Arsenic Induced Breast Cancer Risk in Population of Bihar, India

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Abstract

Globally, ~ 300 million people are exposed to arsenic poisoning while in India an estimated 70 million people are affected by consumption of arsenic contaminated water. The state of Bihar is an endemic belt for arsenic contamination in groundwater affecting over 10 million people with moderate to serious health manifestations. Life threatening disease like cancer is not uncommon now, and carcinoma type of cancer cases are on the rise. Breast cancer incidences in the state follows closely with more and more women getting affected. Approximately, 23% of the cancer types in women are related to breast cancer, diagnosed at a fairly advanced stage (III or IV) of the disease. The etiology of the disease is not clearly known though faulty lifestyle and genetic makeup cannot be ruled out. The present study attempts to derive a relation between increasing breast cancer cases with the sustained arsenic intake in the second most populated state of the country. Pathologically confirmed, female breast cancer patients (n = 55) from across the state were included in the study. Sampling of the breast tissue, blood, hair and toe nail was carried out by the surgical oncology department of the institute. As a part of cross-sectional study, (n = 12) female breast benign cases were taken as the control group and their biological samples were also collected. All the samples as per the protocol of NIOSH were digested and analysed by graphite furnace atomic absorption spectrophotometer. For the epidemiological parameter study, their age, type of malignancy, stage and demographic information was compiled. The results were correlated with the arsenic concentration in groundwater as per their endemic status and anomalous values on GIS platform. The role of geological studies to establish the morpho-stratigraphic control and aquifers with higher concentration was brought to use. The results are fairly indicative of the high correlation of anomalous concentration of arsenic with the sample population with diagnosed malignancy as compared to the control group. The maximum arsenic concentration observed in the biological samples in blood was 1856µg/L, in breast tissue 446.4µg/Kg, in hair 1296·9µg/Kg and in toenail 621·83µg/Kg respectively. The scattered plots correlate the relationship between age of the breast cancer patients with arsenic contamination, while the geospatial distribution positively correlates with the districts with increased arsenic endemicity in a predominantly alluvium dominated country.

The high arsenic contamination in the biological samples of the breast cancer patients is an indicative marker to the possible relation of the disease to arsenic, as compared to the control population exposed to a much lesser toxicity. Consumption of water with more than 10 ppb arsenic contamination over a sustained longer time span has possibly exposed the population to a larger threat to disease as inferred from the findings. The disease breeding as a silent killer with reduced or unnoticeable symptoms upto a fairly advanced stage has further accentuated the problem. The present study thus endeavours to identify a significant relation of the disease with sustained intake of arsenic consumed through water and food products laden with anomalous concentration.

Introduction

Cancer is a multifactorial genetic disease that causes uncontrolled growth of abnormal cells in the body. It is now the most common cause of death globally and still baffles the world of medicine by the life-

threatening health challenges posed by it. It is estimated that 01 in 5 people develop cancer during their lifetime and 1 in 8 men and 1 in 11 women die from this disease.[1] It is reported to affect population representing all ages, from children to older age group and not limited to territorial boundaries. According to the report published by International Agency for Research on Cancer (GLOBOCAN 2020),[2] the mortality rate due to the disease rose to 10 million people worldwide. Globally, Breast cancer is a commonly diagnosed cancer among women (159 out of 185 countries). Breast cancer accounts for about 11·7% (2,261,419) of all new cancer cases diagnosed among women in 2020, and 6·9% (684,996) of all cancer related deaths among women in 2020 worldwide.[3] According to GLOBOCAN 2020,[2] breast cancer is responsible for increased mortality among women in India, with an estimated 13,24,413 of all new cancer cases and 13·5% (1,78,361) of new breast cancer cases.[4] India was ranked third amongst the countries with highest number of breast cancer cases in 2018 affecting all age groups (19 to 85). Women in older age groups are at increased risk to breast cancer and even more with a family history.[5,6] Life style factors such as low parity, lack of breast feeding, alcohol consumption and physical inactivity are the chief causes of breast cancer among urban population of India.[7] The breast cancer etiology includes age, family history, personal history, dense breast tissue, previous chest radiation, obesity, increase in exogenous or endogenous estrogen hormone, smoking, drinking alcohol, consumption of high-cholesterol food, red and processed meat and physical inactivity.[8] Several other triggering factors for causing the disease are yet to be known.

