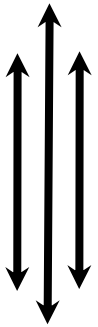
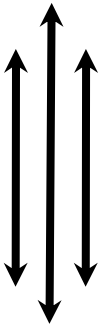
Prevalence Study of Arsenicosis Symptomatic Patients in Ramgram Municipality, Nawalparasi, Nepal



FINAL REPORT



Submitted by
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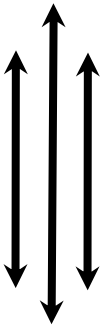

Submitted to
Nepal Health Research Council
Ram Shah Path, Kathmandu, Nepal
July 2008

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ABBREVIATIONS AND ACRONYMS

mg/l	-	milligram per liter
µg/L	-	microgram per liter
AAN	-	Asia Arsenic Network
As	-	Arsenic
ATSDR	-	Agency for Toxic Substances and Disease Registry
CBS	-	Central Bureau of Statistics
CCA	-	Chromated Copper Arsenate
CDES	-	Central Department of Environmental Science
CDG	-	Central Department of Geology
DHM	-	Department of Hydrology and Meteorology
DOI	-	Department of Irrigation
DWSS	-	Department of Water Supply and Sewerage
ENPHO	-	Environment and Public Health Organization
FAO	-	Food and Agriculture Organization
GDWQ	-	Guidelines for Drinking-Water Quality
IARC	-	International Agency of Research on Cancer
IOE	-	Institute of Engineering
IOM	-	Institute of Medicine
JRCS	-	Japan Red Cross Society
KTH	-	Royal Institute of Technology, Sweden
MCL	-	Maximum Contaminant Level
MIT	-	Massachusetts Institute of Technology
NASC	-	National Arsenic Steering Committee
NP	-	Nagarpalika
NRC	-	National Research Council
NRCS	-	Nepal Red Cross Society
ppb	-	Parts per billion
ppm	-	parts per million
RVWRMP	-	Rural Village Water Resources Management Project
SES	-	Socio Economic Status
U.S.	-	United States
UN	-	United Nations
UNF	-	United Nations Foundation
UNICEF	-	United Nation's Children Fund

USEPA	-	United State Environmental Protection Agency
VDC	-	Village Development Committee
WHO	-	World Health Organization

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EXECUTIVE SUMMARY

Arsenic contamination in drinking water has been a global concern. It has affected many countries of the world including India and Bangladesh. The Terai region of Nepal has similar geography to these countries and similar practice of extraction of groundwater for drinking. So, contamination of ground water with arsenic and prevalence of arsenicosis might be similar. The disease is chronic in nature and most of the time the patients do not have any symptom until they are detected through a screening survey. This study tries to diagnose arsenic case by common dermatological manifestations called melanosis and keratosis in combination with a history of consuming arsenic contaminated water.

The general objective of the study was to find out the prevalence of arsenicosis symptomatic patients among the risk households in Ramgram Municipality, Nawalparasi, Nepal.

The study was conducted from 26th June, 2007 to 28th July, 2007. This was a cross-sectional and descriptive study. Households exposed to 50 ppb to 350 ppb of arsenic concentration in drinking water were selected on the basis of blanket testing data of DWSS (2006). The tools applied were the questionnaires, direct observation, and interview. The Algorithm Chart produced by WHO, 2005 was followed for identifying arsenicosis symptomatic patients. Arsenic contents in hair and nail samples were determined by Atomic Absorption Spectrometer in ENPHO laboratory, Kathmandu. Data were analyzed by means of tables and diagrams, prevalence rate and percentage rate.

Out of 866 populations studied, 28 people (3.2%) showed the symptoms of arsenicosis. The prevalence was 4.5% (17/379) in males and 2.3% (11/487) in females. The maximum prevalence of arsenicosis patients (8.8%) was in ward number 6; whereas, the maximum number of patients (14) was in ward number 8. The maximum prevalence of arsenicosis symptom was that of melanosis on trunks (71.4%) followed by Keratosis on palm (11.1%) and then by Keratosis on sole (7.4%). The maximum numbers of patients (14) showed mild or initial state of arsenicosis. Majority of the arsenicosis symptomatic patients (82.1%, 23/28) replied that television, radio, newspapers, local students and educators were the good sources of knowledge about arsenic. Maximum numbers of patients (53.6%, 15/28) demanded for the maintenance of Kanchan filters and distribution of anti-arsenicosis drugs. Among the two hair and three nail samples from 5 patients, all these samples showed the arsenic concentration more than 2 mg/Kg.

Maintenance of Kanchan Filters, further testing of skin by dermatologists, and distribution of free anti-arsenicosis drugs, beginning of rehabilitation program and providing training of distinguishing arsenicosis and non-arsenicosis to the local educated people are some recommendations for the government and non-governmental organizations.

CONTENTS

CHAPTER -1

INTRODUCTION

1.1 Background

1.1.1 Arsenic

Arsenic (As) is a silver-gray brittle crystalline solid (Budavari *et al.* 1989). It also exists in black and yellow amorphous forms. Arsenic has an atomic weight of 74.9216 and an atomic number of 33. Silver-gray arsenic has a specific gravity of 5.73; a melting point of 817 °C (28 atm) and sublimes at 613°C. The yellow amorphous form of arsenic has a specific gravity of 1.97. Elemental arsenic can be present as a metalloid, although arsenic has an elemental structure similar to non-metals. In high oxidation states, arsenic displays covalent tendencies, while in low oxidation states it shows ionic tendencies (Ferguson, 1990). The valence states of As are: -3, 0, +1, +3, and +5 (Welch *et al.,* 1988). Elemental arsenic (valency 0) is rarely found under natural conditions. The +3 and +5 states are found in a variety of minerals and in natural waters. Many of the chemical behaviors of arsenic are linked to the ease of conversion between +3 and +5 valence states (National Research Council (NRC), 1999). The valence state affects the toxicity of arsenic compounds. While arsine (-3) is the most toxic, the following are successively less toxic: organo-arsines, arsenites (+3), arsenates (+5), arsonium metals (+1), and elemental arsenic (0) (EPA, 2000).

1.1.2 Environmentally Relevant Arsenic Species

Arsenic species are classified as either organic or inorganic (EPA, 2000).

1.1.2.1 Inorganic Arsenic

Inorganic arsenic, with +5 (arsenate) and +3 (arsenite) oxidation states, is more prevalent in water than organic arsenic (Irgolic, 1994; Clifford and Zhang, 1994). The dominant arsenic species depends on pH and redox conditions. In general +5 predominate under oxidizing conditions and +3 predominate under reducing conditions (ATSDR, 1998; Clifford and Zhang, 1994).

1.1.2.2 Organic Arsenic

Organic arsenic compounds such as Monomethylarsonic acid (MMAA), Dimethylarsinic acid (DMAA), Trimethylarsine (TMA), and Trimethylarsine oxide (TMAO) are generally associated with terrestrial settings, however, some are found in water (NRC, 1999). Organic arsenic is produced naturally in the environment in natural gas (ethylmethylarsines), shale oil, in water when microorganisms metabolize inorganic arsenic, and in the human body, as a result of enzyme activity in the liver (USEPA, 1993). Organic arsenical compounds are reported to have been detected in surface water more often than in ground water (NRC 1999).

1.1.3 Sources of Arsenic

There exist two major types of arsenic sources: Natural and anthropogenic.

1.1.3.1 Natural Sources of Arsenic

Arsenic occurs in the environment in rocks, soil, water, air, and in biota. Most arsenic in the environment exists in rock or soil (ATSDR, 1998).

1.1.3.1.1 Earth's Crust

Arsenic is the twentieth most abundant element in the earth's crust (ATSDR, 1998; NAS, 1977). Concentrations of arsenic in the earth's crust vary, but average concentrations are generally reported to range from 1.5 to 5 mg/kg (ATSDR, 1998; Cullen and Reimer, 1989; NAS, 1977). Arsenic is a major constituent of many mineral species in igneous and sedimentary rocks. Among igneous rock types, the highest arsenic concentrations are found in basalts. Sedimentary rocks particularly iron and manganese ores often contain higher average arsenic concentrations than igneous rocks (Welch *et al.,* 1988).

1.1.3.1.2 Soil and Sediment

Arsenic concentrations in soils depend in part on the parent materials from which the soils were derived, although they may be enriched by other sources, including anthropogenic sources. Typical natural concentration ranges are 0.1 to 40 mg/kg, with an average concentration of 5-6 mg/kg (NAS, 1977). The level of arsenic in soil derived from basalts tends to be higher than in soils of granitic origin, and concentrations of 20 to 30 mg/kg may be found in soils derived from sedimentary rocks (Adhikari, 2005b). Arsenic may be transferred to surface water and ground water

through erosion and dissolution. Because arsenic can be fixed in inorganic and organic compounds in soil, soil may also be a sink for arsenic (EPA, 2000).

1.1.3.1.3 Geothermal Waters

Geothermal water can be sources of arsenic in surface water and ground water. Flow of arsenic-enriched geothermal water from hot springs may result in high concentrations of arsenic in surface water systems (ATSDR, 1998).

1.1.3.1.4 Other Sources

Natural emissions of arsenic associated with volcanic activity and forest and grass fires are recognized to be significant. Indeed, volcanic activity appears to be the largest natural source of arsenic emissions to the atmosphere. But the relative contributions of volcanic sources, other natural sources, and anthropogenic sources to the atmosphere have not been definitively established (ATSDR, 1998).

1.1.3.2 Anthropogenic Sources of Arsenic

From man-made sources, arsenic is released to terrestrial and aquatic environments and to the atmosphere. The anthropogenic impact on arsenic levels in these media depends on the level of human activity, the distance from the pollution sources, and the dispersion and fate of the arsenic that is released (EPA, 2000).

1.1.3.2.1 Wood Preservatives

Chromated copper arsenate (CCA) is the most widely used wood preservative. CCA is an inorganic arsenic compound and consists of arsenic, chromium and copper. Different arsenic compounds are used as active ingredients in CCA, including arsenic acid (H_3AsO_4), arsenic pentoxide (As_2O_5), and sodium arsenate (Na_2HAsO_4) (Reese, 1998).

1.1.3.2.2 Agricultural Uses

Past and current agricultural uses of arsenic and arsenic compounds include lead arsenate, arsenic trioxide, sodium arsenate, calcium arsenate, copper acetoarsenite (Paris Green), copper arsenate, and magnesium arsenate in various pesticides, herbicides, insecticides, defoliants, and soil sterilants (Azcue and Nriagu, 1994). Arsenic compounds (Roxarsone and Arsanilic acid) are currently used in raising livestock as feed additives

and for disease prevention (Azcue and Nriagu, 1994). The, most widely applied Organoarsenical pesticide is Monosodium Methanearsonate (MSMA), which is used to control broadleaf weeds (Jordan *et al.,* 1997).

