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Original research article

ARSENIC BODY- BURDEN IN CORONARY HEART DISEASE CASES OF WEST BENGAL, INDIA.

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ABSTRACT

Background: Coronary Heart Disease is a big concern for the health of the residents of India. Environmental contaminants, air pollutants, synthetic chemicals, and the mineral content in drinking water can affect the heart by altering the heart rate, contractility and excitability of heart muscle or by causing atherosclerosis. **Objective:** The aim of this study is to observe the level of arsenic exposure and clastogenic effect of arsenic in coronary heart disease cases of West Bengal. **Methods**: A total 511 cases were screened. Our study group included 100 nos of coronary heart disease cases and 70 no of healthy controls from West Bengal. Multivariate Logistic Regression and Student's *t* - test was used for statistical analysis of the parameters. The clastogenic effect of Arsenic was also examined by performing Cytogenetic Analysis. **Results:** Multivariate logistic regression analysis has revealed that a patient's age, Low density lipoprotein level and hypertension played a significant role in the development of CHD. Hair arsenic level in CHD cases (0.7±0.2) µg/kg was significantly high ($p<0.01$) than that of the controls (0.20±0.09) µg/kg. Mitotic index (4.4±0.21) was significantly low ($p\leq 0.05$) and chromosomal aberration (0.61±0.13) was significantly higher ($p\leq 0.005$) in CHD cases. **Conclusion**: This study has revealed the facts that age, low density lipoprotein level, hypertension are the three significant risk factors of CHD. It was found that, the hair arsenic content of the CHD cases in this region was having the arsenic body burden. Clastogenic effect of arsenic on coronary heart disease cases.

Key words: Coronary Heart Disease; West Bengal; Arsenic Exposure; Clastogenic effect

INTRODUCTION

Cardiovascular disease is the leading cause of mortality worldwide[1] .Atherosclerosis is the most common form of cardiovascular disease; it usually manifests clinically as the coronary artery disease, peripheral arterial disease. The major risk factors are smoking, alcohol intake, hyperlipidemia, diabetes, hypertension, obesity, positive family history. Unhealthy diet, sedentary and stressful

lifestyle, environmental pollution are taking a toll on the heart health of millions of Indians. High level of low density lipoprotein (LDL) increases the risk of coronary heart disease (CHD) by narrowing or blocking arteries which carry blood to the heart. Increased levels of high density lipoprotein lower the risk of CHD. High levels of triglycerides which make up a large part of the body's fat and also found in the blood stream are also associated with the increasing risk of CHD[2]. There are some environmental factors like air pollution, presence of synthetic chemicals, heavy metals and pharmaceuticals that cause or exacerbate preexisting cardiovascular disease[3]. Arsenic content in drinking water can affect the heart since it enhances the atheroma formation by involving cholesterol metabolism and favoring fatty deposition beneath the surface of endothelial cells lining of the arterial wall, which causes atherosclerosis[4, 5]. Long term exposure to inorganic arsenic is a risk factor for cardiovascular disease[6,7]. Arsenic increases the production of reactive oxygen which has been identified as the initial step towards the arsenic induced endothelial cell proliferation[8]. Arsenic increases the level of lipid peroxides and super oxide radicals in blood[9, 10]. Arsenic toxicity may alter the endothelial function and nitric oxide metabolism, resulting in decreased cell growth[11,12]. Arterial thrombosis and platelet aggregation may get induced by arsenic toxicity[13,14]. Major incidences of arsenic toxicity have been found in Taiwan, Antofagasta in Chile, Mexico and Argentina[15]. The contamination of ground water with arsenic in West Bengal (W.B., India) has been recognized as a major public health hazard[16]. Total nine districts of W.B. have been identified as Arsenic affected areas. Approximately 63% of total the population of W.B. reside in these areas. People living in these areas, are exposed to arsenic through drinking water and those who are not from these areas get exposed through food composites[17]. Several studies conducted around the world have reported that the risk factors also depend on the genetic background of different ethnic groups[18]. Arsenic has long been known to cause chromosomal damage, but most investigators have been unable to induce direct gene mutation[19]. The reports on the arsenic exposure of coronary heart disease patients of West Bengal are very limited. The clastogenic effect of arsenic on human cell is represented in this study. The extent of dependency of CHD on different risk factors is also investigated.

