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Hematological and Free Radicals Changes among People of Arsenic Endemic Region of Buxar District of Bihar, India

Rahman MS[1], Kumar A[2]*, Kumar R[2], Ali M[2], Ghosh AK[2] and Singh SK[1]

[1]*Department of Biotechnology, Anugrah Narayan College, Patna, Bihar, India*
[2]*Department of Research, Mahavir Cancer Institute and Research Centre, Patna, Bihar, India*

Abstract

Groundwater arsenic is developing as a major problem in floodplain of the Ganga. The large scale shift in the water resource allocation from surface water to groundwater in India exposed millions of people to the arsenic through groundwater. Arsenic induced toxicity in human blood cells has been evident from the several cases like Megaloblastic Dyserythropoietic Anemia, Pancytopenia. The present study is aims to know about the toxic effect of arsenic on human health by exploring blood arsenic concentration, some free radical assessment [Malondialdehyde (MDA), Glutathione Peroxidase (GPx)] and hematological parameters (RBC, Hb, HCT, MCV, MCH, MCHC and WBC) evaluation. The subjects belong from our recently explored arsenic hit area of Bihar. The result reveals maximum blood arsenic concentration as 706.1 µg/L while the mean blood arsenic concentration was 83.04 ± 10.60 µg/L in adult male and 70.47 ± 18.49 µg/L in adult female which was calculated corresponding to 228.105 ± 18.942 µg/L mean arsenic concentration in their drinking water. The elevated levels of MDA and GPx represent anti-oxidative stress in the subjects. All the hematological parameters like WBC count, RBC count, hemoglobin percentage and other RBC indices were found at significant abnormal levels. It concludes that arsenic exposure to the population residing in the arsenic hit area leads to hematological changes and low immunity in them and indicating that they are at very high risk.

Keywords: Blood arsenic; Groundwater; Free radicals; Hematology; Buxar district; Simri village

Introduction

The metalloid named arsenic (atomic number 33) is well known for its toxic effects [1,2]. Arsenic, arsenic trioxide, arsenic pentoxide, arsenous acids, arsenic acid and their salts (arsenites and arsenates) are carcinogenic substances (Carcinogen Category 1) [3-6] as well as these also have toxic effects on the epidermal, nervous and the vascular system [7,8]. Groundwater arsenic is developing as a major problem in floodplain of the Ganga. The large scale shifting of the water resource allocation from surface water to groundwater in India exposed millions of people to the arsenic through groundwater. The assessment of groundwater of many areas from West Bengal and Bihar [9-12] have been revealed to be with high arsenic contamination and the exposure of local residing populations to the high arsenic contaminated ground water have resulted in very extreme environmental health conditions [13,14].

Arsenic after ingestion into the human body through groundwater it first encounter with the tract, hepatic system and blood cells and plasma as a channel of transportation to the other organ. Arsenic toxicity is evident in Gastrointestinal and Hepatic system [15-18], Renal system [19], Respiratory system [20], Reproductive system [21] including all vital body systems as described above. Arsenic introduces oxidative damage which further initiates cytotoxicity. Oxidative stress arises when reactive oxygen species (ROS) are generated, which can react with cellular constituents such as thiols and lipids that results in the alteration of the antioxidant defense systems and cell membrane disruption [22,23]. Arsenic interacts with protein sulfhydryls (ProSH) (cell membrane, cytoplasmic proteins, etc.) as well as with nonprotein sulfhydryls (glutathione, cysteine, lipoic acid, acetylCoASH, etc.) in biological systems. Arsenate and arsenite interact with rat red blood cell sulfhydryls that provides the possible mechanism through which arsenic cause damage to the blood cells [24]. Arsenic induced toxicity in human blood cells has been evident from the several cases like Megaloblastic Dyserythropoietic Anemia [25] and Pancytopenia [20]. In chronic arsenic toxicity neutropenia occurs [26].

Presently, arsenic contamination in the groundwater of Bihar is reported from 18 districts, threatening more than 10 million people in the state [27]. The present study is aimed to know about the toxic effect of arsenic on human health by exploring some free radical assessment and hematological parameters evaluation. The subjects belong from our recently explored arsenic hit area of Bihar [11,14].

Materials and Methods

Ethical approval

Ethical approval was obtained from the Institutional Ethics Committee (IEC) of Mahavir Cancer Sansthan and Research Centre, Patna, India with IEC No.MCS/Research/2015-16/2716 Dated 08/01/2016.

