that arsenic polluted drinking water is a worldwide health concern. According to [5,6] a naturally occurring element, inorganic arsenic can dissolve in groundwater and lead to human exposure if the polluted water level is used as a source of drinking water or to irrigate crops that accumulate arsenic such as rice. This is extremely problematic in Bangladesh, where public health interventions intended to decrease the occurrence of waterborne disease switched the primary drinking water source from surface water to groundwater[7] . [8] evaluated that 46% of the population in Bangladesh is exposed to arsenic concentrations above the World Health Organization's drinking water recommendation of 10 µg/L, and 27% are exposed to levels above the Bangladesh government's recommendation of 50 µg/L. According to [9] there are at least 19 other countries, including Taiwan, Mexico, Chile, Argentina, Vietnam, Laos, India, China, Romania and the United States, that have level of groundwater that are naturally contaminated with arsenic at levels exceeding the World Health Organization drinking water recommendation. Many of these water levels are positioned below densely populated regions, leading to millions of people being chronically sensitive to arsenic as reported by[4].

Ingested arsenic is absorbed by villi of gastrointestinal tract and then transported into the blood via blood capillaries. Inhaled arsenic is absorbed by the lungs and trasported into the blood. Finally, the arsenic present in the blood, is transported through the transport mechanism of plasma membrane into the cells of the lungs, liver, spleen, kidney, intestine, skin, vascular and lymphatic systems, as well as reproductive and nervous systems as explained by[10,3]. After absorption through the lung and the gastrointestinal tract, 95 to 99% of the arsenic is accumulated in erythrocytes, bound to the globin of haemoglobin and is then transported to the other parts of the body. About 70% of the arsenic is discharged mainly through urine. Most arsenic entered into the body is converted by the liver to less toxic methylated form that is efficiently excreted in the urine as reported by[11,12]. According to [13] chronic oral exposure to inorganic arsenic (0.05-0.1 mg/kg/day) causes neurological and hematological toxicity in humans but not in monkeys, dogs, and rats exposed to arsenite or arsenate at doses of 0.72 to 2.8 mg/kg/day.

Thus, it is clear from the above literature that arsenic has diverse effects on the various system of mice, rat, goat, and humans. But very few literatures are available on the impact of arsenic on the ovary and the blood in mice and human.

II. MATERIALS AND METHODS

In the present experiment, 3 months old normal female Swiss albino mice (*Mus musculus*) were selected. These mice were kept in the polypropylene cages containing paddy husk at temperature 26 ± 2°C; the humidity was maintained at 50 ± 10% and in controlled light (12 hrs light and 12 hrs dark). Animals were maintained in ideal conditions as per the ethical guidelines of the CPCSEA, (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008) Government of India and Institutional Animal Ethics Committee (IAEC). All the mice were segregated into two groups, each group containing five mice: a control group and arsenic treated group. The inorganic form of arsenic, sodium arsenite (Sigma) was administered to the arsenic treated mice group (except the control group) at the dose of 1.8 mg/kg body weight for 1 month, 2 months, 3 months, 4 months and 5 months by gavage method.

Patients were enrolled from Mahavir Cancer Sansthan & Research Centre (MCSRC), Patna, India, after the Ethical clearance from the Human Ethical Committee, MCSRC, Patna. The consent of the ovarian cancer patients were taken prior for the purpose of the

study and they were selected for further research work as per their inclusion and exclusion criteria. Blood samples were collected from normal women and arsenic exposed ovarian cancer patients, who came from Arsenic hit districts of Bihar, such as Munger, Bhagalpur, Vaishali, Patna & Buxar for their treatment at Mahavir Cancer Sansthan, Patna, with their prior consent of the subjects.