MATERIALS AND METHODS

Study Subjects

A total 1011 cases were screened for this study from cardiology OPD (Out Patients Department) of Ramakrishna Mission Seva Pratishthan. Out of the 511 cases clinically diagnosed by the physician of cardiology OPD, 100 cases with CHDs were chosen for this study. The studied population belonged to ages ranging from 20 to 70 years. Patients were having some major complaints of Ischemic heart disease (IHD), Myocardial infarction (MI), Unstable angina (UA). Patients are not having any congenital heart disease. Experienced cardiologist verified the diagnosis of these cardiac problems with the presence of at least two of the following criteria: (i) Characteristic chest pain, (ii) elevated cardiac enzyme, (iii) Electrocardiographic changes. Cases with minor complications like chest pain due to acidity, hypertension without any cardiac problem and cases who came for routine check up before any surgery were excluded. Those patients who had renal and hepatic insufficiency and those were taking some hormonal drugs were excluded from this study. Cardiac cases were divided into two groups according to drug intake. Out of 100 cases 50 cases were under statin therapy (group A), remaining 50 cases were not under statin therapy (group B). In cardiology OPD, patients were from different districts of West Bengal and their main source of drinking water was either tube well water or well water. For the 58% of the cases, arsenic content in drinking water was above 10µg/L (the WHO guideline value) and for the remaining 42%, the same was above 50µg/L (the Indian standard value). 70 healthy control cases were from non-arsenic affected area. Seventy populations based

controls, which were free of clinical CHD (substantiated by 12 lead rest ECG and history), were included in the study. Moreover, the source of drinking water was also a criterion for selecting the controls. For controls, the main source of drinking water was municipality supplied filtered water.

Detailed history was taken from all cases and control with the help of a questionnaire prepared as per the guidelines of the World Health Organization[20]. A questionnaire, which included questions on lifestyle factors (smoking), personal factors (age, sex, source of drinking water, family history of CHD etc.), health status (state of health, diabetic, hypertensive), was filled-up. Fasting blood glucose≥120mg/dl considered to be diabetic. Studied samples having systolic blood pressure of >160mmHg and diastolic blood pressure of >90mmHg, were defined as hypertensive. All patients gave their written consent prior to the participation, and the study procedures followed the guidelines of the Institutional Ethical Committee.

Lipid analysis

Overnight fasting blood was collected from the patients and healthy controls. 2-3ml blood was collected from each patient. Lipid levels Triglycerides (TGL) by GPO-PAP method, Total Cholesterol (T.Chol) by CHOD, High Density Lipoprotein Cholesterol (HDL-C), level were studied using AGAPPE (India) diagnostics kit with the help of Johnson & Johnson Vitros Eci autoanalyser. Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein (VLDL) was calculated using the formula:

VLDL=TGL/5, LDL= T.Chol-HDLC- VLDL.

Short-term leukocyte cultures

Short-term leucocyte culture was carried out by the method of Sharma and Talukder[21]. A total of 4ml of peripheral venous blood was collected from each donor under aseptic condition with the help of a sterile disposable needle and transferred to heparinized vial. For each subject, duplicate cultures were maintained. Leucocyte-rich plasma (0.5ml) was added to 5ml of culture media (RPMI 1640, Sigma, St. Louis, USA) supplemented with 20% fetal bovine serum (Sigma) and phytohemagglutinin M (0.04 mL/mL of culture media, GIBCO BRL). The cultures were incubated at 37^0C. At 70 hours of culture, colchicine (0.2mL of 0.04% mL) was added. Two hours later, cells were centrifuged at 1000rpm for 10 min, treated with pre-warmed KCL (0.075 M) for 15 min, centrifuged at 1000 rpm for 10 min and fixed in methanol: acetic acid (3:1). The fixed cell suspension was laid on clean grease-free and glass slide air-dried. The preparation was stained with aqueous Giemsa. All slides were coded and 1000 blast cells were scored to determine the mitotic index per individual and 100 metaphase plates were scored randomly for chromosomal aberrations (chromatid and chromosome types) per individual.

Estimation of arsenic concentration

Biological samples (hair and nails) were collected from both patients and controls. Before estimation, the hair and nail samples were digested with 5ml of concentrated nitric acid and 3ml of concentrated sulfuric acid. Flow injection-hybrid generation-atomic absorption spectrometry (FI-HG-AAS) at 327nm was used for estimation of arsenic in the collected biosamples. A Perkin-Elmer model 3100 AAS with a Hewlett-Packard-Vectra 386/25N computer with GEM software, Perkin-Elmer EDL System-2 and arsenic lamp (lamp current 400mA) were used for this purpose.