Location

The study was conducted in India in Simri and Tilak Rai Ka Hatta village of Buxar district of Bihar (25°38'17.6" N 84°06'49.4" E and 25°41'36" N, 84°07'51" E respectively). The population of the Simri village is approximately 17,670 in 2011 and 2621 households [28]. The population of the Tilak Rai Ka Hatta village is approximately 5,348 and 340 households in 2011 [28]. The Simri village is divided into seven strips named as Bakulaha Patti, Bhan Bharauli, Khaira Patti, Ramo Patti, Halwa Patti, Doodhi Patti, and Gope Bharauli. The Simri village is situated approximately 1.65 km away from the banks of the river

***Corresponding author:** Arun Kumar, Department of Research, Mahavir Cancer Institute and Research Centre, Patna, Bihar, India, Tel: +919334740800; E-mail: arunk31@rediffmail.com

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Ganga. Tilak Rai Ka Hatta village is a flood plain region of river Ganga (Figure 1). Phulwarisharif, Patna (25°34'33"N 85°4'6"E) was selected as control area.

Blood sample collection and analysis

Blood sample collection: 5 ml Blood samples, of each test subjects (n=195) and control subjects (n=20), were taken from the medial cubital vein in the arm using disposable syringes and transferred to EDTA and heparinised vaccutainer as per the guidelines [29]. The blood samples were carried in the ice packs at 4 °C to the laboratory and analyzed within 12 h of collection. A short health related questionnaire was also conducted in between the blood sample collection process.

Blood arsenic estimation: After the collection, all the blood samples were double digested using concentrated HNO_3 on hot plate under fume hood and estimated for arsenic as per the protocol of [30] through Graphite Furnace Atomic Absorption Spectrophotometer (Pinnacle 900T, Perkin Elmer, Singapore).

Lipid peroxidation: Thiobarbituric acid reactive substances (TBARS), as a marker for Lipid Peroxidation (LPO), were determined by the double heating method [31]. This method is a spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this purpose, 2.5 ml of 100 g/L trichloroacetic acid (TCA) solution was added to 0.5 ml serum in a centrifuge tube and incubated for 15 min at 90°C. After cooling in tap water, the mixture was centrifuged at 3000 g for 10 min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/L TBA solution in a test tube and again incubated for 15 min at 90°C. The solution was then cooled in tap water and its absorbance was measured using Thermo Scientific UV-10 (UV-Vis) spectrophotometer (USA) at 532 nm wavelength.

Glutathione peroxidase estimation: Glutathione Peroxidase (GPx) activity was measured by the kinetic assay using the "continuous spectrophotometric rate determination reaction" and expressed as unit per liter in plasma (U/L). One unit of GPx represents 1 µmol oxidized NADPH per min [32,33].

Hematological parameters assessment: The red blood cells (RBC) and white blood cells (WBC) counts were determined by the improved Neubauer hemocytometer method. The hemoglobin (Hb) concentration was determined by using the Sahli's hemoglobinometer. Hematocrit (HCT) was determined directly by microhematocrit tubes. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was computed according to Dacie et al. [34]. All tests were performed in duplicates.

Statistical analysis

Data were analyzed with statistical software (GraphPad Prism 5) and values were expressed as Mean ± SEM. The mean and standard error of mean (S.E.M.) were calculated for all values. Differences between the groups were statistically analyzed by one-way analysis of variance (ANOVA) using the Dunnett's test while scattered graphs were plotted through another statistical software (IBM SPSS Statistics 25) using linear regression analysis method.

Results

Blood arsenic level assessment

Total 195 test blood samples were analyzed from the entire village in which the maximum arsenic concentration in blood sample reported was 704.7 µg/L in adult male and 706.1 µg/L in adult female (Figure 2).