1.1.3.2.3 Industrial Uses and Releases

Arsenic and arsenic compounds are used in a variety of industrial applications. Arsenic metal is used in the production of posts and grids for lead-acid storage batteries, and is used in the formulation of some copper alloys (Reese, 1998). Crystalline gallium arsenide is a semiconducting material used in computers, optoelectronic devices and circuits, and other electronic applications. Industrial processes including the burning of fossil fuels, combustion of wastes (hazardous and non-hazardous), pulp and paper production, glass manufacturing, and cement manufacturing can result in emissions of arsenic to the environment (USEPA, 1998). Coal-burning power plants may emit aerosols and fly ash that contain arsenic (Adhikari 2005b). Waste disposal practices also may have impact upon arsenic concentrations in ground water and surface water at waste disposal sites (EPA, 2000).

1.1.3.2.4 Mining and Smelting

Mining of the arsenic content rocks and minerals for various purposes may emit arsenic to the atmosphere. Sulfide-bearing rocks are often mined for gold, lead, zinc, and copper, and arsenic is frequently found as an impurity in the sulfide ores of these metals. In mining areas, the arsenopyrite (FeAsS), mineral orpiment, realgar, and arsenic-rich iron oxides are the sources of dissolved arsenic (Welch *et al.,* 1988).

1.2 Health effects of chronic Arsenic poisoning

Following long-term exposure (generally 5 to 15 or more years) to arsenic, the first physical changes are usually observed on the skin. Typically this manifests in the appearance of small black or white marks (Melanosis), then thickening of the skin on the palms and the feet (Keratosis), followed by skin lesions and eventually skin cancer Internal cancers are a late phenomenon, and usually take more than ten years to develop. In advanced stages of Arsenocosis, parts of the body develop gangrene, making the victims appear similar to leprosy patients. The early symptoms of Arsenicosis (eg. Melanosis) appears to be reversible and/or can be arrested if exposure to arsenic- contaminated water is avoided (UNF, 1999, Egis *et al.,* 2000).

With history of chronic arsenic exposure and arsenical skin lesions, other indicators of chronic Arsenicosis are weakness, anemia, and peripheral neuropathy, hepatomegaly with portal zone fibrosis (with/without portal hypertension), chronic lung disease and peripheral vascular disease. Infrequent manifestations, which have been reported to occur by some investigators in people giving a history of chronic As exposure and which may be As unrelated are: conjunctivitis, Keratitis, rhinitis, cardiovascular disease, gastrointestinal disease, hematological abnormalities, cerebrovascular disease, dysosmia, perceptive hearing loss, cataract, nephropathy, solid edema of the limbs, and diabetes mellitus (Guha Mazumder *et al.,* 1998).

1.3 Diagnosis of chronic Arsenic Poisoning

Although chronic arsenic toxicity produces varied non-malignant manifestations as well as cancer of skin and different internal organs, dermal manifestations such as hyperpigmentation and hyperkeratosis are diagnostics of chronic arsenicosis (Guha Mazumder 2000).

Arsenical hyperkeratosis appears predominantly on the palms and the plantar aspect of the feet, although involvement of the dorsum of the extremities and the trunk have also been described. An indurated, gritlike advance to form raised, punctuated, 2-4 mm wart like keratosis are readily visible (Tay, 1974).

A history of arsenic exposure through inhalation or ingestion is helpful in corroborating a diagnosis of arsenicosis. Spotty raindrop pigmentation of the skin distributed bilaterally and symmetrically over trunks and limbs is the best diagnostic feature of arsenical hyperpigmentation. The duration of the patient's arsenic exposure with the date of onset of symptoms does not follow a particular time frame. Hence, a history of chronic arsenic exposure for more than 6 months is essential for diagnosis of arsenic related skin manifestation (Guha Mazumder, 2000).

Diagnostic criteria for Blackfoot disease include objective signs of ischemia, i.e., absence or diminution of arterial pulsation, pallor on elevation or rumor on dependency of ischemic extremities, and various degrees of ischemic changes in the skin, as well as subjective symptoms of ischemia, i.e., intermittent claudication, pain at rest, and ischemic neuropathy (Guha Mazumder, 2000).

Skin cancer of chronic Arsenicosis have lesions that are frequently multiple and involve covered areas of the body, contrary to non arsenical skin cancer which usually presents as a single lesion and which occur in exposed parts of the body (Tseng, 1977; Zaldivar *et al.,* 1981).

1.4 Global distribution of arsenic contamination

The extent of the arsenic problem world-wide is as yet unknown. Before arsenic was identified as the unambiguous cause of wide-scale health problems in Bangladesh, such occurrences were considered relatively isolated. Although the exact global scenario of the problem is yet to be revealed the problem globally has been found to exist in different parts of the world. A number of large aquifers in various parts of the world have been identified with problems from Arsenic occurring at concentrations above 50 µg/l often significantly so, the most noteworthy occurrences are in parts of Argentina, Bangladesh, Chile, Northern China, Hungary, India (West Bengal), Mexico, Romania, Taiwan and many parts of the USA, particularly the south-west. These include natural sources of contamination as well as mining–related sources. Arsenic associated with geothermal waters has also been reported in several areas, including hot springs from parts of Argentina, Japan, New Zealand, Chile, Kamchatka, Iceland, France, Dominica and the USA. Mining related Arsenic problems in water have been identified in many parts of the world, including Austria, Ghana, Greece, India (Madhya Pradesh), South Africa, Thailand and the USA.

1.5 Exposed population in Nepal

According to the population census data 2001, the population of Nepal is 23.4 million. Out of this about 10.4 million people (45% of total) live in 20 Terai-districts of Nepal, about 8 % of total tubewells were found contaminated, in average. Since ninety percent of the Terai population (9.4 million) is supposed to use tubewells for their drinking and others purpose, eight percent of its population, i.e. 0.75 million is estimated as the exposed population. If the estimation is made according to individual district-population and its exposed-population, this total exposed-population lowers to 0.46 million. In case of the affected area one can estimate the total Terai area that is 30000 km^2 as the arsenic-affected area. So far, only few studies about prevalence of Arsenicosis have been done in Nepal. From the studies, it is estimated that about 2.6 % of the total population, exposed to arsenic contaminated water with a concentration more than 50 ppb, have a prevalence of Arsenicosis (RVWRMP, 2004).

The present estimation of number at risk population from arsenic in drinking in Nepal is 0.3 million which is 3.4% of the total population. Similarly, the number of Arsenicosis patients identified so far counts 8,600 (World Bank, 2005).

Table 1: Districts by vulnerability and uncertainty levels

Districts	Total VDCs/ Municipalities	Arsenic Tested VDCs	Vulnerability			
			Low	Moderate	Moderately High	High
Kanchanpur	20	20	1	16	3	0
Kapilbastu	78	78	2	70	6	-
Nawalparasi	74	53	13	20	11	9
Parsa	83	86	6	74	4	2
Saptari	115	115	7	103	4	1
Sarlahi	100	95	15	54	15	11
Siraha	111	108	13	72	15	8
Rautahat	97	97	4	62	23	8
Total	678	652	61	471	81	39
Percentage			9.4%	72.2%	12.4%	5.9%

(Source: NASC, 2006)

1.6 Arsenic Contamination of Groundwater in Nawalparasi District

Nawalparasi is one of the 20 Terai districts of Nepal where the problem of arsenic contamination in tube-wells is detected. Water from shallow tube-wells is one of the major drinking water where almost 31,930 have been installed by different agencies including private tube-wells. Out of total tube-wells only 1092 i.e. 3.4% samples have been tested where 18% of the tube-wells were found to have arsenic concentration above the national standard of 50 ppb (DWSS and UNICEF, 2002). A small health survey conducted by NRCS covering 20 contaminated tube-wells has shown the prevalence of arsenicosis in the district as 3.33%. Since the study was based on only 20 contaminated tube-wells dug by NRCS, its findings couldn't be generalized. Apart from these NRCS has also conducted activities like establishments of arsenic information center at affected areas of the district, distribution of household filters, construction of Iron-Arsenic Removal Plants, human resource development etc.

Nawalparasi district consists of moderately high vulnerability level with medium uncertainty level.

Table 2: Summary of known Arsenic occurrence in Groundwater in Nawalparasi District as of June, 2006

Sources of data	Total numbers of tests	Arsenic concentration (ppb)			Maximum concentration (ppb)	Percentage exceeding	
		0-10	>10-50	>50		10 ppb	50 ppb
DWSS/UNICEF	30,939	23,156	4,061	3,722	1,200	25.16%	12.03%
NRCS	716	324	286	106	436	54.75%	14.80%
RWSSSP	442	336	45	61	341	23.89%	13.80%
DOI	95	25	20	50	-	73.68%	52.63%
KTH	27	3	6	18	409	88.89%	66.67%
Total	32,219	23,844	4,418	3,957	1,200	25.99%	12.28%

(Source: NASC, 2006)

The table 2 shows Summary of known Arsenic occurrence in Groundwater in Nawalparasi District as of June, 2006. According to this table, out of 32,219 total numbers of tests, 3,957 tubewells were found to be more than 50 ppb arsenic concentration.

Analyzing the latest data provided by Department of Water Supply and Sanitation (DWSS, 2006), 905 (12.3% of total 7362) tubewells were found to have arsenic contamination above 50 ppb. The highest percentage of tubewells with arsenic level above 50 ppb was found out to be in Ramgram Municipality (629 out of 1417, 44.7%).

Considering the National Standard of Nepal (50ppb) as safe, the population consuming water above this value is considered a population at risk. The total number of population at risk was found to be 40.87% (out of 7910 frequency) in Ramgram municipality in a study (Pathak, 2006).

1.7 Maximum permissible level of arsenic in drinking water

The World Health Organization (WHO) has fixed a guideline for maximum concentration of arsenic in water is tolerable for human body. According to this guideline, the concentration 0.01 mg/liter (10 µg/L or 10 parts per billion or 10 ppb) arsenic in water is safe and tolerable (WHO, 2005). In Nepal, the maximum permissible level of arsenic in water has been fixed at 0.05 mg/L (50 µg/L or 50 ppb). This is just the provisional guideline and it is not compulsory to follow because it is not Standard Guideline. The worldwide discussion of the Guideline Value for arsenic varies from country to country because of economic considerations, the technical ability to measure below 50 µg/L in the field, the health aspects, and focus on coverage rather than quality, lack of expertise and knowledge of the implementing agents. There is still a risk of drinking of water with the amount of arsenic recommended by Nepal as safe dose. Debate also remains over whether a threshold Arsenic concentration exists below which the element is effectively safe (Smith *et al.,* 1992).

1.8 Rationale of the study

Due to increasing extraction of arsenic contaminated groundwater in Terai region is causing health problems in Terai dwellers. Nawalparasi is one of the mostly affected districts and the study site Ramgram Municipality is severely affected part within Nawalparasi district. Therefore, People of Ramgram Municipality are at risk of arsenicosis.

Nepal should be alert to prevent the arsenicosis cases for which it is important to assess the Arsenicosis symptoms among people of Terai community. Therefore, this study will be significant, as it reveals prevalence of arsenicosis and its awareness among people to prevent melanosis, keratosis within the study area of Nawalparasi district.

There can be no state of positive health and well-being without safe drinking water. Each country is obliged to provide safe drinking water to its people. Safe drinking water is a basic element of primary health care. Therefore, the Government of Nepal has formed a National Steering Committee on Arsenic problem consisting 19 members to coordinate and solve the problem recently. The 10^{th} five-year plan aims to provide safe drinking water for 85% population. Drinking water facility has been already available to 71.6% at the last of 9^{th} plan in Nepal. However, 28.4% population wants basic drinking water facility (NPC, 2003).