Statistical analysis

An exhaustive search of various subsets of explanatory variables (age, sex, family history of CHD, hypertension, diabetes, smoking, Total cholesterol, triglyceride, low density lipoprotein, high density lipoprotein, very low density lipoprotein) was carried out in the cases corresponding to without statin group and control. The stepwise logistic regression analysis was performed with the SPSS statistical package (version 16) to determine the independent risk factor for CHD. Multivariate logistic regression was used to find the Odds Ratio (O.R.) and 95% CI was used to assess the extent of risks of

independent variables on CHD. Intergroup comparisons of risk factors were performed using analysis of variance. Student's t test was also used for other parameters. The value of $p<0.05$ was considered as statistically significant.

RESULTS

The mean age of cardiac cases was found 50.06±1.25 years & control cases with 52.27±1.96 years. The baseline characteristic of the sample population was described in Table 1. In CHD cases the percentage of male (79%) sample was higher than women (21%). Frequency of smokers was significantly higher ($p<0.001$) in the cardiac cases than control. Presence of past family history of coronary heart disease was significantly higher ($p<0.01$) in cardiac cases than control. The prevalence of hypertension ($p<0.001$) and diabetes ($p<0.01$) were also significantly higher in cardiac cases than control cases.

In Table 2, a different set of biochemical parameters was compared between three groups i.e. group A and group B vs. healthy control group. Total Cholesterol level of cardiac cases in both of the groups i.e. of group A (162.81±6.23) and group B (204.79±10.47) were significantly higher ($p<0.005$) and ($p<0.0005$) respectively than healthy control cases. The same scenario was repeated for triglyceride level of Group A (129.21±8.84) & Group B (218.63±9.24) were significantly higher ($p<0.05$) and ($p<0.0005$) respectively than healthy control cases (149.14±8.818). But for both the parameters, a significance level of group B was higher than group A while compared with controls. Level of low density lipoprotein was significantly higher in group A (99.65±4.86) ($p<0.0005$) and group B (142.33±4.77) ($p<0.0005$) than healthy control (78.52±2.50). On the other hand it was found that the level of high density lipoprotein was significantly higher in control cases (44.44±1.29) ($p<0.001$) than group B (37.30±0.612). No significant difference was observed between group A and control cases. Very low density lipoprotein level was significantly higher in control cases (26.3±1.82) ($p<0.02$) than group A (21.10±0.216) but no significant difference was found in between group B (28.92±3.57) and control cases. Multivariate logistic analysis was performed in 120 cases (cardiac cases group B=50, control cases=70). Of the different variables, age, LDL and hypertension remained in the final model. The final model is,

Log (Probability of heart disease/Probability of no heart disease $= b0+b_1\times LDL+b_2\times Age+b_3\times$ Hypertension

According to the logistic model (Table 3), patients age ($p< 0.025$), LDL level ($p<0.004$) and hypertension ($p<0.019$) played a significant role in the development of CHD. All regression coefficients of independent variables were positive which indicates the positive relationship of risk factors with CHD. Age's OR (1.166), showing that there are 1.166 times more chances to have the CHD with one year increases in age. An increase in LDL by 1µg/dl, the risk of heart disease increases by an estimated factor by 1.358. In the presence of hypertension there are 41.596 times more chances to get the disease of CHD. The remaining explanatory variables do not contribute to the model significantly after taking into account the effects of LDL, age and hypertension. It may be due to low sample size.

The arsenic level of biological sample of cardiac cases was compared with healthy control. There was a significant ($p<0.01$) differences in the amount of arsenic content in cardiac cases (0.7±0.2) µg/kg & in control (0.2±0.09) µg/kg (Table 4). Mean arsenic content of different diagnosis cases like Ischemic Heart Disease (IHD), Myocardial Infarction (MI) and Unstable Angina (UA) were 0.8±0.12µg/kg, 1.0±0.39µg/kg and 0.2±0.05µg/kg respectively (Table 5).

The mitotic indexx was significantlyhigherh ($p\leq$. .005) in healthy control than patient, it was 5±0.24. Chromosomal Aberration was significantly higher ($p\leq 0.05$) in more patient than healthy control, it was 0.61±0.13 (Table 6). Mitotic index observed in Ischemic Heart Disease (IHD) was 4.4±0.29, Myocardial Infarction (MI) was 4.3±0.3 and Unstable Angina (UA) was 6.3±1.22.

Chromosomal Aberration of IHD & MI was 0.435±0.16 and 0.842±0.22 respectively (Table 7).

Table: 1. Baseline clinical characteristics of cardiac and control cases.