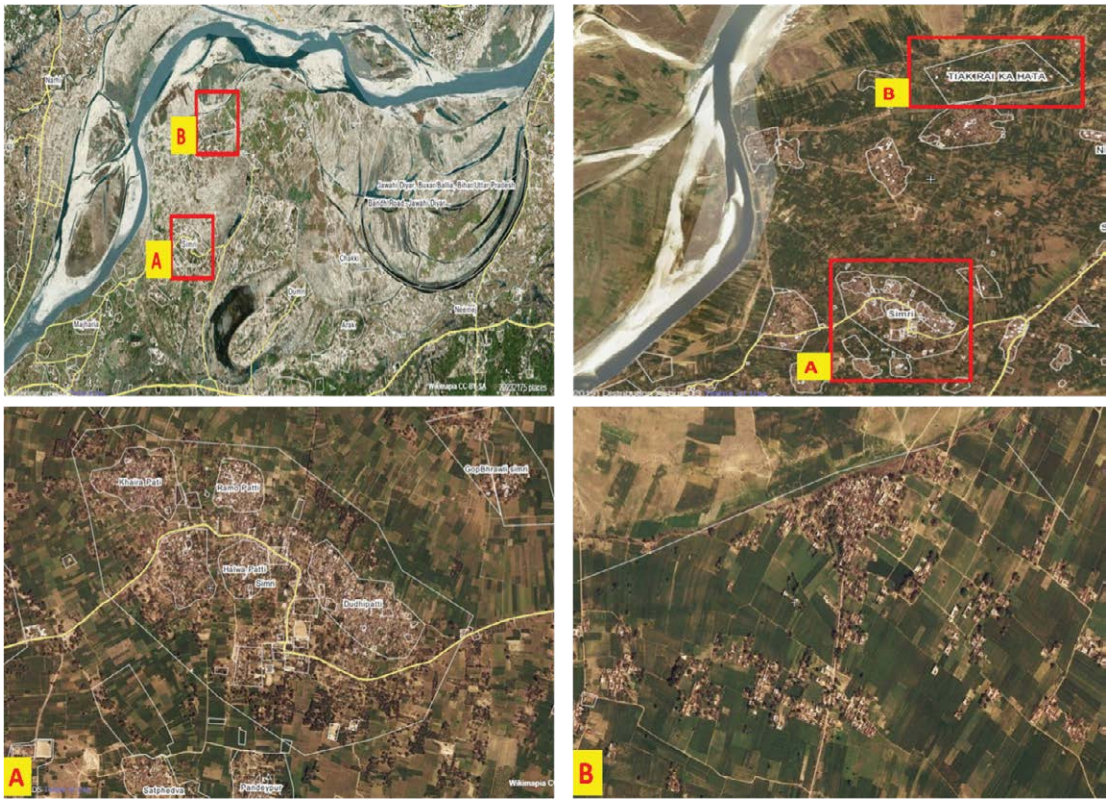

Figure 1: (A) Simri village (B) Tilak Rai ka Hatta village.

Free radicals assessment

Malondialdehyde: Total 195 test blood samples were analyzed from the entire village for malondialdehyde (MDA) levels in which the maximum level reported was 16.00 nmol/ml in adult male and 14.00 nmol/ml in adult female (Figure 3 and Table 1).

Correlation coefficient between blood arsenic level and MDA level of the subjects: The study showed significant positive correlation between blood arsenic levels and MDA levels of the individuals (Males r=0.142 and Females r=0.022, P<0.05, Figure 3).

Glutathione peroxidase: Total 195 test blood samples were analyzed from the entire village for Glutathione Peroxidase (GPx) levels in which the maximum level reported was 418 U/L in both adult male and female (Figure 4).

Correlation coefficient between blood arsenic level and GPx level of the subjects: The study showed significant positive correlation between blood arsenic levels and GPx levels of the individuals (Males r=0.147 and Females r=0.209, P<0.05; Figure 4).

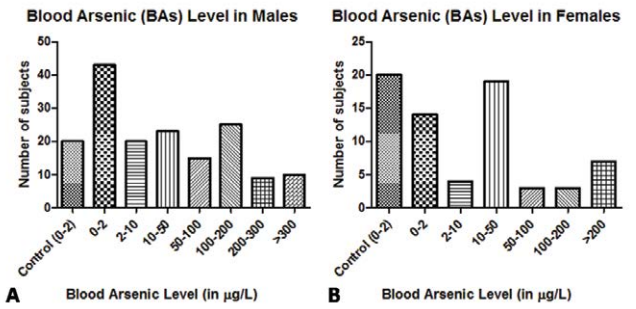

Figure 2: Arsenic concentration in blood samples of village people of Simri and Tilak Rai ka Hatta (A) Adult Males, (B) Adult Females analyzed through GF-AAS (ANOVA-Dunnett's Test, P<0.05).

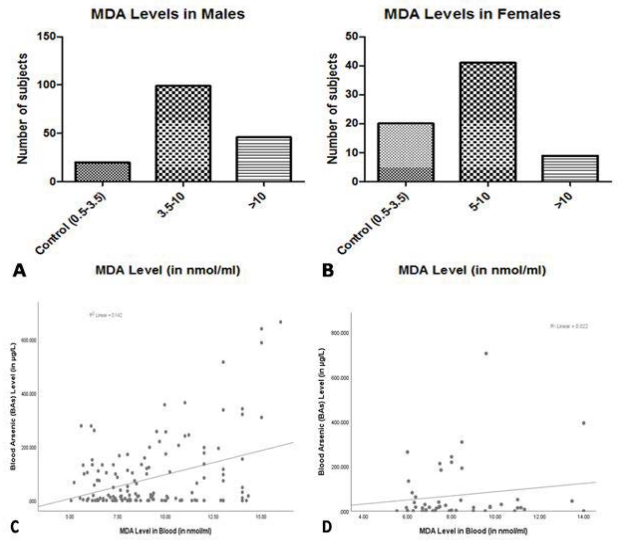

Figure 3: (A & B) MDA levels in blood samples of village people (Males & Females, respectively) of Simri and Tilak Rai ka Hatta (ANOVA- Dunnett's test, P<0.05), (C & D) The correlation coefficient between blood arsenic level and MDA level of the test subjects (Males r=0.142 and Females r=0.022, respectively & P<0.05).