The drinking water facility on the basis of different notices, quality, quantity, accessibility, supply time and reliability, classifying high, middle and basic level and facilities of high level for 25% population, middle level for 60% population and basic level for 15% population, facilities of sewage disposal and roughage management service in city area, confirmation of drinking water and sanitation, providing good domesticated toilet facility in rural area are the long term aspects of the "20 years Drinking Water Facility Related Long-Term Aspects" program implemented from 9th plan (NPC, 2003).

In the unit 25 (Drinking Water and Sanitation), in subunit 4, in sub-subunit 5 of 10th plan (Program and Operation Management), many objectives such as collection of rain water, helpful programs have been aimed. The helpful programs include the following points:

- For the sustainable development of drinking water and sanitation areas, direct and quantitative advantageous planning, establishment, maintenance, relief, quality relief, metering, leakage control, increase of public awareness, management notice of perfect-drinking water planning will be conducted.
- Conduction of water testing programs will be conducted at the national level joining with the different areas and organizations to determine the amount of arsenic in tubewells water in Terai regions.
- If alternative water resources are not available while searching in these areas, available water will be managed for the distribution after treatment.

Thus, it is important to complete the aims of 9th and 10th plan of Nepal in the field of drinking water and sanitation by the research on the water sources, arsenic and its health effects.

As there are only very few study regarding awareness of arsenicosis among different levels of people in a Municipality, Ramgram Municipality in Nawalparasi district has been selected. It is the area where people might have been highly infected with arsenic. One part of preventive measure is knowledge assessment aiming for strengthening health workers to fight against this burden of the country whereas the other part is assessment of the socio-economic dimensions of the arsenic hazard mitigation. The present study has attempted to cover both important dimensions to address the arsenic issues.

CHAPTER-2

AIMS AND OBJECTIVES

2.1 General

The general objective of the study was to find out the prevalence of arsenicosis among the risk household in Ramgram Municipality, Nawalparasi, Nepal.

2.2 Specific

- To identify Arsenicosis symptomatic patients in Ramgram Municipality
- To analyze the symptomatic effects among the people of various socioeconomic status.
- To assess the level of awareness about the arsenic and its mitigation among the users of arsenic contaminated drinking water.
- To review and recommend suitable preventive measures.

CHAPTER-3

LITERATURE REVIEW

3.1 Review of Past Work on Arsenic in Nepal

DWSS and WHO (1999) carried out the first Nepalese studies on arsenic in groundwater followed by NRCS & JRCS (2000). Both studies provided evidence of arsenic contamination in Terai region of southern Nepal. Furthermore, an initial health survey in 2001-2002 found evidence of arsenic-related dermatosis and elevated amounts of arsenic in human hair and nail samples in four districts where tubewell drinking water contained arsenic above 50 ppb (parts per billion). This initial evidence of arsenic contamination and associated health effects led to the creation of the National Arsenic Steering Committee (NASC) to help coordinate efforts by government and non-government agencies to address the potential problems of arsenic contamination in the rapidly growing region of southern Nepal.

Sharma (1999) carried out study on possible contamination of groundwater with arsenic in Jhapa, Morang and Sunsari, Eastern Terai. Out of 268 water samples tested for arsenic, 244 of them were found safe (below WHO guideline; 10 ppb), 2 of them showed a concentration level higher than 50 ppb. Most of the contaminated sample sites lied within active flood plains near Koshi River.

Tandukar (2000) conducted a study to explore the severity of arsenic contamination of groundwater in Rautahat District, Central Terai, Nepal. The results showed that some samples exceeded WHO drinking water quality standard and few of them exceeded India and Bangladesh standards. High arsenic was found to be associated with high iron content. Arsenic contamination was found to be higher in shallow aquifer and most of the contaminated tubewells were located in active flood plain of River Bagmati. The concentration of arsenic in groundwater of the study area did not remain constant throughout the year. Villagers were using arsenic contaminated water without taking any precautions and without having any knowledge about the severity and ultimate effects of arsenic. Hence people, especially women of the affected area, should be made aware of Arsenicosis, its prevention and precautions to be adopted.

NRCS and ENPHO (2001) collaboratively conducted a household survey on the health impact of Arsenic contaminated ground water in Parsa under the drinking water quality improvement program. A cross-sectional and descriptive study of the exposed population was done in Parsa District to assess the arsenic related health problems and to recommend appropriate measures to prevent or contain the problem. A total of 473 households with 3,579 populations were surveyed with a questionnaire. Out of that, a population of 2,732 present at the time of the survey was observed to detect the Arsenicosis-like skin problems. Overall the prevalence of the Arsenicosis among the exposed population was 1.8 percent. Recommendations made were to continue an ongoing water testing and survey or research programs, replacement of water supply with non-contaminated sources (surface water or deep bored tube-wells) or control programs, appropriate nutritional education, soothing treatment and follow-up observation of detected cases.

DWSS and UNICEF (2002) conducted a cross-sectional and descriptive study of the exposed population was done in Nawalparasi district to assess the arsenic related health problems and to recommend appropriate measures to mitigate the problem. A total of 148 households with 1102 populations were surveyed with a structured questionnaire. Out of that, 961 individuals present at the time of interview were observed to detect arsenicosis like skin lesions of which 49 were found to be affected. Overall arsenicosis prevalence rate was 5.1%. Males were more affected than females and most of them were in between 15-49 years. Mealnosis was observed frequently in trunk and keratosis on palm and sole. Hair sample analysis revealed that 98.5% of the exposed population had higher level of arsenic in hair than the reference value.

NRCS and ENPHO (2003) made a cross-sectional, descriptive study on Arsenic status of the tubewells installed by NRCS in Rautahat district. The study was carried out from October 2001 to May 2002 with the objectives of identifying Arsenic related health problems, analyzing mitigation measures adopted by NRCS and providing appropriate strategy as recommendations for prevention and management of Arsenicosis. Altogether 815 NRCS installed tubewells from 25 VDCs had been considered for the study, of which 157 tubewells were found to have Arsenic concentration above acceptable level (>50 ppb). A total of 1,338 households with 7,441 persons exposed to Arsenic contaminated drinking water (> 50 ppb) for more than 2 years had been considered for the study. A total of 167 were confirmed to have symptoms, Melanosis on the trunk and Keratosis on the palm. The prevalence rate of Arsenicosis was 2.2% among the exposed population.

Sah *et al.* (2003) carried out a study on possible natural sources of arsenic poisoning of ground water in Terai Plain of Nepal. The study found out that aquifer sediments of Terai Plain did not contain sulphide minerals like pyrite, arsenopyrite and galena but it contains Fe-concretions, Fe-coatings rich in arsenic and possibly that they represent the immediate source of arsenic in groundwater of Terai Plain. There is less possibility of groundwater contamination by arsenic from the recharge zone.

Bhattacharya *et al.* (2003) investigated the groundwater chemistry in the central part of the Terai Alluvial Plain (TAP) in Nawalparasi district. TAP groundwaters were near neutral to alkaline, with predominantly reducing character and high HCO_3^- low SO_4^{2-} and NO_3^- concentrations. Elevated HCO_3^- levels possibly resulted due to the oxidation of organic matter, low SO_4^{2-} levels reflected sulfate reduction. Elevated NH_4^+ concentrations in these groundwaters suggested dissimilatory nitrate reduction in the aquifers. Total arsenic (As) levels in groundwater varied from 1.7g/L to as high as 404 g/L with dominance of As (III) species and elevated levels of dissolved Fe and Mn. Arsenic was mobilized in groundwater as a result of desorption of As-oxyanions adsorbed onto Fe-and Mn oxides as well as reductive dissolution of these surface reactive phases from the sediments along with release out as in anoxie groundwaters.

Bhagat (2003) carried out a cross sectional descriptive study in Rampurkhap VDC, Rautahat District. Arsenic concentration of 126 sample of drinking water source in the study area was selected randomly using lottery method and arsenic field-test kit was used to analyze arsenic concentration. The risk group was assessed for the awareness of arsenic problem by using structured questionnaire. About 6.2% people were found aware of arsenic and its problem. Only 16.6% health personnel were found aware of arsenic and its effect on human health. All the households consuming water from the sources were considered as risk households with family members of 1044. Prevalence of arsenicosis symptoms and signs was found 1.8%.

Pandey (2004) investigated the effect of air space between the diffuser basin and the resting water level on removal of arsenic by the Arsenic Biosand Filter. Four filters from Tilakpur VDC of Nawalparasi district were selected for the research. Altogether 150 water samples were collected and flow rate of each sample was taken. The collected samples were tested for arsenic by using ENPHO arsenic field test kit. Besides, the social acceptance of the filter was evaluated through questionnaire and informal survey. The result obtained from the research was compared with the hypothesis set. According to the comparison, the result and conclusion were made. The result obtained from the research was not according to the hypothesis set and this thesis described the different reasons not satisfying the hypothesis.

Kanel *et al.* (2004) conducted a study that was directed to investigate the occurrences of arsenic contamination and its mechanism to release in groundwater in Gaur Municipality, Rautahat District, Nepal. The groundwater was found to be rich in iron, manganese and bicarbonates, which supported iron reduction hypothesis as the main mechanism of mobilization of arsenic in the groundwater. The arsenic concentration was varied from 0 to 62 µg/L in groundwater samples from shallow tubewells. Among analyzed samples, 2 % exceeded 50 µg/L concentration and 36 % were between 10-50 µg/L concentrations and rest of samples (62%) were below 10 µg/L concentration. The high arsenic concentration found in large number of tubewells indicated that several million people were consuming arsenic contaminated water (without any pretreatment) at serious risk of arsenic poisoning. However, there was no counter treatment of arsenic diseases and arsenic remediation is the only one option to save the lives of millions of people.

Ahamed *et al.* (2004) carried out a study in two rural villages of Nawalparasi district, where the existence of arsenic contamination has been reported. Almost all tubewells in one of the two villages exceeded (ranging from 0.104 mg/L to 1.702 mg/L) the maximum permissible limit for arsenic in drinking water in Nepal (0.05 mg/L), and only a few tubewells (19.5%) in the other village (Kunuwar Big, KB) were below this level (0.004 mg/L to 0.972 mg/L). Prevalence rates of Arsenicosis diagnosed on the basis of the presence of dermatological manifestations were 11.3% (56 out of 495 examined) and 6.5% (34/525) in two villages, respectively, with an overall prevalence rate of 8.9%.

World Bank (2005) mentioned that the current estimation of number at risk population from arsenic in drinking in Nepal is 0.3 million, which is about 3.4% of the total population. Similarly, the number of Arsenicosis patients identified so far counts 8,600.

Maharjan *et al.* (2005) conducted a community-based, dose-response study on Arsenic contamination in three communities in Terai in lowland Nepal. The arsenic concentration of all the tubewells in use (n=146) and the prevalence of arsenic-induced skin manifestation among 1,343 (approximately 80% of the inhabitants) subjects indicated the existence of a highly contaminated area in Terai. The overall prevalence of Arsenicosis among the subjects $\geq$15 years old was 6.9%, which was comparable to those found by the same examiner in arsenic-contaminated areas in Bangladesh, and that males had prevalence a twice as high as females, which could not be explained by the difference in the exposure level.

