For occupational and residential exposure 35 metals are of main concern, out of these 23 of them are called the heavy like antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc (Glanze, 1996). Interestingly, small amounts of these elements in our diet and environment are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity (poisoning). Arsenic is a naturally occurring metalloid, ubiquitously present in the environment. Arsenic contaminated groundwater is one of the most serious problems worldwide (WHO, 2001; IARC, 2004). Arsenic poisoning or arsenicosis is a condition that is caused by consuming, absorbing or inhaling more than the optimum levels of arsenic, which is a semi-metallic element that is capable of forming an array of poisonous compounds.

Arsenic has been used as a healing agent since Greek physicians such as Hippocrates and Galen popularized its use. Arsenic compounds became available as solutions, tablets, pastes, and in injectable forms. Fowler’s solution, a 1% arsenic trioxide preparation, was widely used during the 19th century. As recently as 1958, the British Pharmaceutical and Therapeutic Products handbook edited by Martindale, listed the indications for Fowler’s solution as: leukemia, skin conditions (psoriasis, dermatitis and eczema), stomatitis and gingivitis in infants, and Vincent’s angina. Fowler’s solution was also prescribed as a health tonic. Chronic arsenic intoxication from the long term use of Fowler’s solution caused haemangiosarcoma (Regelson W, 1968), angiosarcoma of the liver,(Lander JJ, 1975; Neshiwat LF, 1992) and nasopharyngeal carcinoma (Prystowsky SD, 1978). Arsenic was the primary treatment for syphilis until World War II. Arsphenamine (neoarsphenamine), a light yellow compound containing 30% arsenic was used intravenously to treat syphilis, yaws, and some protozoan infections (Ratnaike RN, 2003).

Arsenic trioxide (As$_2$O$_3$) is now widely used to induce remission in patients with acute promyelocytic leukaemia, based on its mechanism as an inducer of apoptosis (programmed cell death) (Shen ZX, 1997; Bergstrom SK, 1998; Soignet SL, 1998; Fenaux P, 2001; Zhu J, 2002). Arsenic induces apoptosis by releasing an apoptosis-inducing factor (AIF) from the
mitochondrial inter-membrane space from where it translocates to the cell nucleus (Lorenzo HK, 1999). AIF then affects apoptosis, resulting in altered nuclear biochemistry, chromatin condensation, DNA fragmentation, and cell death. AIF has been isolated and cloned and is a flavoprotein with a molecular weight of 57 000 (Susin SA, 1999).

Arsenic continues to be an essential constituent of many non-western traditional medicine products. Some Chinese traditional medications contain realgar (arsenic sulphide) and are available as pills, tablets, and other preparations. They are used for psoriasis, syphilis, asthma, rheumatism, haemorrhoids, cough and pruritus, and are also prescribed as a health tonic, an analgesic, anti-inflammatory agent, and as a treatment for some malignant tumors (Wong SS, 1998; Ko RJ, 1999; Shen ZY, 1999). In Korea arsenic is prescribed in herbal medicine for haemorrhoids (Mitchell Heggs CA, 1990).

However rather than an intended ingredient, arsenic is more often a contaminant, sometimes with mercury and lead (Ong ES, 1999; Wong ST, 1998). The Department of Health Services of California screened 251 products in retail herbal stores and detected arsenic in 36 products (14%) in concentrations from 20.4 to 114 000 parts per million (ppm) with a mean of 145.53 ppm and the median 180.5 ppm (Ko RJ, 1999). A study in Singapore identified 17 patients during a five year period with cutaneous lesions related to chronic arsenic toxicity, and in 14 (82%) patients toxicity was due to arsenic from Chinese proprietary medicines while the other three consumed well water contaminated with arsenic (Wong SS, 1998). In India, herbal medicines containing arsenic are used in some homoeopathic preparations (Kew J, 1993) and hematological malignancies (Treleaven J, 1993).

Most sea foods contain elevated concentration of arsenic mainly in the form of organic arsenic compound of which arsenobetaine species found in major, while arsenosugars are common in seaweed and bivalves. Arsenocholine is also found in certain kind of fish and shrimps (Lau BP, 1987). Inorganic arsenic compounds have been widely used in wood preservatives (chromate copper arsenate), herbicide, insecticide, pigments, glass and medicines. Combustion of coal is considered as one the main source of arsenic pollution in air. The
concentration of arsenic in sea water is usually under detection limit to 4µg/L (WHO/IPCS, 2001).