Free Radicals Assessment				
Parameters	Adult Males		Adult Females	
	Control area* (N=20)	Arsenic endemic area** (N=145)	Control area* (N=20)	Arsenic endemic area** (N=50)
Blood Arsenic Level (in µg/L)	2.27 ± 0.11	83.04 ± 10.60	2.25 ± 0.09	70.47 ± 18.49
MDA Level (in nmol/ml)	2.12 ± 0.17	9.23 ± 0.22	2.36 ± 0.21	8.25 ± 0.29
GPx (in U/L)	230 ± 7.51	292.72 ± 6.37	225 ± 8.89	300.14 ± 10.26
*Mean Arsenic level in groundwater of control area=4.272 ± 0.451 µg/L ** Mean Arsenic level in groundwater of Arsenic endemic area=228.105 ± 18.942 µg/L P<0.01 values are mean ± S.E.				

Table 1: Free radicals assessment of the village population of Simri and Tilak Rai ka Hatta.

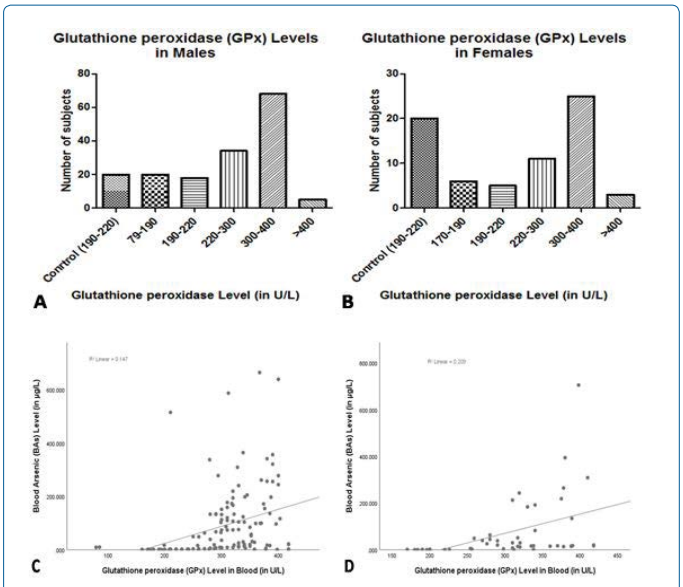

Figure 4: (A & B) GPx levels in blood samples of village people (Males & Females, respectively) of Simri and Tilak Rai ka Hatta (ANOVA- Dunnett's Test, P<0.05), (C & D) The correlation coefficient between blood arsenic level and GPx level of the test subjects (Males r=0.147 and Females r=0.209, respectively & P<0.05).

Hematological assessment

Hemoglobin: Total 195 test blood samples were analyzed from the entire village for Hemoglobin levels in which the minimum level reported was 6.00 g/dL in adult male and 7.00 g/dL in adult female (Figure 5).

Correlation coefficient between blood arsenic level and Hemoglobin level of the subjects: The declining trend was observed between blood arsenic levels and Hemoglobin levels of the male individuals (Males r=0.029 and Females r=0.002, P<0.05, Figure 5).

Red Blood Cell (RBC) Count: Total 195 test blood samples were analyzed from the entire village for RBC levels in which the minimum level reported was 1.21×10^6 µL in adult male and 2.10×10^6 µL in adult female (Figure 6 and Table 2).

Correlation coefficient between blood arsenic level and RBC count of the subjects: The declining trend was observed between blood arsenic levels and RBC count of the individuals (Males r=0.005 and Females r=negative, P<0.05; Figure 6).

Hematocrit (HCT): Total 195 test blood samples were analyzed from the entire village for Hemoglobin levels in which the minimum level reported was 16% in adult male and 19% in adult female (Figure 7).

Correlation coefficient between blood arsenic level and Hematocrit value of the subjects: The declining trend was observed between blood arsenic levels and Hematocrit value of the male individuals (Males r=0.029 and Females r=0.003, P<0.05; Figure 7).

Mean Corpuscle Volume (MCV): Total 195 test blood samples were analyzed from the entire village for MCV value in which the minimum level reported was 47.7 fL in adult male and 55.23 fL in adult female (Figure 8).

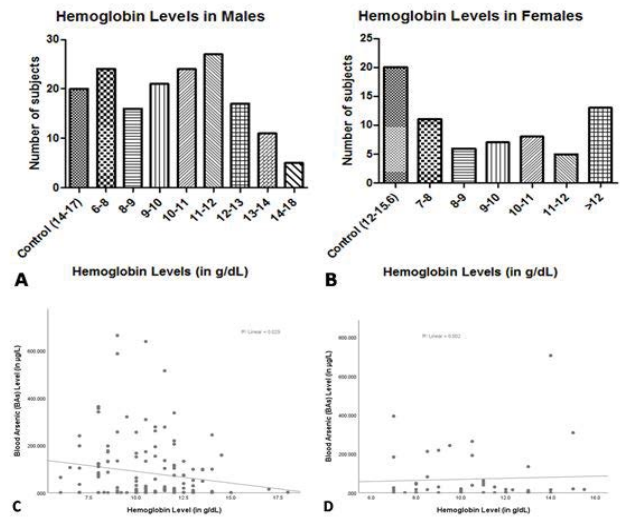

Figure 5: (A & B) Hemoglobin levels in blood samples of village people (Males & Females, respectively) of Simri and Tilak Rai ka Hatta (ANOVA-Dunnett's Test, P<0.05), (C & D) The correlation coefficient between blood arsenic level and Hemoglobin level of the test subjects (Males r=0.029 and Females r=0.002, respectively & P<0.05).