Adhikari (2006) carried out a cross-sectional study to sketch out the impacts of arsenic contamination on human health through drinking water in Balchanpur Area (Dumariya VDC), Rautahat District. 144 people from 31 families, among 63 males, 10 (15.87%) had Melanosis in trunk, where as, out of 81 females, 1 (1.23%) had Melanosis in limb. The highest number of patients (5 out of 11, 45.45%) were found to be consuming the water of the tubewell for 10 years. The overall prevalence of Arsenicosis symptomatic patients among the risk household of Balchanpur area was found to be 7.63% (11 out of 144). From the Dumariya Balchanpur litholog analysis, Arsenic testing in groundwater of the site showed that aquifer lying at 10-11 m and 16-18 m were contaminated, whereas, aquifer lying at 36-45 m was safe from arsenic contamination. It was observed

that villagers were using arsenic contaminated water without taking any precautions and having very little knowledge about the severity and its ultimate effects on human health. The proper mitigation measures are recommended for combating the situation.

Adhikari (2005b) carried out a cross-sectional study to sketch out the impacts of arsenic contamination on human health through drinking water in Santpur VDC, Rautahat District. One hundred and twenty four persons were examined from 36 risk households who used to drink water from 42 tubewells. The overall prevalence of Arsenicosis symptomatic patients among the risk household of Santpur VDC was found to be 15.3% (19 out of 124). The study recommended that proper investigations needed to be carried out to define the various clinical manifestations of Arsenicosis.

Pathak (2005) conducted a study on detection and management of Arsenicosis in the selected local communities of Terai regions of Nepal. The study revealed that in Rautahat, 93.33% of the health workers were not familiar with Arsenicosis cases and that of Bara was 70%. Out of 250 household sampling done, 4.78% of population is affected from Arsenicosis problem. The study revealed that maximum populations were being affected above 25 years of their age.

NHRC (2006) conducted the evaluation study of health impacts due to Arsenic contamination in the selected communities of Terai regions in Nepal. The objective of the study was to evaluate the health impacts of arsenic contamination on human health through drinking water in Ramgram and Lahan municipality and Swathi, Hakpara Santpur, Dumariya VDC of Nawalparasi, Siraha and Rautahat districts respectively. The study sites were selected on the basis of high vulnerability and low uncertainty level. The tools and techniques applied were the Questionnaires, Direct Observation, Focus Group Discussion (FGD), interview and computer software programming. A total of 312 people were selected from 312 risk households who used to drink water from arsenic contaminated tubewells. The overall percentage of risk tubewell (>50 ppb as level) in selected areas was found to be 12.3% in which 84(3.0%) in Lahan, 101(34.6%) in Hakpara, 629(44.4%) in Ramgram Municipality, 23(14.7%) in Swathi, 39(2.7%) in 29(2.4%). The total number of population at risk was found out to be 11204. The overall knowledge of arsenic among these risk household were found to be low (42%, totally unaware). Two third of the respondent (75.5%) of the community didn't use to purify drinking water. Analysis of community view towards mitigation revealed the following results: 72.3% of respondents preferred Arsenic filters as the immediate mitigation option and 59.3% of them preferred deeper tubewells as the long term mitigation option. Most of the respondents got

information about arsenic contamination in the tubewell from the testing campaign of DWSS 68.2% in Nawalparasi, 45.1% in Rautahat, 81.0% in Siraha District. The study found out that 67.9% (212 out of 312) respondents said that there was no any institution to respond the alternative source of drinking water free from arsenic. Majority of the respondents (74.6%, 232 out of 311) preferred governmental participation in solving the arsenic crisis. A significant percentage (39.2% out of 51) of the health workers suggested the need of trainings for the health workers in Arsenic and Arsenicosis.

Pradhan (2006) examined the arsenic state and classified water availability in the Narayani Irrigation Command (NIC) area. The first level included total tubewell samples for all three districts of Parsa, Bara and Rautahat lying in the command area of Narayani Irrigation System. At the second level, the data and information on the water samples of the tubewells were gathered at VDC of those districts. The analysis of the data has been schematically presented through Geographical Information System (GIS) map. Based on the samples, the households consuming arsenic contaminated water above Nepal Interim Standard (50 μg/L) were classified as risk households. Of the total 2389 tubewells samples in the study, 4.5% were above the Nepal standard and 28.3% above WHO guidelines. The distribution of arsenic levels in the tubewells is more or less similar pattern in all three districts of the study region. The dependency on groundwater was higher in areas where the availability of surface water was low. There existed an inverse relationship between arsenic level and water availability index. The arsenicosis prevalence rate for all three districts was less than 1 percent. The farm households locating in the downstream were more exposed to arsenic problem than those in the upstream of the command area.

CHAPTER- 4

MATERIALS AND METHODS

4.1 Study area

Nawalparasi is located in Lumbini zone of the Western Development Region of Nepal, which covers a total area of 2,162 square kilometers. It is bounded by Palpa and Tanahun districts on the north, Rupandehi and Palpa districts on the West, Chitwan and Tanahun districts on the east and Chitwan districts and Uttar Pradesh state of India on the South. Elevation of the district ranges from 91m-1936m above the sea level. Geographically the area lies between the latitude $7^{\circ}21'00''$ to $27^{\circ}47'00''$ North and the longitude $83^{\circ}36'00''$ to $84^{\circ}25'00''$ East and confined to the Terai plain. Climatically, it lies in tropical, sub-tropical and mid-temperate zones and experiences an average annual rainfall of 1588.4 ml.

As other Terai districts of Nepal, Nawalparasi is topographically made up of alluvial soil deposited by the river like Narayani, Jharahi, Girubari, Binayi, Arunkhola etc and its tributaries. Most of the area in Nawalparasi consists of flood-plain areas. Being a flood plain; the depth of groundwater is relatively low. The climate prevailing over the districts is characterized by hot & humid summers & cool winters. There are 1 district level hospital, 3 primary health centers, 10 health posts & 63 sub-health posts.

The tubewell & tap water (which is also provided through deep boring ground water) putting together represent 94% of the total households among all sources

Table 3: Proportions of the households of Nawalparasi district accessed to different sources for drinking water

Sources	Percentage
Tap water	25%
Well	4%
Tubewell	69%
River	0.2%
Others	1.8%

SOURCE: CBS (2001)

There are 73 VDCs and 1 municipality (Ramgram) in the district. Parasi Bazar is the district headquarters. According to the CBS (2002), the population of the districts was 612,361**.** The females (306,685) outnumber males (305,676). The density of population for the district as a whole is 2833 persons per square kilometer, which is one of the thickly populated districts of Nepal with a total of 101,867 households and average household size of 8. Majority of the population of the district speak Bhojpuri, Tharu and Nepali languages.

Health facilities in the district seem poor. There is only one government hospital is the district which is located in Ramgram municipality. There are five Primary health centre, 6 Ayurvedic clinics, 8-health post, and 63 sub-health post in the district.

The total population of the Ramgram municipality accounts for 22,630 where 11,570 are males and 11,060 are females in total household 3,893. Considering the population and total household, average household size of the municipality is 5.81 family members per household. About 54.1 percent of the total population of this municipality is literate.

For this study, we selected 104 households heads from ward numbers 1, 2, 4, 5, 6, 7 and 8 of Ramgram Municipality purposively. These wards numbers were selected on the basis of accessibility to go for the field survey.

STUDY AREA

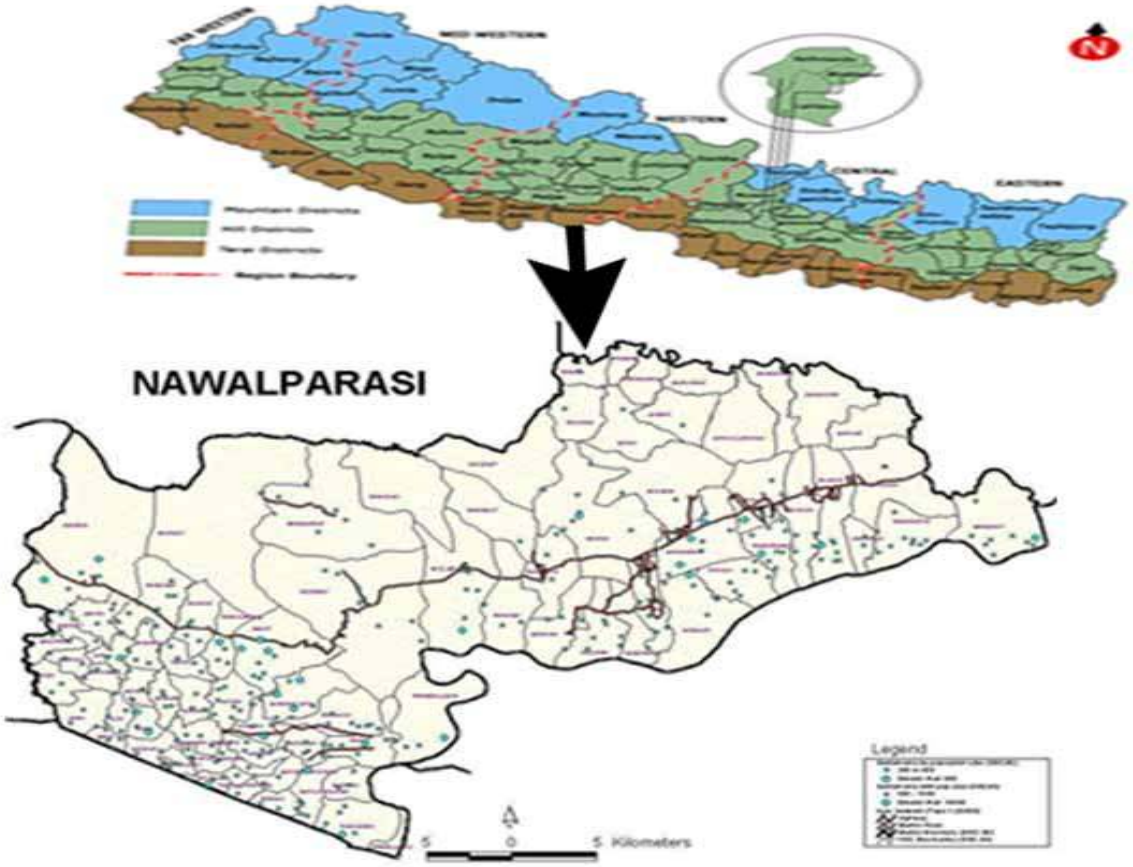


Figure : Geographical Map of Nepal (Above)
Geographical Map of Nawalparasi District (Below)

4.2 Study Design

This is a cross-sectional and descriptive study conducted in the study area to assess the existing water use and to find out the arsenicosis symptoms among the human population of Ramgram Municipality, Nawalparasi. Some of the literatures show the seasonal change in the concentration of Arsenic in water in different time of blanket survey; however, this study is clearly based on the survey of DWSS, 2006. We have evaluated it as standard tool for our study. Households exposed to 50 ppb or more than this value of arsenic concentration in drinking water were selected on the basis of blanket testing data of DWSS (2006). In this regard, 104 households using tubewell water with the above arsenic concentration in the community in different wards were selected. The targeted household was defined as a risk household in this study. The schematic diagram of the study design is predicted in the Figure 2.