	Case(n=100)	p value	Control (n=70)
Age Range(20-70)	50.06±1.25	NA	52.27±1.96
Gender (n, %)			
Male	79(79%)	NA	43(61.43%)
Female	21(21%)	NA	27(38.57%)
Lifestyle Factors (n, %)			
Smoking	60(60%)	<0.001**	13(18.57%)
Family History	66(66%)	<0.01**	26(37.14%)
Diabetes	39(39%)	<0.01**	12(17.14%)
Hypertension	67(67%)	<0.001**	23(32.861%)

p=calculated as a chi square test. NA= not applicable.

Table: 2. An association of the Lipid profile of coronary heart disease cases with control cases.

Total cholesterol (mg/dl)	Case	p-value	Control
Group A	162.81±6.23	<0.005	141.78±5.36
Group B	204.79±10.47	<0.0005	141.78±5.36
Triglyceride (mg/dl)			
Group A	129.21±8.84	<0.05	149.14±8.185
Group B	218.63±9.24	<0.0005	149.14±8.185
LDL (mg/dl)			
Group A	99.65±4.86	<0.0005	78.52±2.50
Group B	142.33±4.77	<0.0005	78.52±2.50
HDL (mg/dl)			
Group A	42.57±1.53	NS	44.44±1.29
Group B	37.31±0.612	<0.0005	44.44±1.29
VLDL(mg/dl)			
Group A	21.10±1.216	<0.01	26.3±1.82
Group B	28.92±3.57	NS	26.3±1.82

p=calculated as a chi square test. NS= Not Significant

Table: 3. Variables in the equation

Regression Coefficient	Beta	Standard Error	95% confidence intervals (CI)	p-value	Odds ratio (OR)
b_o	38.322	13.653	-	0.005	0.000
b_1	0.306	0.107	1.102-1.674	0.004	1.358
b_2	0.153	0.068	1.020-1.332	0.025	1.166
b_3	3.278	1.400	0.002-0.587	0.019	41.596

Analysis comparing CHD risk factors among case and control subjects

Table: 4. Arsenic content in hair of cardiac cases & control cases

Arsenic content in hair sample	**Arsenic (µg/kg) (Mean±S.E.)**
Patient	0.7±0.2*
Healthy control	0.2±0.09

P was calculated by student t test, *(p<0.01)

Table: 5. Distribution of arsenic level according to different diagnosis cases

Types of CHD cases	**No. of cases**	**Arsenic (µg/kg) (Mean±S.E.)**
IHD	51	0.8±0.12
MI	35	1.0±0.39
UA	14	0.2±0.05

IHD=Ischemic Heart Disease, MI= Myocardial Infarction, UA=Unstable Angina

Table:6. Chromosomal study of patient and healthy control

Type of cases	**MI (Mean±S.E.)**	**CA (Mean±S.E.)**
Patient	4.4±0.21	0.61±0.13**
Control	5±0.24*	0.23±0.08

MI=Mitotic Index, CA=Chromosomal Aberration, *p≤0.05, **p≤0.005

Table: 7. Distribution of Chromosomal study according to differential diagnosis cases

Types of CHD cases	**No. of cases**	**MI (Mean±S.E.)**	**CA (Mean±S.E.)**
IHD	51	4.4±0.29	0.435±0.16
MI	35	4.3±0.3	0.842±0.22
UA	14	6.3±1.22	0.00

IHD=Ischemic Heart Disease, MI= Myocardial Infarction, UA=Unstable Angina

DISCUSSION

Around 29.8 million people with CHD were reported in 2000 in India, out of a total estimated population of 1.03 billion people, or a nearly 3% overall prevalence[22]. Heart disease is a leading cause of illness disability & death in industrialized countries[23]. The roles of environmental pollution in the etiology of different disease was an important consideration[24,25]. The risk factors are family history of coronary heart disease, smoking, hypertension, and diabetes mellitus, Serum lipid levels comprising of TGL, T.Chol, HDL, LDL, and VLDL level were compared between cardiac cases and healthy control. Environmental pollution like arsenic exposure was also observed. There are some toxic compounds e.g. oxides of nitrogen, sulfur dioxide and suspended particles which are involved in air pollution, are powerful pro-oxidants that enhance the oxidation of lipoproteins; and oxidized lipoproteins, particularly LDL cholesterol, are powerful inducers of artherosclerosis[26]. Various past studies indicated that smoking is prevalent in coronary heart disease patients. Here, the number of smokers were significantly higher (p<0.001) in CHD cases than in control. Presence of diabetes mellitus and hypertension were significantly higher in cardiac cases than healthy control. Lipid level Triglyceride, total cholesterol and LDL level were significantly higher in cardiac cases as compared to healthy control. In India, various cases – control

studies have reported a high level of total cholesterol, Triglycerides, Low Density Lipoprotein in patients having Coronary Heart Disease The study of Kumar et al., Wasir et. al., Vashist et.al. reported that level of Total Cholesterol were 20% - 40% higher in CHD cases compared to hospital based controls ($p < 0.05$)[27,28,29]. The result of this study is in good agreement with the past studies. Some diet and lifestyle related risk factors were very important in increasing the CHD epidemic in India[30,31,32]. Multivariate Logistic Regression Model was used to assess the extent of risks of independent variables on CHD. It is observed that the risk of CHD significantly increases with increase in serum LDL level, hypertension and age.