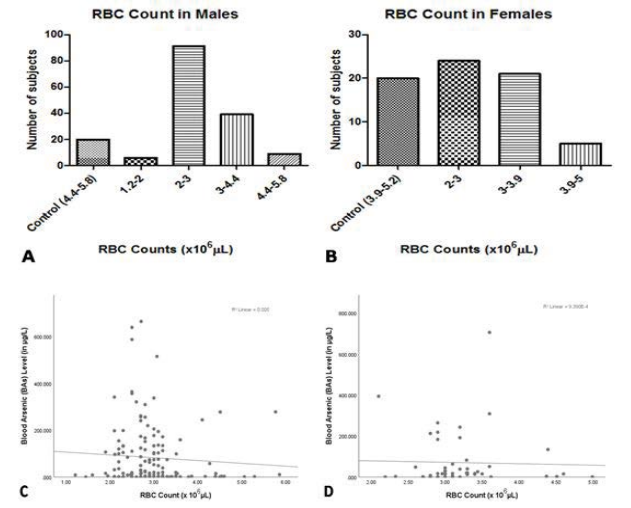

Figure 6: (A & B) RBC count in blood samples of village people (Males & Females, respectively) of Simri and Tilak Rai ka Hatta (ANOVA- Dunnett's Test, P<0.05), (C & D) The correlation coefficient between blood arsenic level and RBC count of the test subjects (Males r=0.005 and Females r=negative, respectively & P<0.05).

Hematological Parameters				
Parameters	Adult Males		Adult Females	
	Control area* (N=20)	Arsenic endemic area** (N=145)	Control area* (N=20)	Arsenic endemic area** (N=50)
WBC (× 10^3 µL)	7.87 ± 0.26	5.73 ± 0.23	6.89 ± 0.29	5.64 ± 0.33
RBC (× 10^6 µL)	4.42 ± 0.13	2.94 ± 0.06	4.16 ± 0.12	3.18 ± 0.08
Hb (g/dL)	14.71 ± 0.22	10.71 ± 0.18	13.52 ± 0.28	10.55 ± 0.33
HCT (%)	41.26 ± 0.47	30.24 ± 0.54	39.41 ± 0.73	29.64 ± 0.95
MCV (fL)	107.65 ± 1.92	106.80 ± 2.53	95.62 ± 2.20	93.95 ± 2.89
MCH (pg)	39.81 ± 0.76	37.83 ± 0.87	35.47 ± 0.82	33.46 ± 1.00
MCHC (g/dL)	38.76 ± 0.21	35.48 ± 0.04	36.54 ± 0.16	35.67 ± 0.08
*Mean arsenic level in groundwater of control area=4.272 ± 0.451 µg/L ** Mean arsenic level in groundwater of arsenic endemic area=228.105 ± 18.942 µg/L P<0.01 values are mean ± S.E.				

Table 2: Hematological parameters of the village population of Simri and Tilak Rai ka Hatta.

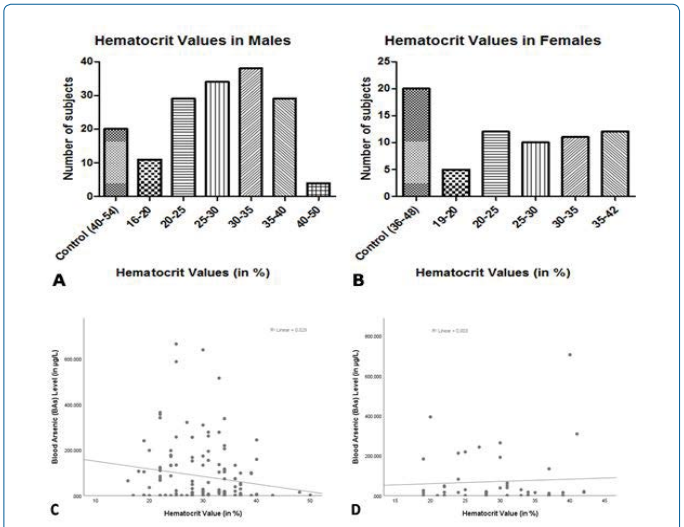

Figure 7: (A & B) Hematocrit value in blood samples of village people (Males & Females, respectively) of Simri and Tilak Rai ka Hatta (ANOVA-Dunnett's Test, P<0.05), (C & D) The correlation coefficient between blood arsenic level and Hematocrit value of the test subjects (Males r=0.029 and Females r=0.003, respectively & P<0.05)

Correlation coefficient between blood arsenic level and MCV value of the subjects: The declining trend was observed between blood arsenic levels and MCV value of the male individuals (Males r=0.011 and Females r=0.002, P<0.05; Figure 8).