Figure 2: Study Design

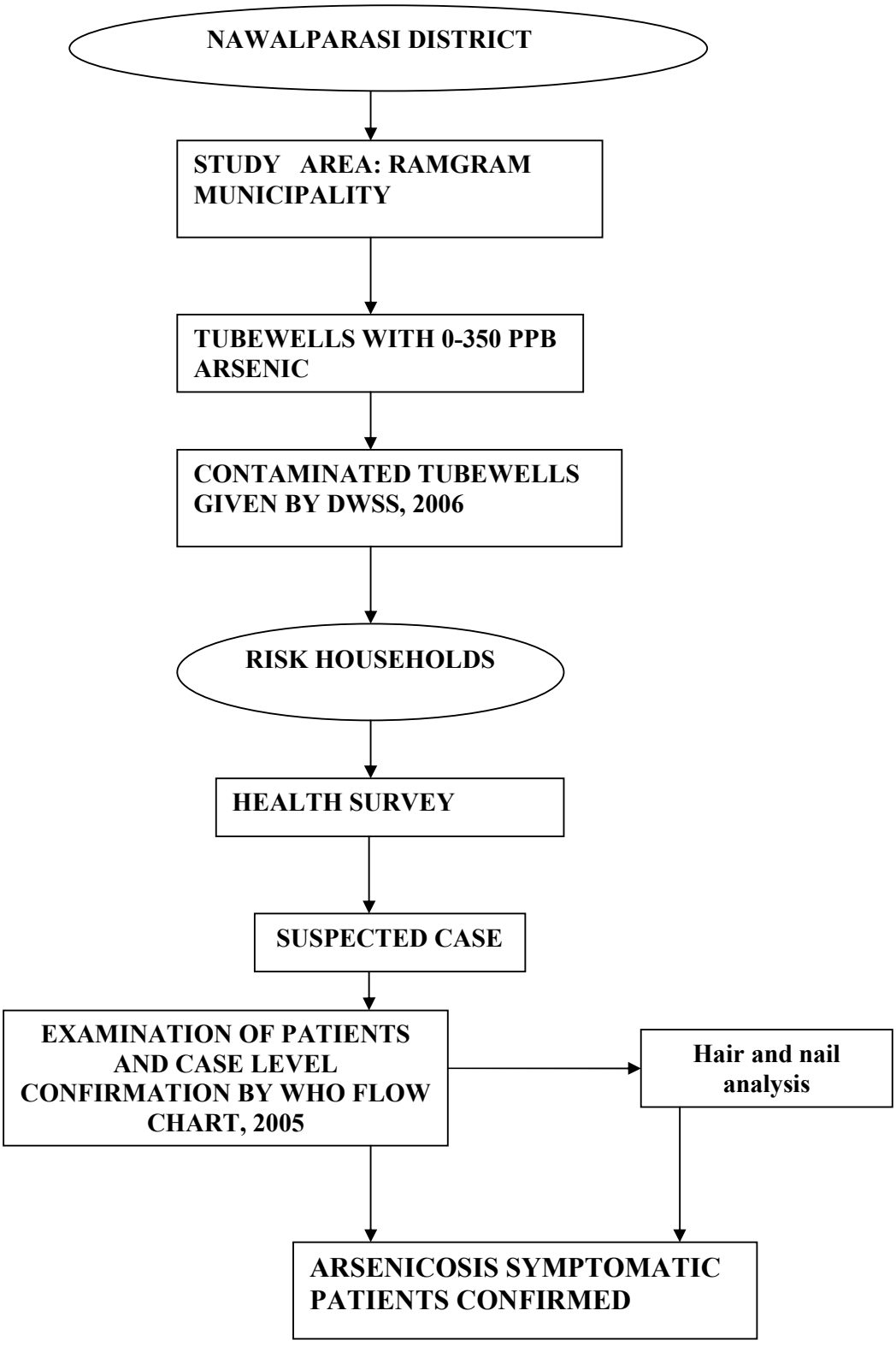
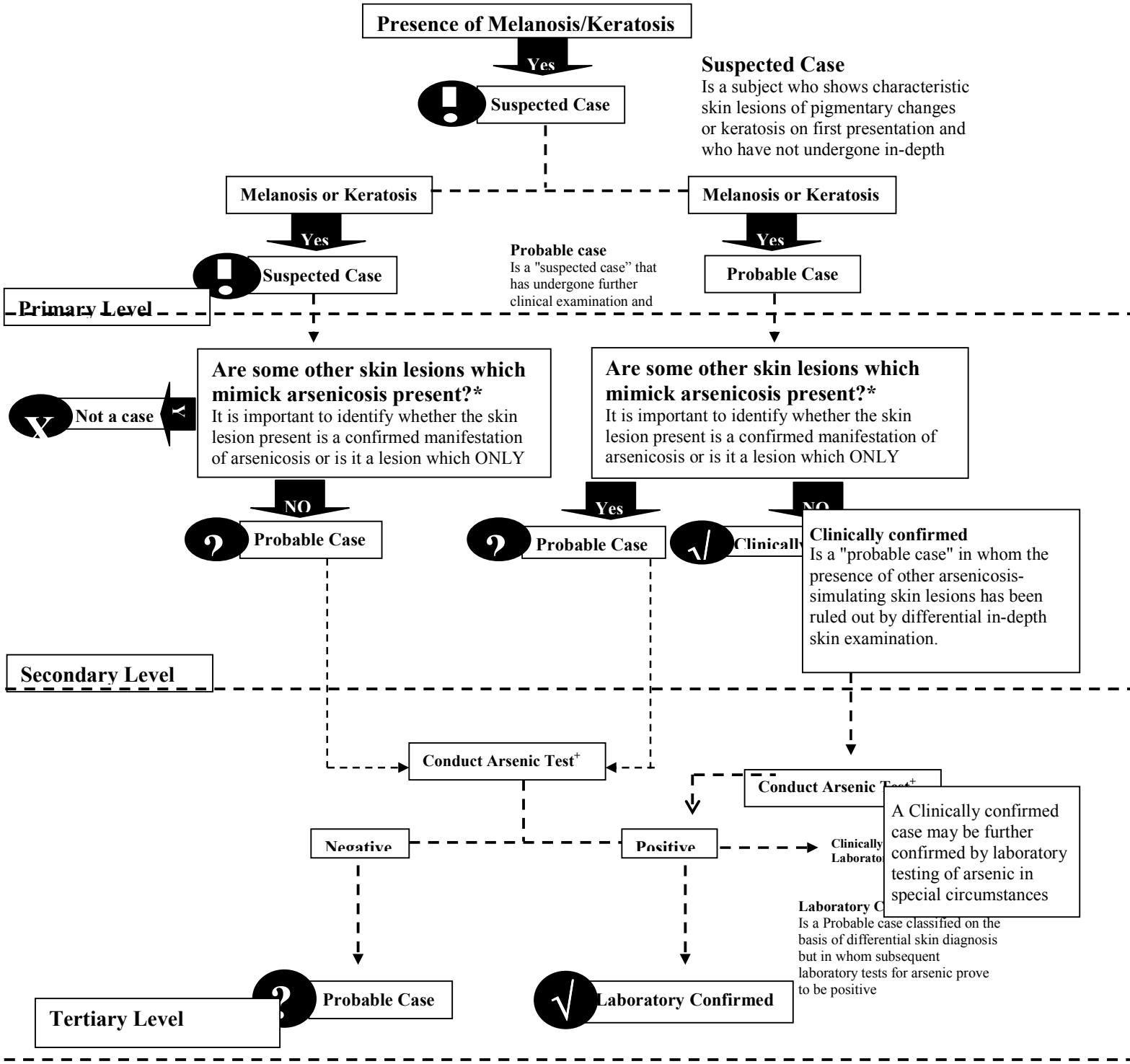


Figure 3: Flow chart: Case Definition Algorithm



4.3 Study population

Those households from the one municipality comprised about 866 populations whose family members were found to consume water from 83 tubewells. This comprised 104 households.

4.4 Unit of analysis

It consists of the following units:

- Individuals who were interviewed
- Individuals who were observed.
- Individuals who were symptomatic.
- Symptomatic and asymptomatic individuals whose nails and hair samples were tested for the arsenic concentration.

4.5 Research Tools

The research tools consist of questionnaire sheets and clinical examination of the exposed population. A detailed questionnaire was used to conduct the interview with the head of each household. The questionnaire contained questions regarding the sources of drinking water, socio-economic conditions of the households, perception of arsenic contamination of drinking water. The sample households were given the questionnaire. All the questions were asked in Nepali since most villagers neither speak nor understand English. Data were cross-tabulated and interpreted to explore the respondents' water use trend, knowledge and perception of the arsenic hazard, its mitigation process, and its variation among various socio-economic groups. For the collection of relevant information, structured questionnaire sheets were used.

Questionnaire sheet to collect household information, drinking water sources, clinical examination for the characteristic skin manifestations of chronic arsenic exposure and mitigation measures etc.

4.6 Inclusion and exclusion Criteria for the community and health survey

4.6.1 Inclusion criteria

- Head of the family were the key informants for assessing the knowledge about arsenic and its human health impacts among the community.
- Family members included in the list of DWSS data were included.

4.6.2 Exclusion criteria

- Members of household other than mentioned in the DWSS (2006) were excluded.
- Visitors or guests of household were excluded in all kinds of data collection.

4.7 Ethical considerations

Before data collection, the purpose and the procedure of this study were explained to the household head or key informant and other interested family members. Participation of the subjects in all kinds of data collection was voluntary. Confidentiality of each case was maintained.

4.8 Data Collection

4.8.1 Interview

All the 104 households consuming water from the 83 tubewells were purposively visited for the interview. These household heads were selected as respondents; however, interview was taken from other members of the same house as other information such as water collecting habit, water drinking habit etc.

4.8.2 Physical examination

Only those family members from the 104 households present at the time of contact person's interview were clinically examined by the trained health workers. Hence, out of 866 individuals, only 689 could be observed. Furthermore, the subjects with the observed skin changes were classified as below:

- Melanosis on palm (spotted/diffused)

- Melanosis on sole (spotted/diffused)
- Melanosis on trunk(spotted/diffused)
- Keratosis on palm (spotted/diffused)
- Keratosis on sole (spotted/diffused)
- Keratosis on trunk (spotted/diffused)

The type of skin changes along with the duration and sides of the observed lesions were recorded in the sheet. The diagnosis was confirmed if a patient with keratosis and hyperpigmentation was found to have been drinking water with a high arsenic content over a period of years. The diagnosis of Arsenicosis patient was entirely based upon the WHO Algorithm (WHO, 2005). Senior health advisor also examined some of the cases for the confirmation in the follow-up visit.

4.8.3 Hair and nail samples collection and analysis

Hair samples from the persons having arsenicosis like skin changes were taken for the laboratory analysis and tested in the human body since arsenic residue in hair is used as an indicator of absorbed arsenic in the human body since arsenic residue in hair is said to be deposited in the hair surface for a long time and resists from washing.