Sporadic breast cancer is a result of accumulation of molecular abnormalities in normal breast tissue. The overexposure to estrogens and other carcinogens associated with poor dietary habits is an important reason for the development of sporadic breast cancer. Breast cancer develops from the progressive accumulation of mutations in “driver” and other genes that gives proliferative advantage to the cells. It has been shown that 50% – 70% of women who develop breast cancer have no identifiable risk factors.[9] Supporting the notion that many women are in fact accumulating carcinogenic genomic changes in their breast tissue due to unknown risk factors.

Studies have indicated that, several prominent molecular changes in normal breast tissues, including loss of heterozygosity, or allelic imbalance from small segmental deletions at loci of potential tumor suppressor genes, are the major molecular changes that occurs in breast cancer. DNA methylation of tumor suppressor genes and other genes, telomere shortening, potentially overexpression of Her-2/neu and p53 mutations, telomerase expression, widespread abnormalities in gene expression, and loss of cell cycle control are other common molecular abnormalities that are responsible for the potential development of breast cancer. Apart from loss of function mutation of p53 gene, mutation in other tumor suppressor genes such as $p16^{INK4A}$, BRCA-1 and BRCA-2 are considered to be the high-risk genetic factors for breast cancer development.[10–13]

In the recent times, due to geogenic cause, the arsenic poisoning in ground water has increased many folds in the state of Bihar in endemic proportions.[14–18] This has increased the disease burden in the arsenic exposed population of Bihar. Arsenic poisoning leading to cancer has been studied globally, and a

relationship between arsenic and cancer has been established.[19,20] Khanjani et al., (2017)[21] has also stated that exposure to arsenic may increase the risk of breast cancer and may be attributed to the high breast cancer incidence.[22–24]

The present study, aims to find the relationship between arsenic contamination and breast cancer risk in the cancer patients of Bihar. This study is a maiden effort globally to establish the direct relationship of arsenic with breast cancer risk.

Materials And Methods

Ethical Approval: The study was approved from the Ethics Committee (IEC) of Mahavir Cancer Sansthan and Research Centre with the approved IEC No. MCS/Research/2015-2016, dated 24/08/2016, (agenda serial no. 15). Written consent were obtained from all the studied subjects as per the norms of the ethics committee.

Location: The study was carried out in Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India during June 2016 and January 2019. A total of 55 pathologically confirmed breast cancer patients were selected for the study, while for the cross-sectional design, 12 subjects with pathologically benign breast disease were selected representing the control group.

Selection of the subjects for the study:

(a) Cancer patients: Approximately, 15,000 confirmedcancer cases are registered in the institute annually. Out of which, about 23% (n=3450) are of confirmed breast cancer cases. Pathologically confirmed, female breast cancer patients (n=55) from across the state were included in the study. All the selected patient's blood, hair, toenail and breast tissue samples were provided by the surgical oncologist involved in this study for the estimation of arsenic in these samples.

(b) Control Subjects: As a part of cross-sectional study, (n=12) female breast benign cases were taken as the control group and their biological samples were also collected. Their epidemiological data were also recorded for the study. For the epidemiological parameter study, their age, type of disease and their demographic information was compiled. All the samples as per the protocol of NIOSH were digested and analysed by Graphite furnace atomic absorption spectrophotometer.

Biological sample collection from the breast cancer patients with the breast benign subjects:

a) Blood sampling: About 5ml of blood by volume were collected from the peripheral vein of the arm of the patients using disposable syringes and thereafter transferred to the heparinised vacutainer as per the IUPAC guidelines.[25] The blood samples were stored at a temperature of -80 degree centigrade for the future retrieval and use.

b) Hair andtoenail sampling: About 200mg of hair sample of the patients from the hair root was collected. Likewise,200mg of the toenail samples were also collected. The samples were cleaned through

sterile tissue papers and kept in the zipper packs. The hair and nail samples were stored at a temperature of -80 degree centigrade for the future retrieval and use.

c) Breast tissue sampling: The patients who went for the breast surgery (mastectomy) or breast benign lump removal (lumpectomy) were identified for the study.Their breast tissue (~1g) provided by the surgical oncologist was well packed in zipper bags. The breast tissue samples were stored at a temperature of -80 degree centigrade for the future retrieval and use.