Mean Corpuscle Hemoglobin (MCH): Total 195 test blood samples were analyzed from the entire village for MCH value in which the minimum level reported was 17.06 pg in adult male and 20.35 pg in adult female (Figure 9).

Correlation coefficient between blood arsenic level and MCH value of the subjects: The declining trend was observed between blood arsenic levels and MCH value of the male individuals (Males r=0.011 and Females r=negative, P<0.05, Figure 9).

Mean Corpuscle Hemoglobin Concentration (MCHC): Total 195 test blood samples were analyzed from the entire village for MCHC

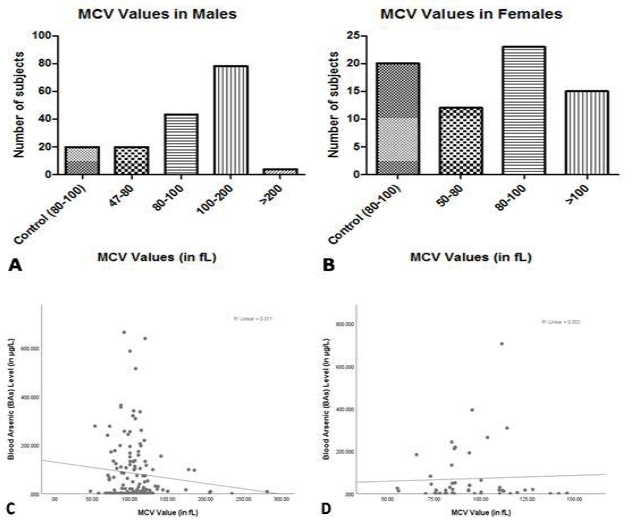

Figure 8: (A & B) MCV value in blood samples of village people (Males & Females, respectively) of Simri and Tilak Rai ka Hatta (ANOVA-Dunnett's Test, P<0.05), (C & D) The correlation coefficient between blood arsenic level and MCV value of the test subjects (Males r=0.011 and Females r=0.002, respectively & P<0.05).

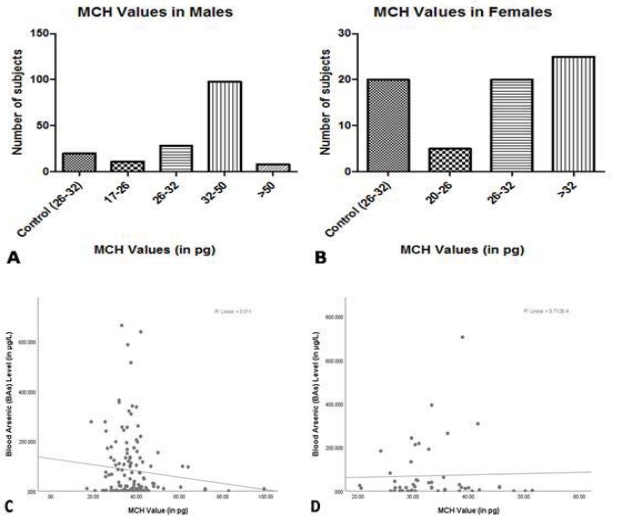

Figure 9: (A & B) MCH value in blood samples of village people (Males & Females, respectively) of Simri and Tilak Rai ka Hatta (ANOVA-Dunnett's Test, P<0.05), (C & D) The correlation coefficient between blood arsenic level and MCH value of the test subjects (Males r=0.011 and Females r=Negative, respectively & P<0.05).

value in which the minimum level reported was 34.62 g/dL in adult male and 34.85 g/dL in adult female (Figure 10).

Correlation coefficient between blood arsenic level and MCHC value of the subjects: The declining trend was observed between blood arsenic levels and MCHC value of the female individuals (Males r=0.006 and Females r=0.033, P<0.05; Figure 10).

White Blood Cell (WBC): Total 195 test blood samples were analyzed from the entire village for WBC levels in which the minimum level reported was 2.25×10^3 µL in adult male and 2.45×10^3 µL in adult female (Figure 11).

Correlation coefficient between blood arsenic level and WBC count of the subjects: The declining trend was observed between blood arsenic levels and WBC count of the individuals (Males r=0.019 and Females r=0.006, P<0.05, Figure 11).