The value in hair and nails is not known with certainty. On review of the literature it can be assumed that arsenic concentration of greater than 1 mg/kg of dry hair and arsenic concentration of more than 1.5 mg/kg of nail may be considered as indicative of exposure to an unsafe dose of arsenic within the preceding 11 months (WHO, 2005).

An appropriate acid-washed plastic container was used. About 30 hairs in female and 60 hairs in male were collected. About the 5 cm long hairs were collected mainly from the areas close to the scalp by using a steel scissor. The hair was washed with arsenic-free water using a shampoo that did not contain arsenic. The hairs were kept in the plastic container after labeling.

The patients were asked to wash hands with arsenic-free water, cleaning the undersurface of nails. About 2 mm of nail clippings were taken and put in a plastic container washed with acid. The samples were labeled. A series of three steps were involved in the determination of arsenic content in hair and nail samples by AAS.

In this way, a total of 5 nail and hair samples were collected in which 3 nail samples were collected from 3 patients and 2 hair samples were collected from the 2 patients.

4.8.3.1 Washing

The collected samples were washed with few drops of biodegradable detergent (Extran Merck, MA 03 phosphate free) in magnetic stirrer for five minutes. Again it was washed with distilled water. The pH was checked, and finally the sample was washed with acetone, made dry in hot oven at a temperature of 50-60 degree centigrade and stored in a polythene bag and labeled properly.

4.8.3.2 Digestion

The sample was weighed 0.02-0.05 gm and kept in a 25 ml beaker. About 8-10 ml of concentrated HNO_3 was added and the beaker was placed on a hot plate around 60-80 dc for 48-78 hours until the color turned from deep brown to pale yellow. When the sample became 1 ml, it was filtered through multipore membrane (0.45M) filter, washed with distilled water to volume 2 to 5 ml.

4.8.3.3 Measurement

Arsenic content was then determined by Atomic Absorption Spectrometer.

4.9 Data Processing and Analysis

4.9.1 Data Editing

Data were edited as soon as possible to detect errors, mission and to make sure that the data were accurate, uniform and well arranged.

4.9.2 Coding

Information was coded so that they were easily classified and tabulated.

4.9.3 Classification and Tabulation

All the data were classified according to the need of the objectives and tabulation was done for summarizing the data and displaying statistically.

4.9.4 Data Analysis

Data were analyzed by means of tables and diagrams, prevalence rate and percentage rate.

4.10 Validity and Reliability

- The collected hair samples were preserved and sealed in a plastic bag to prevent loss or damage or external contamination.
- All forms were checked for completeness and accuracy and repeated visits on the next day were arranged for the missing family members for observation.
- The fieldwork was carried out under the supervision of the health consultant. The suspected cases with arsenicosis were re-examined by him.
- A total of three hair samples and two nail samples were tested in ENPHO laboratory using Atomic Absorption Spectro-photometry for the conformation of diagnosis.

4.11 Limitations of the study

- This study is limited to the households selected based upon the information provided by the DWSS.
- Because of the domestic conflicts and time limitation the regular arrangement of field visit was disrupted.
- Hair and nail samples were found to be limited due to unavailability of patients in our second visit, ethical reason and time limitations.

CHAPTER -5

RESULTS

5.1 Characteristics of the respondents

In the present study, a total of family members of 866 from the 104 households were studied. A small numbers of persons in some houses were absent during the studied period, however, we included them in the present study on the basis of interview on their family members about the presence or absence of any arsenicosis symptoms. In this study, a total of 379 males and 487 females were involved in the study (Table 4).

Table 4: Age-and sex-wise distribution of population in the research

Age-groups	Males	Percentage	Females	Percentage	Total	Percentage
≤20	117	30.9	125	25.7	242	27.9
21-40	101	26.6	233	47.8	334	38.6
41-60	74	19.5	82	16.8	156	18.0
≥61	87	23.0	47	9.7	134	15.5
Total	**379**	**100.0**	**487**	**100.0**	**866**	**100.0**

5.2 Age-and Sex-wise prevalence of arsenicosis symptoms

In the present study, out of 866 populations studied, 28 showed the symptoms of arsenicosis and the prevalence was 3.2%. Out of 379 males, 17 (4.5%) males were infected with arsenicosis and out of 487 females, 11 (2.3%) females were infected with arsenicosis. The persons of age groups 41-60 were highly infected in the present study (Table 5).

Table 5: Age-and Sex-wise prevalence of arsenicosis symptoms

Age-groups	Males	Positive	Prevalence	Females	Positive	Prevalence	Total population	Total Positive	Total Prevalence
≤20	117	1	0.9%	125	1	0.8%	242	2	0.8%
21-40	101	7	6.9%	233	5	2.1%	334	12	3.6%
41-60	74	9	12.2%	82	2	2.4%	156	11	7.1%
≥61	87	0	0.0%	47	3	6.4%	134	3	2.2%
Total	**379**	**17**	**4.5%**	**487**	**11**	**2.3%**	**866**	**28**	**3.2%**

5.3 Ward-wise prevalence of arsenicosis symptomatic patients

The study was conducted in seven wards including 1, 2, 4, 5, 6, 7 and 8 of the Ramgram Municipality. The highest prevalence of arsenicosis symptomatic patients was found in the ward number 6 (8.8%), whereas it was the lowest in ward number 7 (1.4%) (Table 6). No case of arsencosis was found in ward number 1 and 5.

Table 6: Ward-wise prevalence of arsenicosis symptomatic patients

Ward numbers	Numbers of households	Numbers of members	Numbers of Arsenicosis symptomatic patients	Prevalence of arsenicosis symptomatic patients
2	13	89	3	3.4%
4	7	87	4	4.6%
6	11	68	6	8.8%
7	9	72	1	1.4%
8	46	433	14	3.2%

5.4 Age-, sex- and ward-wise distribution of arsenicosis symptomatic patients

The studied arsenicosis symptomatic patients were divided into different age-groups in different ward numbers. The number of male patient was more than female in ward numbers 2, 6, and 8, whereas the number of female was more than male in ward number 7. There was equal number of male and female patient in ward number 4 (Table 7).

Table 7: Age-, sex- and ward-wise distribution of arsenicosis symptomatic patients

Ward numbers	Age-groups	Male Positive	Female Positive	Total Positive	Percentage %
2	≤20	0	0	0	0.0%
	21-40	1	1	2	66.7%
	41-60	1	0	1	33.3%
	≥61	0	0	0	0.0%
	Total	**2**	**1**	**3**	**100.0%**
4	≤20	0	0	0	0.0%
	21-40	1	1	2	50.0%
	41-60	1	1	2	50.0%
	≥61	0	0	0	0.0%
	Total	**2**	**2**	**4**	**100.0%**
6	≤20	0	0	0	0.0%
	21-40	2	1	3	50.0%
	41-60	2	0	2	33.3%
	≥61	0	1	1	16.7%
	Total	**4**	**2**	**6**	**100.0%**
7	≤20	0	0	0	0.0%
	21-40	0	0	0	0.0%
	41-60	0	0	0	0.0%
	≥61	0	1	1	100.0%
	Total	**0**	**1**	**1**	**100.0%**
8	≤20	1	0	2	14.3%
	21-40	3	2	5	38.5%
	41-60	5	1	6	46.2%
	≥61	0	1	1	7.7%
	Total	**9**	**5**	**14**	**100.0%**

5.5 Sex-wise prevalence of different arsenicosis symptomatic patients

In the present study, the patients who had the symptoms of melanosis on trunks were highly infected. MT was followed by KP and then by KS. Other symptoms seem to be low significant in percentage (Table 8).

Table 8: Sex-wise prevalence of different arsenicosis symptomatic patients

Symptoms	**Numbers of males**	**Numbers of females**	**Total numbers**	**Percentage %**
Melanosis on Trunk (MT)	14	6	20	**71.4%**
Melanosis on Limbs (ML)	0	1	1	**3.7%**
Keratosis on Palm (KP)	1	2	3	**11.1%**
Keratosis on Sole (KS)	1	1	2	**7.4%**
ML+MP	1	0	1	**3.7%**
MT+KS+KP	0	1	1	**3.7%**
Total	**17**	**11**	**28**	**100.0%**

5.6 Status of skin lesion(s) as compared to before in arsenicosis symptomatic patients

In the present study, 4 persons stated that their lesions got improving, 8 persons said that their lesions had been going to deteriorating, 1 person said that there was no change of the lesion, and 14 persons said that they didn't know about the conditions of their skin lesions as compared to before (Table 9).

Table 9: Status of skin lesion(s) as compared to before in arsenicosis symptomatic patients

Conditions of skin lesion(s)	**Total number of patients**	**Percentage**
Improving	4	14.2
Deteriorating	8	28.6
No change	1	3.6
Don't know	15	53.6
Total	**28**	**100.0**

5.7 State of arsenicosis symptoms in different patients in different ward numbers

In the present study, out of 28 arsenicosis symptomatic patients, 14 showed mild or initial state, 11 showed moderate state and 3 showed severe state of arsenicosis (Table 10).

Table 10: State of arsenicosis symptoms in different patients in different ward numbers

Stage of arsenicosis symptoms	**Total number of patients**	**Percentage**
Mild or initial	14	50.0
Moderate	11	39.3
Severe	3	10.7
Total	**28**	**100.0**

5.8 Distributions of arsenicosis symptomatic patients using water having different level of arsenic contamination for different periods of time

In the present study, out of 28 arsenicosis symptomatic patients, 9 persons were found to consume different arsenic concentrated water for 16-20 years. Similarly, maximum persons (9) were found to consume 151-200 ppb arsenic concentrated water (Table 11).

Table 11: Distributions of arsenicosis symptomatic patients using water having different level of arsenic contamination for different periods of time

Concentration (ppb)	Period of consumption (Years)					Total
	0-5	6-10	11-15	16-20	21-25	
0-50	0	0	1	0	0	**1**
51-100	0	0	0	0	0	**0**
101-150	2	2	1	2	0	**7**
151-200	0	5	2	2	0	**9**
201-250	0	1	0	4	1	**6**
251-300	1	0	0	1	0	**2**
301-350	0	0	3	0	0	**3**
Total	**3**	**8**	**7**	**9**	**1**	**28**

5.9 Occupation of the arsenicosis symptomatic patients

In the present study, out of 28 patients, 24 patients (85.7%) were agriculture workers or farmers. The least number of patients was from student (1) and service holder (1) (Table 12).

Table 12: Occupation of the arsenicosis symptomatic patients

Occupation	**Males**	**Females**	**Total**	**Percentage**
Farmers	14	10	24	85.7%
Businesspersons	2	0	2	7.1%
Service holders	1	0	1	3.6%
Students	0	1	1	3.6%
Total	**17**	**11**	**28**	**100.0%**

5.10 Awareness of arsenicosis symptomatic patients towards arsenicosis

In the present study, out of 28 patients, maximum, i.e., 23 patients (82.1%) said the knowledge about arsenic and its sources but didn't know about its effects in skin.

