Ground Water Arsenic Poisoning in “Tilak Rai Ka Hatta” Village of Buxar District, Bihar, India Causing Severe Health Hazards and Hormonal Imbalance

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Abstract

In the recent times, arsenic poisoning due to contaminated ground water in the middle Gangetic plain has resulted to lots of health related problems in the population. In Bihar (India), about 16 districts have been reported to be affected with arsenic poisoning. In the present study, Tilak Rai Ka Hatta village, a flood plain Diara region of river Ganga in Buxar district was targeted for the ground water arsenic assessment and health related problems assessments among the village population. Altogether, 120 water samples were randomly collected for arsenic estimation and their results were statistically analysed. While for assessment of health related problems in the village people, 120 biological samples (blood) were collected from the same households. The study showed high contamination of arsenic in the ground water as three fourth region of the entire village had arsenic levels more than 100 ppb. The typical symptoms of hyperkeratosis in the palm and sole, melanosis of the skin in the village people were highly prevalent. In the population, there was prevalence of cancer and infertility cases also. The assessment of blood samples exhibited severe hormonal imbalance among the village population. Present study thus concludes that, arsenic poisoning in entire village has caused severe health hazards to the village population. It has not only caused skin diseases or cancer but the entire population has threat over the infertility due to hormonal imbalance. So, a proper strategy is immediately required to cater the severity of the arsenic poisoning in this village.

Keywords: Arsenic poisoning; Tilak Rai Ka Hatta village; Buxar district; Ground water; Health assessment; Hormonal imbalance

Introduction

In the recent times, the potable water demand in urban as well as rural areas has increased a lot due to fast growing population in India. The Upper, Middle and Lower Ganga flood plains are the most severely dense populated areas of India. The entire land area is highly fertile and agricultural practices are the main occupation of the village people. The primary states of the upper, middle and lower Ganga plains are Uttarakhand, Uttar Pradesh, Bihar and West Bengal. The upper Ganga plain areas are from the states of Uttarakhand and Uttar Pradesh; middle Ganga plain areas are from the Eastern Uttar Pradesh and Bihar state, while the lower Ganga plain covers the entire West Bengal state [1].

The present study area is located in Buxar district which is south to the Middle Ganga plains and these areas are densely populated since the pre-historic times because of its religious importance as well as the highly fertile agricultural land.

Arsenic is abundant in the crust of the earth and is found in all environments. It is found in soil, minerals, surface and groundwater. Both natural and anthropogenic sources are responsible for the distribution of it throughout the environment [2]. The excessive withdrawal of ground water for agricultural practices was well practiced in 1990s which lead to geological changes in Gangetic plain. This lead to the lowering down of the water table in summer season and oxygen to enter into the aquifer causing oxidation of arsenic rich iron sulphide and then contaminating the entire aquifer with arsenic [3-5].

Today, it is estimated that more than 5 million people in the Bihar state are drinking water with arsenic concentrations greater than 50 μg/l [6] and presently the ground water arsenic contamination has spread to 16 districts of the state [6-9]. In India, problems with arsenic in groundwater in West Bengal (Lower Ganga plain region) were first recognised in the early 1980s and the health effects are now reasonably well documented [1,10,11]. Due to groundwater arsenic contamination, a large number of populations in the arsenic hit area are suffering from melanosis, leuco-melanosis, keratosis, hyperkeratosis, dorsum, non-petting oedema, gangrene, skin cancer and skin lesions in sole and palm [12-16]. Tubewell revolution since 1980’s, has led to serious arsenic menace causing severe health hazards in the population in the recent years [17,18]. The village people of these contaminated regions are still drinking arsenic contaminated water and are not aware of this fact as well as its consequences. The demographic evaluation of arsenic in the ground water has not been done extensively especially in the Buxar district where arsenic poisoning in ground water has a meagre reporting.