Arsenic exposure occurs from inhalation, absorption through the skin and, primarily, by ingestion for example, contaminated drinking water. Arsenic in food occurs as relatively non-toxic organic compounds (arsenobentaine and arsenocholine). Seafood, fish, and algae are the richest organic sources (Edmonds JS, 1987). These organic compounds cause raised arsenic levels in blood but are rapidly excreted unchanged in urine (Buchet JP, 1996; Han B, 1998). Arsenic intake is higher from solid foods than from liquids including drinking water (Thomas KW, 1999; Tripathi RM, 1997). Organic and inorganic arsenic compounds may enter the plant food chain from agricultural products or from soil irrigated with arsenic contaminated water (Tamaki S, 1992).

The major site of absorption is the small intestine by an electrogenic process involving a proton (H\(^+\)) gradient (Gonzalez MJ, 1997). The optimal pH for arsenic absorption is 5.0 (Silver S, 1984), though in the milieu of the small bowel the pH is approximately 7.0 due to pancreatic bicarbonate secretion (Ratnaike RN, 2000).

The absorbed arsenic undergoes hepatic biomethylation to form monomethylarsonic acid and dimethylarsinic acid that are less toxic but not completely innocuous (Thompson DJ, 1993; Aposhian HV, 1997). About 50% of the ingested dose may be eliminated in the urine in three to five days. Dimethylarsinic acid is the dominant urinary metabolite (60%–70%) compared with monomethylarsonic acid (Hopenhayen-Rich C, 1993). A small amount of inorganic arsenic is also excreted unchanged. After acute poisoning electrothermal atomic absorption spectrometry studies show that the highest concentration of arsenic is in the kidneys and liver (Benramdane L, 1999).

In chronic arsenic ingestion, arsenic accumulates in the liver, kidneys, heart, and lungs and smaller amounts in the muscles, nervous system, gastrointestinal tract, and spleen (Benramdane L, 1999). Though most arsenic is cleared from these sites, residual amounts remain
in the keratin-rich tissues, nails, hair, and skin. After about two weeks of ingestion, arsenic is deposited in the hair and nails.

Arsenic through reduction-oxidation reactions can be released from soil and rock into the surrounding aquifers and contaminate ground water with arsenic. The extent of arsenic contaminated groundwater is worldwide. The region with high concentration of drinking water in arsenic studied so far include Bangladesh, China (Taiwan and inner Mongolia), India (West Bengal), Australia, Argentina, Chile, Mexico, The USA (Nevada, California and Arizona), Hungary, Romania, Ghana and Vietnam (BGS, 2001; WHO, 2001; WHO, 2003). In some areas of Japan, Mexico, Thailand and some African countries major source of Arsenic contamination occurs due to mining, smelting and other industrial activities. Many other countries including Sweden, have initiated screening of public and private ground water sources for arsenic concentration.

Arsenic contamination affects different geological domains in India, which are virtually free of any industrial, mining or thermal-water activities and represent natural geological settings (Smedley PL, 2002). Extensive groundwater arsenic pollution affects low-lying Bengal Delta, covering southern parts of West Bengal (India) and major parts of Bangladesh (Singh SK, 2006). The lowland floodplain and deltaic basins of the Ganga and Brahmaputra rivers are entrenched over the Pleistocene terraces, where sedimentation was mainly during the early-mid Holocene sea level rise. Arsenic-contaminated aquifers are pervasive within lowland organic-rich, clayey deltaic sediments in the Bengal Basin and locally within similar faces in narrow, entrenched channels and floodplains within the Middle Ganga Plain,(Smedley PL, 2002; Singh AK, 2006; Mukherjee A., 2006) covering parts of the states of Jharkhand, Bihar and eastern Uttar Pradesh. In West Bengal, contaminated area is mainly confined to the east of the Bhagirathi River (also known as Hoogly River) (Acharya SK, 2001). The Damodar River basin located mainly between the Chhotanagpur and the Hazaribagh plateaus is exclusively confined to the Peninsular India. Thus, groundwater arsenic contamination in India cannot be ascribed to the Himalayan Rivers alone as postulated by some workers (Acharya SK, 2007). Arsenic contamination also affects
Introduction

Terai basin, Nepal. Tubewell water samples from several areas in the North Eastern Region, e.g., Assam, Arunachal Pradesh, Nagaland, Manipur, Tripura contain arsenic concentration higher than the limit of 50µg/L (Acharya SK, 2010). In contrast to these contaminated alluvial tracts, groundwater arsenic contamination affects local isolated areas, in the Ambagarh Chowki Block, Chhattisgarh, central India (Nickson R, 2007). The first report of high arsenic in drinking water and vegetables in India was reported from Chandigarh area (Datta DV, 1979), where non-cirrhotic portal fibrosis was endemic. This occurrence may not be natural and arsenic concentration was correlated mainly with use of superphosphate that analyzed a very high content of arsenic (Murti CRK, 1987). As contamination in alluvium of the Middle Ganga Plain and the Damodar fan delta flanking the Bhagirathi Ganga delta. The recommended permissible limit of As-concentration in potable water is lowered from 50 to 10µg/L in India. Infrastructure for water supply and analysis at this low concentration of arsenic is generally inadequate in India to implement this guideline (Acharya SK, 2000).