The blood samples were digested with an acid mixture containing Triton 'X' solution and nitric acid in the ratio of 1:10, over a hot plate at 200°C. Digested mixture was cooled and the final volume was made up to 10 ml with diluted nitric acid. The estimation was analyzed by atomic absorption spectrophotometer (Perkin Elmer; CT06481-7794, U.S.A.) with graphite furnace.[14,15]

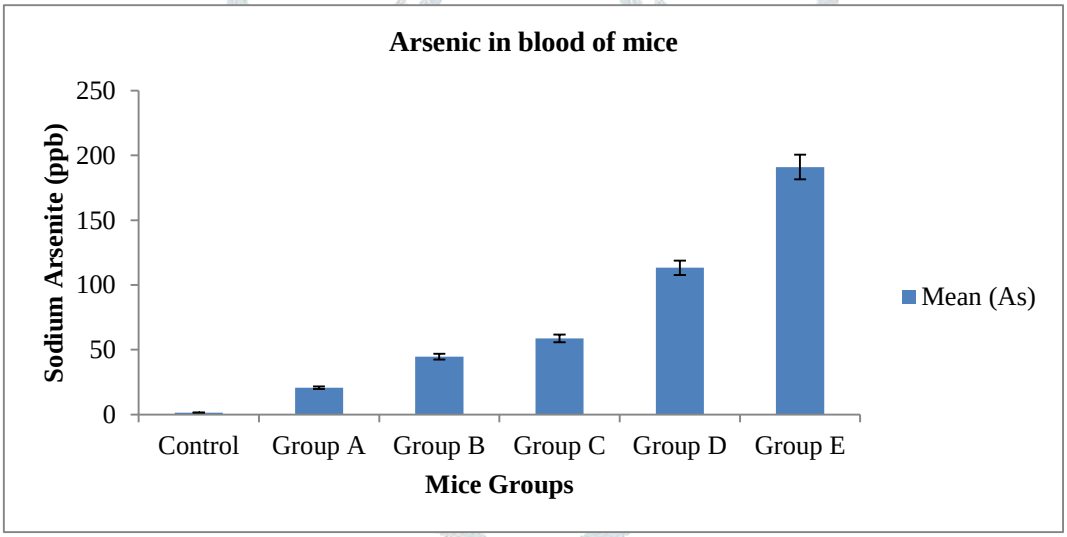
III. STATISTICAL ANALYSIS

For two groups comparison, independent samples Unpaired 't'-test was used (GraphPad Software, USA). Level of significance between the groups was considered at $p < 0.05$ and $p < 0.01$ in mice as well as in women.

Results

Table-1: Sodium arsenite levels in the blood of control and Sodium arsenite treated female *Mus musculus*

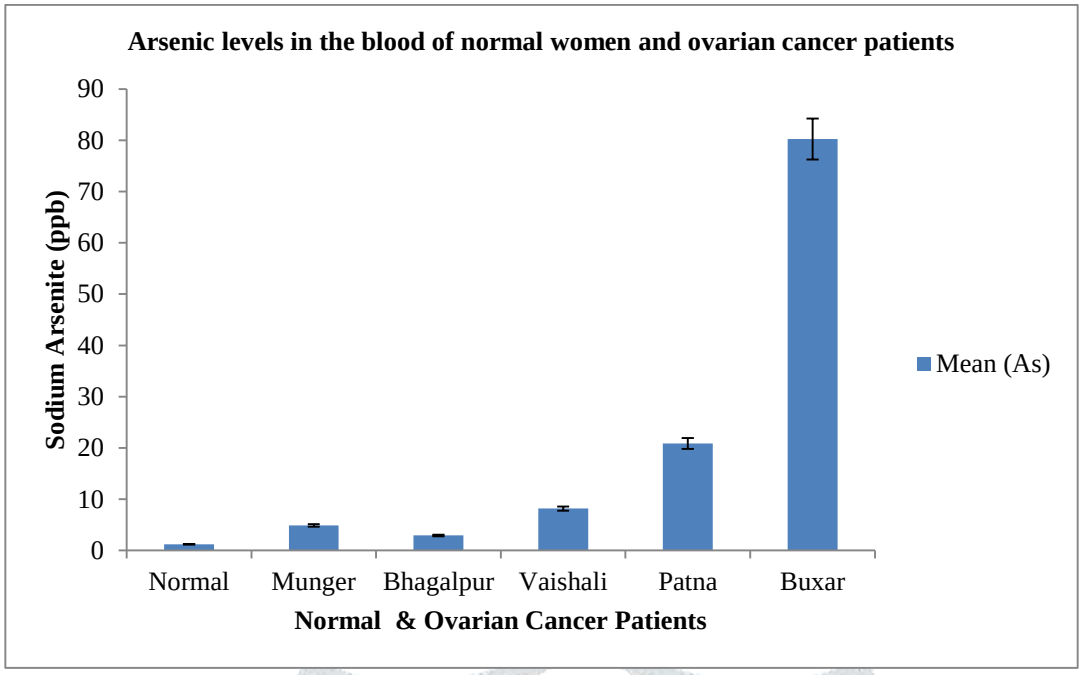
Groups	Dose Duration	Sodium arsenite levels (ppb) Mean ± SD	p-Value
Control	Control	1.39 ± 0.16	
A	1 month	[a]20.66 ± 9.56	p < 0.05
B	2 months	[b]43.99 ± 7.98	p < 0.01
C	3 months	[b]58.69 ± 7.01	p < 0.01
D	4 months	[b]113.22 ± 8.45	p < 0.01
E	5 months	[b]190.01 ± 15.87	p < 0.01