Discussion

In the present study, it has been observed that the maximum blood arsenic concentration was found to be 706.1 µg/L in the population of arsenic hit area of Bihar while they had been drinking very high arsenic contaminated groundwater of around 228.105 ± 18.942 µg/L mean concentrations and 1929 µg/L was the maximum arsenic concentration

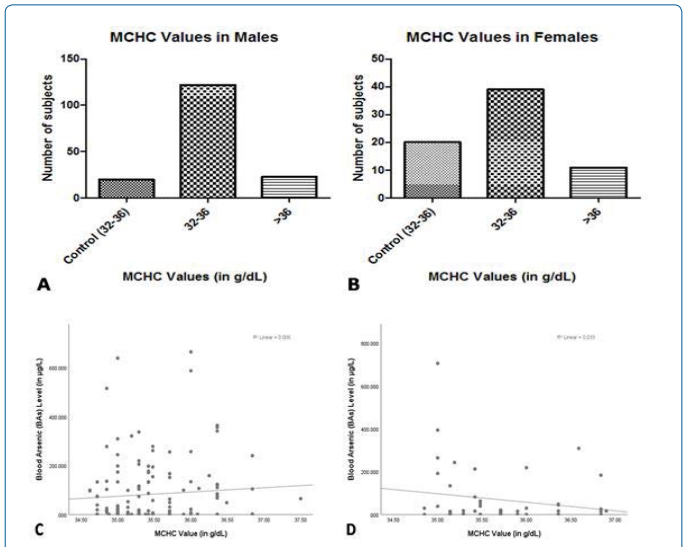

Figure 10: (A & B) MCHC value in blood samples of village people (Males & Females, respectively) of Simri and Tilak Rai ka Hatta(ANOVA-Dunnett's Test, P<0.05), (C & D) The correlation coefficient between blood arsenic level and MCHC value of the test subjects (Males r=0.006 and Females r=0.033, respectively & P<0.05).

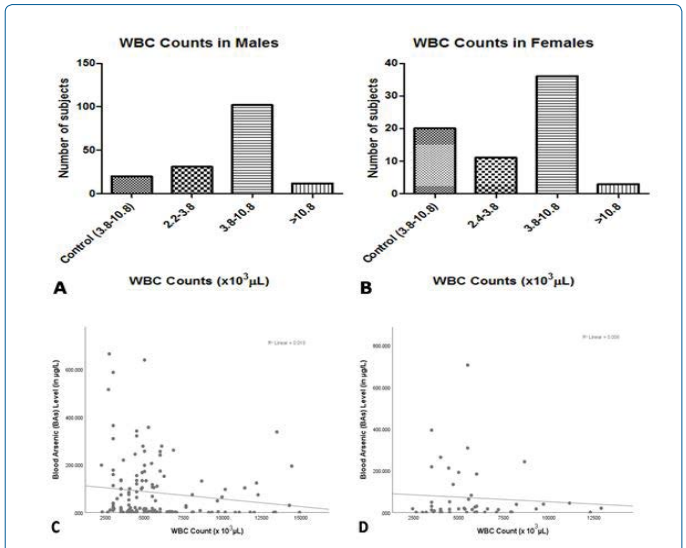

Figure 11: (A & B) WBC count in blood samples of village people (Males & Females, respectively) of Simri and Tilak Rai ka Hatta (ANOVA-Dunnett's Test, P<0.05), (C & D) The correlation coefficient between blood arsenic level and WBC count of the test subjects (Males r=0.019 and Females r=0.006, respectively & P<0.05).

found in their groundwater. This has led to severe health hazards in the population [14]. Higher MDA and Glutathione Peroxidase levels also show the arsenic induced toxic stress in the people. The low level of WBC shows the arsenic toxicity causing low immunity in the population. The RBC counts, hemoglobin level, HCT percentage, MCV, MCH and MCHC are also found to be less than the control value representing the affinity and toxicity of arsenic towards human blood cells, possibly by binding with the α-chain and sulphur groups. The majority of anemia and asthma patients found in that area indicates the health issues that arsenic is causing (Table 3).

The inorganic and organic arsenic in human blood has been merely studied. The blood arsenic reflects exposure for short period followed by absorption and its time dependent. However if the exposure is continuous and steady through drinking water then the blood arsenic has strong correlation with the exposure. Since the half-life of arsenic in blood compared with the half-life of human body makes it difficult to make correlation between blood arsenic correlation and total body arsenic burden or total arsenic concentration ion different organs. Partial speciation of arsenic has been well studied [35,36]. In a similar study people exposed to arsenic in drinking water with concentration 220 µg/L in ppb in northern Argentina had mean blood arsenic concentration of 10 µg/L [37,38]. In California and Nevada, the arsenic concentration in drinking water was 400 µg/L in corresponded to 13 µg/L in human blood while 100 µg/L in drinking water correspondent to 3.5 µg/L in blood [39,40]. In the present study, the maximum blood arsenic concentration 704.7 µg/L in adult male and 706.1 µg/L in adult female was found while the mean blood arsenic concentration 83.04 ± 10.60 µg/L in adult male and 70.47 ± 18.49 µg/L in adult female was calculated corresponded to 228.105 ± 18.942 µg/L mean arsenic concentration in their drinking water.