Table 13: Awareness of arsenicosis symptomatic patients towards arsenicosis

Particulate	**Males**	**Females**	**Total**	**Percentage**
Knows Arsenic, its sources and effects in skin	3	1	4	14.3%
Knows Arsenic and its sources but don't know about its effects in skin	14	9	23	82.1%
Don't know about both As and its health effects	0	1	1	3.6%
Total	**17**	**11**	**28**	**100.0%**

5.11 Sources of Knowledge to arsenic and its effects

In the present study, majority of the arsenicosis symptomatic patients (82.1%, 23 out of 28) replied that television, radio (mostly FM), newspapers, local students and educators were the good sources of knowledge about arsenic. Similarly, 7.1% of the arsenicosis symptomatic patients replied that experts from non-governmental organizations and international non-governmental organizations, projects and researches were the sources of knowledge about arsenic. About 7.1% patients replied that both of the above sources for their knowledge about arsenic (Figure 4).

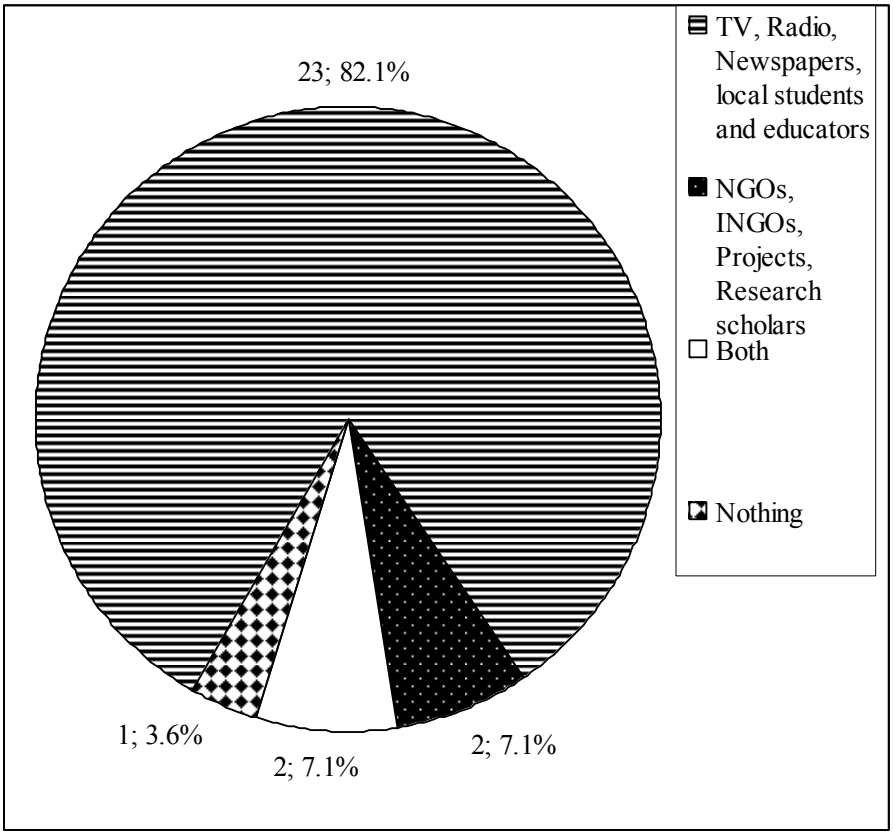


Figure 4: Sources of Knowledge to arsenic and its effects

5.12 Demands proposed by the arsenicosis symptomatic patients

In the present study, maximum numbers of patients (53.6%, 15 out of 28) demanded for the maintenance of Kanchan filters and distribution of anti-arsenicosis drugs. One male person demanded the training of distinguishing arsenicosis from non-arsenicosis symptoms from government and non-government organization so that they could have a good knowledge on the effects of arsenic (Table 14).

Table 14: Demands proposed by the arsenicosis symptomatic patients

Demands	Males	Females	Total	Percentage
Maintenance of Kanchan filters, distribution of antiarsenicosis drugs	11	4	15	53.6%
Maintenance of Kanchan filters	1	5	6	21.4%
Antiarsenicosis drugs	2	0	2	7.1%
Regular health checkup and free antiarsenicosis drugs	2	0	2	7.1%
Training of distinguishing arsenicosis from non-arsenicosis symptoms	1	0	1	3.6%
Nothing	0	2	2	7.1%
Total	17	11	28	100.0%

5.13 Results of hair and nail analysis for Arsenic concentration

In the present study, five samples of nail and hair were taken from the five arsenicosis symptomatic patients. These samples were tested for the arsenic concentration by Atomic Absorption Spectrometer with Hydride Generation in ENPHO Research Laboratory, New Baneshwor, Kathmandu, Nepal.

Hair samples were collected from the one female child of age 4 years that had the melanosis symptoms on trunk. She was suspected case of arsenicosis on the basis of melanosis, because of her lower age. She was found to have highest arsenic concentration among the studied arsenicosis symptomatic patients, i.e., her hair contained 7782 μg/kg concentration of arsenic. Similarly, other arsenicosis patients in this study showed high arsenic concentration in their nail or hair samples (Table 15).

Table 15: Concentration of arsenic in hair and nail in different arsenicosis symptomatic patients

Sex	**Age (years)**	**Samples**	**Symptoms**	**Arsenic concentration (μg/kg)**	**Concentration in mg/kg**
Female	4	Hair	MT	7782	7.782
Male	58	Nail	KS	3432	3.432
Male	29	Nail	KP	4776	4.776
Male	55	Hair	ML + KP	2227	2.227
Male	59	Nail	MT	6678	6.678

CHAPTER-6

DISCUSSION

Arsenic contamination in drinking water has been a global concern. It has affected many countries of the world for example South Africa, America, Canada, Chile, Ghana, Mexico, China, India and Bangladesh. In south Asia region, India and Bangladesh are badly affected.

The Terai region of Nepal has similar geography to India and Bangladesh and similar practice of extraction of groundwater for drinking. Therefore, contamination of ground water with arsenic and prevalence of arsenicosis might be similar. The disease is chronic in nature and most of the time the victims do not have any complaint or symptom until they are detected through a screening survey. The symptoms of arsenicosis are also very difficult to differentiate from other clinical conditions. The present experience to diagnose arsenic case is by external manifestations on the skin called melanosis and keratosis in combination with a history of consuming arsenic contaminated water. The contamination of the underground water has become a great challenge for provision of safe drinking water to the large population of the region.

The study area's primary focus was the Ramgram Municipality, Nawalparasi, Nepal. This area contains many arsenicosis symptomatic patients. The result has disclosed the various facts regarding the health effects of Arsenic poisoning in groundwater. It serves as an evidence of the seriousness of the issue in the context of Nepal.

In the present study, out of total 866 examined people, 28 were found to be arsenicosis symptomatic patients and the prevalence 3.2% was higher than that reported from Nepal (1.3%; Shrestha *et al.* 2004), from Parsa district (1.8%; NRCS and ENPHO, 2001), from Rautahat district (2.1%; NRCS and ENPHO, 2003; 1.8%; Bhagat, 2003), from Nawalparasi, Bara, Parsa and Rautahat (less than 1%; Pradhan *et al.,* 2006) and lower than those reported from Santpur VDC, Rautahat (15.3%; Adhikari, 2005a), from Terai region of Nepal (5.1%; Shrestha *et al.,* 2003), from two villages of Nawalparasi district (8.9%; Ahamed *et al*., 2004), from Nawalparasi district (5.1%; DWSS and UNICEF, 2002), from Terai region of Nepal (6.9%; Maharjan *et al.* 2005). The high prevalence of the present study might be due to the sampling of the arsenic infected tubewells described by DWSS (2006) used by the local people. Besides, other arsenicosis determining factors are nutritional status, addiction, dose response, socio-economic status different methylating capacity among individuals and population groups (Yang and Blackwell, 1961; Vahter and Marafante, 1987; Buchet and Lauwerys, 1985; WHO, 2000; WHO, 2005; Adhikari 2005a).

In the present study, the highest arsenicosis symptomatic patients showed melanosis in trunk (20 out of 28, 71.4%) which is less than the result of study in two villages of Nawalparasi district (95.6%; Ahamed *et al*., 2004). The prevalence of keratosis on palm and sole (5 out of 28, 17.9%) is lower than that of Bara district (22%, NRCS and ENPHO, 2002), Parsa district (70.0%; NRCS and ENPHO, 2001). The first physical changes are usually observed on the skin, following long-term exposure (generally 5 to 15 or more years) to arsenic. Typically, this manifests in melanosis, then thickening of the skin on the palms and the feet (keratosis), followed by skin lesions and eventually skin cancer (UNF, 1999; WHO, 2005).

In the present study, the lowest prevalence (0.8%, 2 out of 242) of arsenicosis symptoms was in the age groups ≤20 because the persons of this age group consume low volume of water per day. The age-groups 21-40 and 41-60 seem to be highly infected. The persons of age-groups 21-40 are mostly outside the housework and they are more likely to drink water. Similarly, the persons of age-groups 41-60 might have consumed arsenic contaminated water for the long duration. Though these groups contain persons with awareness, it was their compulsion to drink the arsenic contaminated water. Besides, consumption of water from the Kanchan filters has been started only from 2 years in the study area. The arsenicosis after 60 years suggest that arsenicosis is related to chronic toxicity, defective immune system, doses and awareness. Similar results have been found which supports the present results (Shrestha *et al.,* 2004; Chiou *et al*., 1995; Tsai *et al*., 1998; Mazumder *et al*., 1998; Tondel *et al*., 1999).

In the present study, the number of male arsenicosis patients (17 out of 379, 4.5%) was found higher than females (11 out of 487, 2.3%). This might be due to the intake of large volume of water in the daily basis by the males of the study area. According to the Bangladesh Observer 2005, in Bangladesh, the average daily intake of arsenic from drinking water by an adult is 532 µg/l with mean arsenic concentration in water 1.33 µg/l and intake of drinking water per day for an adult male is 4 L and for females 3 L (Adhikari 2005a). Similar results have been found in other studies (IOM, 1998; Ahamed *et al*., 1999; NRCS and ENPHO, 2003; Adhikari 2005b).

In the present study, out of 28 patients, 24 patients were found to consume arsenic contaminated water (51-350 ppb) for 11 to 25 years. Arsenicosis develops after the long consumption of arsenic contaminated water. Similar results have been found in other study (Ahamed *et al.,* 2001; Shrestha *et al.,* 2004; Chen *et al.,* 1986; Chiou *et al.,* 1995).

In the present study, 82.8% of the arsenicosis symptomatic patients know Arsenic and its sources but don't know about its effects in skin and 14.3% of the these persons know Arsenic, its sources and effects in skin, whereas, 3.6% of them don't know about both Arsenic and its health effects. The latter rate denotes the knowledge of 4 years old female child. Radio (FM or Frequency

Modulation), television and local magazines seemed to be more effective for the awareness of the people in this area. Very few learn from government and non-government organization sponsored publicities.

In this study, in many houses, Kanchan Filters have been damaged or they could not work properly. The demands of the maintenance of Kanchan Filters, regular health check-up and distribution of free anti-arsenicosis drugs and training on distinguishing arsenicosis and non-arsenicosis symptoms show that all these patients of the study area are enthusiastic in the research and knowledge of arsenicosis.