Graph-1: Showing the mean arsenic concentration in the blood of control and arsenic-treated groups of Swiss albino mice after 1 month (Group A), 2 months (Group B), 3 months (Group C), 4 months (Group D) and 5 months (Group E) respectively.

Table-2: Arsenic levels in the blood of normal group of women and ovarian cancer patients from five arsenic hit districts

Groups/Districts	Arsenic levels (ppb) Mean ± SD	p - Value
Normal	1.2 ± 0.06	
Munger	[b]4.89 ± 1.97	p < 0.01
Bhagalpur	[a]2.92 ± 1.17	p < 0.05
Vaishali	[b]8.18 ± 1.89	p < 0.01
Patna	[b]20.86 ± 5.03	p < 0.01
Buxar	[b]80.24± 10.06	p < 0.01



Graph-2: Showing the mean arsenic concentrations in the blood of normal women and ovarian cancer patients of arsenic hit districts of Bihar, namely, Munger, Bhagalpur, Vaishali, Patna and Buxar.

Sodium arsenite levels in the blood of Swiss albino mice of control group and sodium arsenite administered groups are recorded in Table 1 and depicted in Graph 1. In the Graph, values are expressed as mean ± SD (n = 5). The sodium arsenite level in the blood of control group of mice was observed to be 1.39 ± 0.6 ppb. After treatment of mice with sodium arsenite, the sodium arsenite levels in their blood consistently increased with the duration of the treatment. After 1 month of treatment for Group A it was observed to be 20.66 ± 9.56 ppb ; after 2 months of treatment for Group B it was 44.55 ± 8.46 ppb; after 3 months of treatment in Group C it was 58.69 ± 7.01 ppb; after 4 months of treatment in Group D it was 113.22 ± 8.45 ppb; after 5 months of treatment in Group E it was 191.02 ± 16.17 ppb. "t'' test was performed to compare the levels of sodium arsenite in the blood of mice of control group on one hand and the levels in the blood of mice of different arsenite treated groups on the other hand. It was observed that the levels of sodium arsenite of blood of all arsenic treated groups were significantly ($p < 0.05$ for Group A and for rest of the Groups, $p < 0.01$) higher than that of the control group. Its level kept on increasing with the duration of the treatment.

Arsenic concentrations in the blood of normal women group and ovarian cancer patients groups are recorded in Table 2 and depicted in Graph 2. The concentration of arsenic was observed to be 1.2 ± 0.06 ppb in the blood of normal group of women. The arsenic levels in the blood of ovarian cancer patients were found to be 4.89 ± 1.97 ppb for patients from Munger district; 2.92 ± 1.17 ppb for patients of Bhagalpur district; 8.18 ± 1.89 ppb for patients of Vaishali district; 20.86 ± 5.03 ppb for patients of Patna district; and 80.24 ± 10.06 ppb for patients of Buxar district. The data in Graph-2 were recorded as mean ± SD (n = 10). For the comparison of two-groups, i.e., normal women with arsenic exposed ovarian cancer patients, the level of significance for 't' test was $p \leq 0.05$. The arsenic levels were significantly ($p < 0.05$ for Bhagalpur district and for rest all districts $p < 0.01$) higher in ovarian cancer patients from all sample districts as compared to the normal group of women from those districts. The level was the highest (80.24 ± 10.06 ppb; more than 66 times that of the normal group) for ovarian cancer patients from Buxar District.