The Buxar district is situated between 25° 18’ to 25° 45’ latitude North & 84° 20’ to 84° 40’ longitude East. Its geographical area is 1624 km². The total population of district is 10,87,676 (Rural 9,96,855 Urban 90,821). The population density is 621 person/km² and sex ratio 899 females/1000 males. Buxar is also the most affected arsenic hit area especially the villages located near the river Ganga. Although, the Government funded works are being carried out in almost all the arsenic hit districts of Bihar but, still people are deprived of arsenic free drinking water and food, as this land area is highly fertile where crops of all types are extensively cultivated. Among all the villages of Buxar...
district, Tilak Rai Ka Hatta village in Simri block is the most severely affected area where no recent health related evaluation has been done in the population. Regular health related problems in the village was in the news has ignited the entire team to study this village.

**Materials and Methods**

**Location**

The study was done at Tilak Rai Ka Hatta village (a flood plain region) of Simri Block of Buxar district (25° 41’36”N, 84° 07’51”E). The population of the village was approximately 5,348 in 2011 (Census, 2011 Interim report) [51]. There were approximately 340 households in 2011 (Census, 2011 Interim report). The village bears only a single Primary Health Centre and there is no availability of health check up related facilities for the villagers.

**Arsenic analysis and survey**

The water sample bottles (500 ml polypropylene bottles) were well cleaned and pretreated with 1/10 N hydrochloric acid. 120 water samples were randomly collected in duplicate from hand pumps of the each households situated at every 50 metres of distance in the village and the depth of the handpumps were also recorded. After the collection, water samples were analyzed instantly by utilizing Merckcoquant Arsenic Field test kit (Merck, Germany). For the final confirmation of the same, water samples were analysed using ThermoFisher U.V. Vis- Spectrophotometer following the SDDC (Silverdiethyledithiocarbamate) method against a blank at 520nm.

A survey in the entire village was conducted to estimate the per capita consumption of drinking water through hand pumps by the village people. For this, a questionnaire method was utilised and elderly, adults, youths and children of the village (1530 people) were interrogated related to consumption of drinking water from hand pumps, the depth of the hand pumps and their health related problems. The health related data were extensively collected to know the root cause of their health related problems. The blood samples of 120 village people were collected, where the arsenic contamination in hand pumps were very high. From blood, serum was separated for hormonal assessment of Testosterone, Estrogen and Thyroid hormones by their respective hormone kits. For determining the exact location of the hand pump, hand held global positioning system (GPS) receivers with an estimated accuracy of ± 10 m were utilised.

**Hormonal assessment**

**Testosterone assay**: Using the ELISA method Testosterone kit of AccuBind, Monobind, Lake Forest, CA, USA was utilized for the experiment. The normal range was calibrated and then 25 μl serum samples were taken in the microwell plates. 100 μl of estradiol conjugate was added in each well. After that, it was left for incubation at 37°C in incubator for 1 hour. Then, the wells were washed with 300 μl distilled water for at least 3 times and blotted dry. Then, 100 μl substrate solution was added in each well and was again left for the incubation for 20 minutes for the colour development. Finally, 50 μl stop solution was added in each well to stop the reaction. Reading was taken at 450nm through Merck ELISA reader in pg/ml value.

**Estrogen assay**: Using the ELISA method Estradiol (E2) kit of AccuBind, Monobind, Lake Forest, CA, USA was utilized for the experiment. The normal range was calibrated and then 25 μl serum samples were taken in the microwell plates. 50 μl of Estradiol Biotin reagent was added into each well. After that, this was left for incubation at 37°C inside incubator for 30 min. 50 μl of Estradiol Enzyme reagent was added in each well. Then it was left for incubation at 37°C inside incubator for 90 min. Then, the wells were washed with 300 μl distilled water for at least 3 times and blotted dry. Then, 100 μl substrate solution was added in each well plate and was again left for the incubation for 20 minutes for the colour development. Finally, 50 μl stop solution was added in each well to stop the reaction. Reading was taken at 450nm through Merck ELISA reader in pg/ml value.