Ingested inorganic arsenic has been related to an increased incidence of cardiovascular disease, especially ischemic heart disease and has been reviewed extensively[33]. Many epidemiological studies reported that exposure to fine particles present in ambient air is associated with an increase in cardiovascular mortality. Statistically significant relationships between particulate air pollution and ischemic heart disease, arrhythmias, and heart failure have been reported[34]. Carotid atherosclerosis are associated with ingested inorganic arsenic, showing a significant biological gradient[35]. Zaldivar reported several cases of myocardial infarction and arterial thickening in children who consumed water containing about 0.6 mg/l arsenic (1974)[36].

Two worst arsenic affected areas in the world are Bangladesh and West Bengal, India. According to WHO guideline the maximum permissible limit for arsenic in dirking water is below 50µg/L[37]. Approximately 58% cases were from above 10µg/L and 42% cases were from above 50µg/L water arsenic content area. Among all types of CHD cases, approximately 80% of MI cases were coming from above 50µg/L water arsenic content area. Whereas only 27.45% of IHD cases were coming from above 50µg/L water arsenic content area, the rest of them were from above 10µg/L water arsenic content area. In the human uptake of arsenic mainly occurs via the food chain (dietary sources and drinking water) and occupational exposure[38,39]. It was found that the arsenic concentration of hair of cardiac cases significantly ($p<0.01$) high compared to healthy individuals. Typical background levels of arsenic in hair are in the range of 0.08–0.250 μg g−1 (indicator for toxicity level established at 1 μg As g−1)[40]. In myocardial infarction cases it was in toxic level (1.0±0.39µg/kg).In case of ischemic heart disease arsenic content is not in toxic level but it is in alarming situation that is well above the normal range. Thus it can be say that coronary heart disease cases are having arsenic body burden. Past studies in Bangladesh & Spain also reported raised mortality rates from cardiovascular disease including hypertension, ischemic heart disease and carotid atherosclerosis associated with arsenic exposure[41-47]. The entire study samples were from West Bengal where both the problems that is an occurrence of CHD and ill effects of arsenic are found. Moreover, very few efforts were made previously to find the relation between them in West Bengal.

In our present study some chromosomal aberrations were observed. Patient showed about 2.65 fold increase in chromosomal aberrations as compared to controls (Table 6). Among all types of stable chromosomal aberration (CA) found, chromatid break was the main CA observed. A decreased in the mean mitotic index (MI) was found among the patients in the study. This suggests a slower progression of the lymphocytes from S to M phase of the cell cycles, since every popularly known clastogens disturb the orderly progression of cell division[48]. A previous study by Chakraborty et al (2006) and Mahata et al (2003) explained that the CA was significantly high among the arsenic exposed population of West Bengal comparision with the unexposed healthy control group[19,49].

Very few studies were reported which stated that CA was responsible for CHD. Sajeetha Beegam et al. found some chromosomal aberration among ischemic heart disease patients[50]. CA was higher

in MI patients than other types of CHD cases. Most of the MI cases approximately 80% cases were coming from groundwater arsenic contaminated area. Moreover as per findings above, in the group of myocardial infarction both level of hair arsenic and chromosomal aberration were high. Therefore in line with the above discussion, it may be interpreted that chromosomal aberration in coronary heart disease cases of West Bengal is due to the clastogenic effect of arsenic.

CONCLUSION

It has been observed through this study that the risk of CHD significantly increases with an increase of serum LDL level, hypertension and age. In India, CHD is one of the most imperative reasons of death and the scenario is not at all different in West Bengal too. Moreover, West Bengal is one of the badly arsenic affected areas of India. By studying the hair samples of our CHD cases, we found that they were having arsenic body burden. The clastogenic effect of arsenic was found in the studied cases. In case of MI group, water and hair arsenic levels both were high; it also showed chromosomal aberration higher than other groups. Here, chromosomal aberration played as a bio marker for the effect of arsenic on CHD cases of West Bengal. This study indicated that ground water arsenic contamination of West Bengal might have some impact on occurrence of CHD in this population.

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