IV. Discussion

The work [16,17,18,19] stated that placental exposure in mice through maternal ingestion of sodium arsenite in the drinking water during pregnancy was responsible for causing genetic mutation in the liver, adrenal, and the ovary in three separate studies of the female. [3] reported that the exposure of arsenic in the common population from polluted origins was by ingestion, inhalation or skin absorption. After absorption through the lung and the gastrointestinal tract, 95 to 99% of the arsenic is stored in RBCs, then it is bound to the globin of haemoglobin and after that, it is carried to the other parts of the body. About 70% of the arsenic is excreted mainly through urine. On the basis of the report of [11,12] most arsenic intake

into the body is reformed by the liver to less harmful methylated form that is fully excreted in the urine. According to[2,20,16], arsenic is a multi-site carcinogen in humans, causing tumors in tissues including lung, skin, liver, kidney, and bladder, as well as uterus and prostate.

In the present study, the author found oral administration of Sodium arsenite absorbed by the villi of the gastrointestinal tract (GIT) into the blood. It is supported by[21,22], that the most ingested and inhaled arsenic is well absorbed through the gastrointestinal tract and lung into the blood. Graph 1 showed arsenic concentration in the blood of Swiss albino mice. Arsenic levels were significantly ($p < 0.05$ and $p < 0.01$) increased in the arsenic-treated group of mice compared with the control mice group as recorded in Graph 1. The author's results showed the blood concentrations of arsenic diffused into the ovary, which is possibly via the liver. This is supported by[10,3,23], who have reported that arsenic enters into the human body through ingestion and inhalation and is diffused in organs including the lung, liver, spleen, kidney, intestine, skin, vascular system, lymphatic system as well as reproductive and nervous systems.

In the present study Graph 2 showed increased arsenic levels in the blood of ovarian cancer patients from arsenic hit districts. The level of arsenic significantly ($p < 0.05$ and $p < 0.01$) increased in ovarian cancer patients of arsenic hit districts of Bihar compared with normal women. The Author's data are supported by[24], who stated that the arsenic concentration was found in the human blood and it was responsible for the toxicity of RBCs. [25] explained that arsenic bound with the globin of haemoglobin. The author's findings are also supported by[26], who said that arsenic concentration was found in the human blood of Bangladesh due to the contamination of groundwater. A world leader in telecommunications, Bose was a significant figure behind the creation of modern radio and sonic technology. In 1896 his work was commemorated by IEEE as the oldest "milestone achievement" from Asia. In 1997 the Institute of Electrical and Electronic Engineers of America named Bose as a "Father of Radio Science" [27].

V. CONCLUSION

As a naturally occurring metalloid, arsenic is a dangerous environmental concern globally, as a result of a large number of known poisoning sites and millions of people exposed to drinking arsenic- contaminated water. A better awareness of the mutagenic/carcinogenic mechanisms of arsenic will give a basis for the better intermediary approach in both treatment and prevention. Arsenic-induced creation of ROS and successive reduction of antioxidant cell protections can result in interruption of the antioxidant equilibrium in mammalian tissues. As its sulfhydryl group binding capacity, arsenic can also prohibit the activities of several enzymes, notably those elaborated in the uptake of glucose in cells, fatty acid oxidation and production of glutathione. The toxic and carcinogenic effects on humans exposed to arsenic have been well reported, however, the mechanisms by which arsenic causes health effects, including cancer, cardiovascular disorders, metabolic disease and some other diseases are not well described. To give a deep knowledge of the pathology of arsenic-induced diseases and the toxicology of arsenic in many organs, further study is necessary.

Arsenic is a major environmental pollutant that disturbs the reproductive and developmental toxicity. These toxic results are affected by the forms, sources, and routes, as well as doses and periods of arsenic risk. In vivo investigations proved that inorganic arsenic, like sodium arsenite, arsenic trioxide, and arsenic metabolite, cause reproductive and developmental toxicity. After all, the reproductive and developmental toxicity of arsenic is poorly accepted, and the molecular mechanism of arsenic-induced reproductive toxicity remains not clear. Thus, we more studied some of the probable mechanisms that are concerned by arsenic inducing reproductive toxicity.

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