Many mechanistic studies of arsenic toxicity to humans have been suggested which implicates that free radicals like reactive oxygen species are generated during inorganic arsenic metabolism in living cells [41]. Arsenic induces structural changes in mitochondrial integrity causing damage to mitochondrial membrane and other organelle functions. Mitochondrial alterations are considered to be the primary sites where uncontrolled superoxide anion radicals' formation occurs. Cascade mechanisms of free radicals formation derived through the superoxide radical combined with decreased cellular oxidant defense by treatment with glutathione depleting agents results in increased sensitivity of cell to arsenic toxicity [42]. Many recent studies have provided experimental evidence that arsenic induced generation of free radicals and oxidative stress can cause cellular damage and cell death through activation of oxidative sensitive signaling pathways [43]. Long term arsenic exposure has been reported to cause a malignant transformation of human keratinocytes [44]. Arsenic also transforms the cellular membrane through lipid peroxidation weakening various functions of nucleus like Nrf-2 (Nuclear related factor-2) mediated antioxidant defense, activation coupled with apoptotic resistant, increased expression of casein kinase-2 (Ck 2) and elevated basal Nrf-2 activity. This cause apoptotic resistance and weaken antioxidant response during progression of cancer after exposure to arsenic [45-47]. In the present study similar findings have been observed for anti-oxidative stress in the form of MDA and GPx. The alarming levels of MDA and GPx were found in the blood sample from our current study area denoting the consequences of arsenic toxicity.

The hematopoietic system is also affected by short and long term arsenic exposure through drinking water. Anemia and leucopenia are common effects of poisoning resulting due to acute, intermediate and chronic arsenic exposures. These effects may be due to direct cytotoxic or hemolytic effect on the blood cells and suppression of erythropoies [48-51]. In a study in Bangladesh noticed that humans exposed chronically to 0.07 mg As/kg/day or less had no effects but relatively high doses of arsenic caused bone marrow suppression in humans leading to anemia and leucopenia [52,53]. High concentration of arsine 10 ppm causes death within hours duo to red blood cell hemolysis while low levels of arsenic exposure (0.5-5 ppm) brings the side effects in few weeks [54,55]. Hence, arsine breaks down the inorganic arsenic and methylated derivatives. The mechanism of hemolysis involved depletion of intracellular GSH resulting in oxidation of sulfhydryl group in the hemoglobin from ferrous to ferric and hemocyanin combines with arsenic causing reduced oxygen uptake by cells. In the present study, similar activity was observed in the blood of exposed population. All the hematological parameters like WBC count, RBC count, hemoglobin level and other RBC indices had significant abnormal levels.

Mondal et al. [56] in a study has well established the binding pattern of trivalent arsenic with hemoglobin. The chronic arsenic exposure modifies the conformation of hemoglobin such that two functions occurs simultaneously - as comparatively week binding of molecular oxygen to arsenic exposed hemoglobin and strong binding of trivalent arsenic with the same hemoglobin. This differential binding aspects of structurally and functionally modified hemoglobin plays the vital role in developing diseases pathogenesis in chronic arsenic toxicity. The study also illustrate that binding with arsenical and increased oxidative stress significantly alters the structural and functional activity of hemoglobin indicating the pathway through which arsenic can exerts its toxicity. Hemoglobin is a crucially a tuned protein and any deviation in its structure - function properties will have its implications reflected in the pathogenesis of chronic arsenic toxicity [57-60]. Hence, the study reveals that arsenic poisoning leads to severe hematological abnormalities in the population of arsenic hit areas of Buxar district of Bihar.

Control Area*				
Variables	**Age (in years)**	**Blood Arsenic Level (µg/L)**	**Hb (g/dL)**	**Health Problems (Anemia, Asthma, Weakness)**
Adult Male (N=20)	45.37 ± 2.16	2.27 ± 0.11	14.71 ± 0.22	4 (20%)
Adult Female (N=20)	48.92 ± 1.72	2.25 ± 0.09	13.52 ± 0.28	7 (35%)
*Mean Arsenic level in groundwater of control area = 4.272 ± 0.451 µg/L P<0.01 values are mean ± S.E.				
Arsenic Endemic Area**				
Variables	**Age (in years)**	**Blood Arsenic Level (µg/L)**	**Hb (g/dL)**	**Health Problems (Anemia, Asthma, Weakness)**
Adult Male (N=145)	43.21 ± 1.66	83.04 ± 10.60	10.71 ± 0.18	68 (46.89%)
Adult Female (N=50)	48.84 ± 2.97	70.47 ± 18.49	10.55 ± 0.33	27 (54%)
**Mean Arsenic level in groundwater of Arsenic endemic area=228.105 ± 18.942 µg/L P<0.01 values are mean ± S.E.				

Table 3: Showing level of arsenic toxicity and associated health problems of the village population of Simri and Tilak Rai ka Hatta.

Conclusion

The present study thus concludes that arsenic exposure to the population residing in the arsenic hit area leads to hematological changes and low immunity in them. Furthermore, the population is at very high risk since they are drinking maximum arsenic contaminated water of 1929 µg/L and their maximum blood arsenic level is 706.1 µg/L. Therefore, proper strategy is required to be undertaken by the state government to combat the present problem.

Disclosure of Potential Conflicts of Interest

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

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