**Thyroid stimulating hormone (TSH) assay**: Using the ELISA method Thyrotropin (TSH) kit of AccuBind, Monobind, Lake Forest, CA, USA was utilized for the experiment. The normal range was calibrated and then 50 μl serum samples were taken in the microwell plates. 100 μl of TSH Enzyme reagent was added in each well. After that, it was left for incubation at 37°C inside incubator for 60 min. Then, the wells were washed with 300 μl distilled water for at least 3 times and blotted dry. Then, 100 μl substrate solution was added in each well and was again left for the incubation for 15 minutes for the colour development. Finally, 50 μl stop solution was added in each well to stop the reaction. Reading was taken at 450nm through Merck ELISA reader in μIU/ml value.

**Statistical analysis**: Data were analyzed with statistical software (Graphpad Prism 5) and values were expressed as Mean ± SEM. Differences between the groups were statistically analyzed by one-way analysis of variance (ANOVA) using the Dunnet’s test.

**Results**

**Ground water arsenic assessment in Tilak Rai Ka Hatta village**

The Tilak Rai Ka Hatta is situated near the vicinity of the river Ganga and is the flood plain region of the river (Figure 1). The analysis of 120 water samples from the entire village represented high prevalence of arsenic contamination in ground water as the entire region showed very unique pattern of arsenic contamination in ground water (Figures 2 and 3). The average depth levels of the hand pumps was between 60 to 100 feet (Figure 4) and the arsenic contamination in hand pumps was also highly prevalent in these depths only.

**Health Assessment**

The west region of the village showed high concentration of arsenic in the ground water. The severity of the health related problems was so high that the prevalence of common symptoms of arsenicosis were widely observed in the population of the village. The male and the females showed typical arsenicosis symptoms as hyperkeratosis in their palms as well as in their soles. The severe skin pigmentation that is melanosis in palm and trunk was observed in this particular region (Figures 5, 6 & 7). The commonly observed health related problems in the context of population prevalence was well evaluated in 1530 village people (Table 1). The percentage of skin related problems was hyperkeratosis in palm and sole (28%), melanosis (31%) while in gastrointestinal problems – gastritis (86%), liver related problems (57%), loss of appetite (64%) were observed. The reproductive health in the population was also hampered as male infertility (azoospermia) cases were reported in 9 males while, 6 females reported female infertility case. 137 (9%) females had irregular menstrual cycle denotes the level of health related problems in them. 6 cancer cases were also observed, out of which 4 had died earlier while 2 are still suffering, but none of them tried to had treatment of their cancer in a cancer centre. The cancer cases reported were with Gall bladder – liver cancer and skin cancer cases. The Primary and Middle School children of the village were observed with the severe symptoms of arsenicosis, as hyperkeratosis in palm and sole in 10% (n=250) of
Hormonal Assessment

The hormonal assessment showed declination in the serum testosterone levels. The most fascinating result was that out of 36.6%
(n=30) cases showed severe male infertility as their testosterone values were below 2.0 ng/ml. 2 male subjects also showed the testosterone levels below 0.5 ng/ml (Figure 8). Similarly, in females blood samples collected in their premenopausal phase of their menstrual cycle reported [100% (n=20)] menstrual problems and same females also exhibited symptoms of arsenicosis on their palm and sole (Figure 9). The thyroid abnormality was common in village people as it was well observed in population 48.14% (n=54) which exhibited elevated TSH levels (Figure 10).