In this study, a 4 year girl was found to have highest arsenic concentration (7.782 mg/ kg hair) among the studied arsenicosis symptomatic patients. Her tubewell contained 350 ppb of arsenic and her father also contained the arsenic symptom (melanosis and keratosis). Her hair samples were tested by Atomic Absorption Spectrometer with Hydride Generation. It is the highly sensitive and selective method in the detection of arsenic (WHO, 2005). It is difficult to explain the melanosis symptoms and appearance of such a high content of arsenic in her hair. This might be due to external exposure of arsenic containing air, soil and water during playing. General environmental exposures include ingestion of soil by children and various food items. Arsenic is present at levels ranging from 0.2 to 40 μg/g of soil while in urban air the concentration of arsenic is at levels around 0.02 μg/ m^3 of air (WHO, 2005).

Arsenicosis is prevalent in Ramgram Municipality and it represents the arsenic problem of the people of Western Terai region of Nepal.

CHAPTER-7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

- Out of 866 populations studied, 28 persons showed the symptoms of arsenicosis and the prevalence was 3.2%. Out of 379 males, 17 (4.5%) males were infected with arsenicosis and out of 487 females, 11 (2.3%) females were infected with arsenicosis.
- The highest prevalence of arsenicosis symptomatic patients was in 41-60 years age-groups (7.1%, 11 out of 156).
- Melanosis on trunks was the symptom that was observed in maximum % of the arsenicosis symptomatic patients (71.4%, 20 out of 28) of arsenicosis symptomatic patients.
- Out of 28 arsenicosis symptomatic patients, 9 persons were found to consume different arsenic concentrated water for 16-20 years.
- Maximum percentage of the arsenicosis symptomatic patients (85.7% 24 out of 28 patients) was agriculture workers or farmers.
- The demands proposed by the arsenicosis symptomatic patients were the maintenance of Kanchan Filters, providing regular health check-up, distribution of free anti-arsenicosis drugs and the training of distinguishing arsenicosis from non-arsenicosis symptoms by the government and non-government organization.
- Television, radio (mostly FM), newspapers, local students, educators, experts from NGOs, INGOs, projects and research were the good sources of knowledge about arsenic in the study area.

7.2 Recommendations

- Administration of non-specific nutritional supplements or anti-oxidants directed at hastening recovery or prevention disease progression should be conducted. Some commonly used anti-oxidants include beta carotene, vitamin E and vitamin C.
- The government should launch the program of non-specific therapy against melanosis or keratosis or both that includes the application of keratolytic agents such as 5-10% of salicylic acid and 10-20% of urea-based ointment to the arsenicosis symptomatic patients.
- Government should launch the program of counseling and education to address the psychosocial consequence of the illness and to provide rehabilitation.
- Maintenance of Kanchan Filters, further testing of skin by dermatologists, and distribution of free anti-arsenicosis drugs and providing training of distinguishing arsenicosis and non-arsenicosis to the local educated and interested people are some recommendations for the government and non-governmental organizations.
- Doctors and health workers need to be trained to provide treatment, counseling, assurance and mental support and continuous monitoring to find out the efficacy of handling arsenicosis cases by trained medical doctors.
- The people of ward number 6 and 8 should be further tested for the arsenicosis symptoms. Immediate planning of treatment of this symptom is needed.
- The programs of arsenic and arsenicosis should be produced and delivered on television, radio and newspapers.

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ANNEX-I

DEFINATION, SYMPTOMS AND EVIDENCE OF EXPOSURE OF ARSENIC AND ARSENICOSIS

1. DEFINITION OF ARSENICOSIS

Arsenicosis may be defined as a chronic condition due to prolonged exposure of arsenic above safe level usually manifested by characteristic skin lesions with or without involvement of internal organs and malignancies.

2. ARSENICOSIS CASE DIAGNOSIS PROTOCOL

A. Common dermatological manifestations

a. Pigmentary changes in skin and/or mucous membrane, e.g. hyperpigmentation (melanosis), leukomelanosis. b. Hyperkeratinization, e.g. hyperkeratosis.

B. Common Non-Dermatological manifestations

a. Weakness/asthenia b. Conjunctival congestion (Conjunctivitis). c. Respiratory illnesses, e.g. Chronic cough, Bronchitis, Asthma, etc. d. Peripheral neuropathy, e.g. Tingling, Numbness, Burning sensation and pain.

C. Probable complications

a. Non-pitting oedema. b. Peripheral vascular disease (gangrene). c. Chronic ulcers. d. Bowen's disease (premalignant skin condition). e. Squamous cell carcinoma (SCC). f. Basal cell carcinoma (BCC). g. Hepatopathy. h. Nephropathy. i. Cancer of internal organs, e.g. Urinary bladder, lung, prostate, etc. j. Adverse pregnancy outcomes (spontaneous abortion, still birth, miscarriage etc.). k. Diabetes mellitus. l. Hypertension.

Evidence of Exposure

A. History of exposure

History of consumption of arsenic contaminated water (>0.05mg/L) for a period exceeding 6 months.

B. Evidence of high level of arsenic by biological sample analysis (BSA)

a. Arsenic in nail : >1.0 mg/kg. b. Arsenic in urine (in absence of intake of seafood): > 50µg As/L is indicative of continuing exposure. c. Arsenic in hair: >1.0 mg/kg.

ANNEX-II

OPERATIONAL DEFINITIONS

Pigmentary Changes

A. Hyperpigmentation (melanosis)

a. Mild-Diffuse or spotted blackening of palm, trunk and/or mucous membrane (gum, tongue, buccal mucosa).

b. Moderate- A palpable and visible multiple spotted pigmentation.

c. Severe-Extensive, diffuse or spotted dense pigmentation affecting the trunk and other parts of the body.

B. Leukomelanosis

Depigmentation in hyperpigmented areas characterized by whitish or pallor macules/patches commonly referred to as raindrop pigmentation.

C. Hyperkeratinization of skin (hyperkeratosis)

Hyperkeratinization of skin or hyperkeratosis refers to abnormally rough and dry thickening of palms and soles, which is mostly bilateral.

a. Mild- Just palpable thickening of palms and soles giving a gritty sensation on palpation, in absence of any obvious visible change.

b. Moderate- Palpable and visible multiple spotted or diffuse thickening of palms and soles.

c. Severe- Multiple wart or plaque like elevations (discrete/confluent) on palms and soles, and in addition may be present on other parts of the body.

ANNEX-III

QUESTIONNAIRE

General Information

1. Name:

   _____

2. Gender: Male ☐ Female ☐

3. Municipality/V.D.C:

   _____

4. Ward No. :

   _____

5. District:

   _____

6. Current Age (Years):

7. What is your occupation? (The work that you do most of the time)

   ☐ Day labour ☐ Farmer ☐ Labour

   ☐ Student ☐ Service holder ☐ Businessperson

   ☐ Unemployed ☐ Housewife ☐ Teacher

8 a. How many family members do you have?

   ☐ 1 - 3 persons ☐ 4 – 6 persons ☐ 7 - 10 persons

   ☐ 11 and above

   b. ☐ Total numbers of males: ☐ Total numbers of females

WATER RELATED INFORMATION

9. At present where do you collect your water?

- ☐ Own safe tubewell
- ☐ Pond / River/Canal
- ☐ Others safe tubewell
- ☐ Well
- ☐ Rainwater
- ☐ Others contaminated tubewell
- ☐ Own contaminated tubewell
- ☐ Others…….

a. If you are collecting tubewell water, for what purpose/s do you use?

- ☐ For everything
- ☐ Utensils
- ☐ Washing
- ☐ Only for drinking
- ☐ Cooking
- ☐ Bathing
- ☐ Washing clothes
- ☐ Feeding cows and goats
- ☐ Irrigation
- ☐ Flushing toilets
- ☐ Others…

b. If you drink contaminated water, from how long time you have been drinking?

Years ☐ month ☐ days ☐

c. If you drank contaminated water previously, and not at this time, how long time did you drink?

Years ☐ month ☐ days ☐

10. Do you purify your drinking water?

- ☐ Yes
- ☐ No

a. If yes, how do you purify?

- ☐ By boiling
- ☐ With medicine (Chlorine)
- ☐ Kanchan Filter
- ☐ Normal Filter
- ☐ Others

11. Do you know about arsenic, its sources and health impacts?

- ☐ Know Arsenic, its sources and effects in skin
- ☐ Know Arsenic and its sources but don't know about its effects in skin
- ☐ Don't know about both Arsenic and its health effects

a. If you are known about arsenic, its sources and health effects, what is/ are the source/s of your knowledge?

☐ Television ☐ Radio ☐ Local Educators

☐ Newspaper ☐ Local Students

☐ Experts from Non-Governmental Organizations and International Non-Governmental Organizations, Projects and Researches

☐ Others (specify):…….

22. Do you have any demand from the government?

☐ Yes ☐ No

a. If yes, what kind of demand do you have?

- ☐ Maintenance of Kanchan filters
- ☐ Regular health checkup and
- ☐ Free antiarsenicosis drugs
- ☐ Training of distinguishing arsenicosis from non-arsenicosis symptoms
- ☐ Others (Specify):…….

CLINICAL EXAMINATION SHEET

1. Observed Symptoms (Symptoms can be Multiple):
   - ☐ Melanosis on Trunk (MT)
   - ☐ Melanosis on Soles (MS)
   - ☐ Keratosis on Soles (KS)
   - ☐ Melanosis on Plams(MP)
   - ☐ Keratosis on Palms (KP)
   - ☐ Others (specify):…….

2. What are the uneasy problems with your skin lesion(s)?
   - ☐ Itching
   - ☐ Pain
   - ☐ Only uncomfortable
   - ☐ No problem

3. What is the condition of skin lesion(s) as compared to before?
   - ☐ Improving
   - ☐ Deteriorating
   - ☐ No change
   - ☐ Don't know

4. Stage of symptoms other than Keratosis on Palms?
   - ☐ Mild or Initial
   - ☐ Moderate
   - ☐ Severe

5. Do you have other health problems other than above skin lesions?

- ☐ Yes
- ☐ No

a. If yes, what are the problems? (Answers can be multiple)

- ☐ Respiratory
- ☐ Cough
- ☐ Urinary
- ☐ Weakness
- ☐ Hypertension
- ☐ Tingling or neuropathy
- ☐ Numbness
- ☐ Others (specify):……

PHOTOGRAPHS

Plate 1: Interviewing and discussing with local people

Plate 2: A Kanchan Filter

Plate 3: Examining the melanosis symptoms

Plate 4: Internal Structure of Kanchan filter

Plate 5: A woman showing Keratosis on Palm

Plate 6: The woman showing Keratosis on Palm and Sole

Plate 7: A person with Melanosis symptom

Plate 8: A person with Keratosis

Plate 9: A person with Melanosis symptom

Plate 10: The person with Melanosis symptom

Plate 11: A person with Keratosis symptom

Plate 12: A person with Melanosis symptom

Plate 13: A person with Melanosis symptom **Plate 14:** A person with Melanosis symptom

Plate 15: A person with Melanosis symptom **Plate 16:** A person with Melanosis symptom

Plate 17: A person with Melanosis symptom **Plate 18:** A person with Melanosis symptom

Plate 19: A person with Melanosis symptom

Plate 20: A person with Melanosis symptom

Plate 21: A person with Melanosis symptom

Plate 22: A person with Melanosis symptom