Arsenic problem in Uttar Pradesh was first reported in 2003 by School of Environmental Studies, Jadhavpur University (SOES-JU). The institute reported that concentration of Arsenic more than 50 µg/l in several villages of Ballia, Ghazipur and Varanasi districts of Uttar Pradesh. Bahraich is in one of the places where according to Uttar Pradesh Jal Nigam Board Arsenic concentration is very high (UPJN, 2009). Bahraich arsenic mitigation project (BAMP) is being implemented by University of Miyazaki (UOM), Japan in collaboration with Eco Friends working on affected population for their welfare. The project is being supported by JICA Kyushu, Japan. BAMP office started in September 2008. Therefore, the present study is based on population using arsenic contaminated ground water in Bahraich district.

Non-dermatological features of chronic arsenical poisoning by consuming arsenic contaminated drinking water were first reported in 1961 by Tseng et al. in Taiwan (Tseng, WP, 1977), followed by Rosenberg (Rosenberg, HG, 1974) in Chile and by Datta in India (Datta DV, 1976). Saha’s report is the first report in the world literature of chronic arsenical dermatosis from consuming arsenic-contaminated tube well water. Arsenic and many of its compounds are
especially potent poisons. Arsenic disrupts ATP production through several mechanisms. At the level of the citric acid cycle, arsenic inhibits pyruvate dehydrogenase and by competing with phosphate it uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of NAD+, mitochondrial respiration, and ATP synthesis. Hydrogen peroxide production is also increased, which might form reactive oxygen species and oxidative stress. These metabolic interferences lead to death from multi-system organ failure (see arsenic poisoning) probably from necrotic cell death, not apoptosis. A post mortem reveals brick red colored mucosa, due to severe hemorrhage. Two types of arsenic toxicity are acute toxicity and chronic toxicity (Saha, KC, 1984).

Clinical features manifest in virtually all body systems. Prominent features are nausea, vomiting, abdominal pain and excessive salination, acute psychosis, diffuse skin rash, toxic cardiomyopathy and seizures. Hematological abnormalities occur and renal failure, respiratory failure and pulmonary oedema are common. Neurological manifestations include peripheral neuropathy or encephalopathy. Urinary arsenic concentration is the best indicator of recent poisoning (1–2 days) (Ratnaike RN, 2003).

Absorbed arsenic accumulates in the liver, kidneys, heart and lungs, with smaller amounts in the muscles, nervous system, gastrointestinal tract, spleen, and lungs. Arsenic is deposited in the keratin-rich tissues: nails, hair, and skin. Mee’s lines occur in the fingernails and toenails. There is increased risk of cardiovascular disease, peripheral vascular disease, respiratory disease, diabetes mellitus and neutropenia. Arsenic toxicity also causes cancer of Lung, Liver, Bladder and kidney (Benramdane L, 1999).

Arsenic exposure results in many problems but its effect differ from person to person because of inter individual variation and genetic susceptibility plays a major role. Difference in health is a complex problem of major public health concern because it cannot be explained on poverty, access to health care, behavior, or environmental factors. Their complex etiology is dependent on the interactions between all these factors plus genetics. In many cases diseases are caused by a combination of genetic (nature) and environmental (nurture) factors. Therefore, the
risk of a particular health effect by an individual is the result of a complex interplay between genetic and environmental factors. This phenomenon is also known as a gene-environment interaction. About 99% of human genes are the same from one person to another, but the small fraction that differ can lead to differences in disease rates. Sometimes, a genetic factor alone can cause a health effect. In many cases, a genetic factor alone doesn’t cause disease, but can make an individual more susceptible to an environmental factor. This is often the case, particularly with small changes in the DNA sequence, called single nucleotide polymorphisms (SNPs). SNPs are randomly distributed variations of the building blocks of our genome that make each of us genetically unique. They can occur in any position within or outside of genes and accordingly can have very different effects. Studies have shown role of genetic polymorphisms, especially SNP at occupational as well as environmental exposures, e.g., towards arsenic induced oxidative stress among general population and benzene induced hemato-toxicity under occupational conditions (Breton et al., 2007; Rothman et al., 1997). Differences in disease susceptibility may be due to individual variability in biotransformation of arsenic, and polymorphisms in metabolic genes may contribute to this variability. Because arsenic methylation appears to affect its toxicity, it is essential to identify factors that impact methylation capacity and to better understand risk of disease (Loffredo et al. 2003).