Discussion

In the recent times, arsenic has led to severe health hazards in the Indian population. Although, the first case of arsenicosis was observed in West Bengal in 1980’s [16,18]. The arsenic concentration in ground water had caused severe health related problems in the lower Ganga plains and since 2001 in the lower middle Ganga plain area melanosis, leucomelanosis, keratosis, hyperkeratosis, dorsum, non- petting
symptoms and their percentage in the village population of 1530.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No Problems observed</th>
<th>Total Cases</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperkeratosis in palm and sole</td>
<td>1102 (72%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Melanosis in Palm and trunk</td>
<td>1057 (69%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Other skin problem – irritation</td>
<td>1177 (77%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Anaemia</td>
<td>658 (43%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gastritis</td>
<td>215 (14%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Liver problem</td>
<td>643 (42%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Constipation</td>
<td>934 (61%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>551 (36%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Infertility in male and female</td>
<td>1515 (99%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Irregular Menstrual Cycle</td>
<td>1397 (91%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Asthma or lung problem</td>
<td>1485 (97%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cancer cases</td>
<td>1524 (99.6%)</td>
<td>1530</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 1: Showing Arsenic caused common symptoms and their percentage in the village population of 1530.

The World Health Organisation (WHO) and the U.S. Environmental Protection Agency (EPA) have well recommended threshold of 10ppb for inorganic arsenic concentration in drinking water [19,20]. Unfortunately, millions of people are exposed to toxic levels of Arsenic and are at higher risk for the severe adverse health effects caused by it [21-24]. Although, cancer death risk is associated with daily consumption of 1.6 litres of water with inorganic arsenic (50ppb) has been estimated to be 21/1000 [25]. Arsenic has also been classified as Class I carcinogen by the International Agency of Research on Cancer (IARC) which means that there is sufficient evidence of its carcinogenicity to humans. Skin and several types of internal cancers, including, bladder, kidney, liver, gall bladder, lung and prostate have been found associated with arsenic ingestion [26-30].

In the present study cancer cases were also reported in few numbers.
– skin cancer and Gall bladder cancer. The probability of these two types of cancer cases reported is due to ingestion of arsenic contaminated drinking water that is more than 300 ppb. The common reason for cancer cases is due to the carcinogenic effect of the transformed arsenic into Dimethyl Arseninous Acid (DMA). Furthermore, arsenic is not a direct acting genotoxin or mutagen but elevates the degree of DNA damage and mutations by altering DNA repair [30,31], or also acts as a co-carcinogenic tumor promoter and progressor [32]. Increased generation of oxidative stress by reactive oxygen species (ROS) has led to oxidative signalling, altered cell signalling through other mechanisms, altered cell cycling and apoptotic and differentiation responses, and other mechanisms [33-43].

In this study, peculiar cases of male and female infertility was observed which was also confirmed by the hormonal assessment which showed declined levels of testosterone and elevated levels of estrogen hormones. The study is one of the unique studies ever done in the arsenic hit area of Buxar district of Bihar. It has been well reported that arsenic acts as a potent endocrine disruptor, altering gene regulation by the closely related steroid hormone receptors for glucocorticoids, mineralocorticoids, progesterone, and androgen in a similar manner [44,45]. Furthermore, in the presence of arsenic, the activated receptor is unable to stimulate the correct cascade of signals that usually results from hormone binding, particularly the ability to turn on certain hormone-responsive genes. Metals have not previously been shown to act as endocrine disrupters. Blocking the actions of the glucocorticoid receptor by arsenic in this unique way could explain, at least in part, many of the health effects observed in arsenic-exposed human populations [46-48].

The increased levels of thyroid stimulating hormones (TSH) in the population of the surveyed village are directly correlated with the ingestion of arsenic contaminated drinking water. The population showed clear symptoms of hyperthyroidism as their serum TSH levels were elevated. No proper mechanism has been deciphered till date with arsenic and TSH level elevations. But, a recent study has correlated the similar study [49]. The elevated levels of oestrogen and TSH increase the risk of female infertility due to the failure of ovulation [50].

This, unique study is the first ever study done in this huge population in the severe arsenic hit area of Buxar district. Thus, the present study concludes that, arsenic poisoning in entire village has caused severe health hazards to the village population. It is not only causing skin diseases or cancer but the entire population also has threat over the infertility. So, a proper strategy is immediately required to cater the severity of the arsenic poisoning in this village.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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