Glutathione-s-transferases (GSTs), a super-family of multifunctional enzymes involved in cellular detoxification, conjugate and eliminate electrophilic carcinogens and scavenge free radicals. Role of GSTs M1, T1 and P1 plays very important role. Polymorphisms in these genes may affect the association between arsenic and 8-OHdG. specifically; 8-OHdG lesions are repaired by 8-oxoguanine glycosylase (OGG1) gene (Parl FF, 2005).

A role for glutathione-s-transferase mu (GSTM1) in DNA repair has also been suggested (Lear JT, 2000). A homozygous deletion in the GSTM1 gene results in lack of enzyme and is generally hypothesized to increase the accumulation of cellular DNA damage (Rebbeck TR, 1997). GSTs have also been associated with an increased risk for oxidative stress-related diseases. For example, GSTM1 null genotype was associated with cutaneous basal cell
carcinoma and with solar keratoses (Lear JT, 2000; Carless MA, 2002). Studies have demonstrated that the magnitude of disease risk is greater when the GSTM1 null genotype interacts with other factors (Rebbeck TR, 1997). Because homozygous deletions in GSTM1 have been associated with changes in arsenic methylation capability, GSTM1 genotype may alter arsenic toxicity and therefore modify the association between arsenic and urinary 8-OHdG (Chiou HY, 1997).

There is a growing body of evidence supporting the role of GST, particularly GSTP, in cancer development and chemotherapeutic resistance. The link between GSTP and cancer is most obvious in the over expression of GSTP in many cancers, but it is also supported by the fact that the transformed phenotype of tumor cells is associated with aberrantly regulated kinase signaling pathways and cellular addiction to over expressed proteins. That most anti-cancer drugs are poor substrates for GSTP indicates that the role of elevated GSTP in many tumor cell lines is not to detoxify the compounds, but must have another purpose; this hypothesis is also given credence by the common finding of GSTP over expression in tumor cell lines that are not drug resistant (Tew KD, 2011).

OGG1 is the primary enzyme responsible for the excision of 7,8-dihydro-8-oxoguanine (8-oxoG), a mutagenic base byproduct that occurs as a result of exposure to reactive oxygen species (ROS). OGG1 is a bifunctional glycosylase, as it is able to both cleave the glycosidic bond of the mutagenic lesion and cause a strand break in the DNA backbone. Therefore, functional relevance and commonly occurring genetic polymorphism of these genes (GSTM1, GSTT1, GSTP1 and OGG1) were observed to understand their role in inter individual variation and susceptibility towards arsenic toxicity (Bjoras M, 2002).

**OBJECTIVES OF THE STUDY**

In the present study the population of interest was the exposed to Arsenic residing in Bahraich using and consuming water contaminated with Arsenic. Initially water samples were collected and thereafter confirming presence of higher concentrations of arsenic than permissible
limit, a population based cross sectional study planned keeping in view the entire problem associated due to Arsenic. There are reports from Uttar Pradesh Jal Nigam and NGO reported higher Arsenic level in the water. Therefore, present study was attempted on the population living in this area to explain the affected subjects showing symptoms or not showing symptoms of arsenic toxicity at molecular level to explore the genetic susceptibility of subjects by genotyping of the individuals showing response and not showing response even though both were equally exposed. Thus a genetic polymorphism study which was planned to look for role of genetic factors behind inter-individual variation hypothesizing that the polymorphism of respective genes viz., GSTM1 and GSTT1 (deletion), GSTP1 (A313G) and hOGG1 (C1245G) corresponding to enzymes involved in the detoxification of Arsenic and its toxicity, may modulate individuals susceptibility among exposed population.

Thus the objectives of the study are:

(1) To identify a population getting exposed to Arsenic through literature search.

(2) To correlate the symptoms of toxicity and levels of suspected metal in the affected area.

(3) To collect the blood &/or hairs samples from human subjects for exploring the mechanism of toxicity at the molecular level by studying the specific enzymes of body defense for screening the genotyping of the individuals showing response and not showing response even though both are equally exposed.

(4) To explore the possibility of therapeutic interventions if possible because a proper support of the human subjects of the area will also be sought for.