Estimation of blood arsenic concentration:

For the blood arsenic estimation, 0·5ml of wholeblood sample was taken in 30ml conical flask(glass) to which,5ml of HNO_3 was added and left for overnight reaction. The following day, all the samples were digested on hotplate at 90^oC – 120^oC, allowing volume expansion upto 3ml. Then 5ml volume of HNO_3:$HClO_4$ (6:1) mixture wasadded to the pre-digested solution in the conical flask. The samples were re-digested on the hotplate at 90^oC – 120^oC until the volume of the solution reached to about 2ml. The final volume was adjusted to 10ml with addition of distilled water after rinsing it with 1% HNO_3 and was then filtered through Whatman filter paper no.41 for determination of the final reading on Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS).

Estimation of hair samples for arsenic concentration:

For the hair sample estimation, 100mg of hair samples were weighed and kept in 50ml acid washed glass beaker. To it, 15ml of 0·1% sodium dodecyl sulphate (SDS) solution was added and sonicated for 10 minutes. Hair samples were rinsed with distilled water three times and then decanted. Thereafter, 15 ml of acetone was added to dry the hair sample. The hair samples were transferred in a dry 50ml glass beaker10ml of Conc. HNO_3 added to it and left for the overnight reaction.The following day, samples were digested on hotplate at 90^oC – 120^oC allowing volume expansion upto~3ml. To this solution, 1ml of 30% H_2O_2 was added and the solution was re-digested on the hotplate at 90^oC – 120^oC until the volume of the solution was lowered to 2.5ml. The solution was rinsed with 1% HNO_3 and the final volume was adjusted to 9ml with distilled water and was filtered through Whatman filter paper no.41 for determination of the final reading on GF-AAS.

Estimation of Toenail samples for arsenic concentration:

For the toenail sample estimation, 100mg of nail samples were weighed and kept in 50ml acid washed glass beaker. To it 10ml of 0·1% sodium dodecyl sulphate (SDS) solution was added and sonicated for 15 minutes. Nail samples were then rinsed with distilled water three times and then decanted. Thereafter, 10 ml of acetone was added to dry the nail samples. The nail samples were transferred in a dry 50ml glass beaker and 10ml of Conc. HNO_3:$HClO_4$ (6:1) mixture was added to it and left overnight.
Thefollowing day, samples were digested on hotplate at 90^oC – 120^oC until the volume of the solution reached to approximately 3ml. To this solution 1ml of 30% H_2O_2 was added and the solution was re-

digestedon the hotplate at 90^{o}C – 120^{o}C until the volume of the solution was lowered to ~2.5ml. The solution was rinsed with 1% HNO_3 and the final volume was adjusted to 10ml with distilled water and was filtered through Whatman filter paper no.41 for determination of the final reading on GF-AAS.

Estimation of breast tissue arsenic concentration:

For the breast tissue arsenic estimation, 0.5g of tissue sample were taken in 30ml conical flask (glass) and 5ml of HNO_3 was added to it and then left for overnight reaction. The following day, all the samples were digested on hotplate at 60^{o}C for 2 hours. The samples after cooling were added with 2ml of $HClO_4$. The solution was re-digested on hotplate at 90^{o}C – 120^{o}C for 5-10 minutes until the white fume of $HClO_4$ emitted. The solution was then cooled and 15ml of distilled water was added and was filtered through Whatman filter paper no.41 for determination of the final reading. All the samples were read through Graphite Furnace Atomic Spectrophotometer (Pinnacle 900T, Perkin Elmer, Singapore).

Quality Control: For the entire study, the quality control of the standards, the calibration and the correlation coefficient (@ 0.999) were maintained on the Atomic Absorption Spectrophotometer. The known concentration of arsenic for standard was prepared from the standard stock solution (1000µg/L) purchased from PerkinElmer (CAS No. As 7440-38-2; Lot #: 20-85ASX1; PE #: N9300102), Singapore. The detection limit of arsenic was 0.05µg/L microgram per litre for blood, 0.08µg/L for hair and 0.09µg/L for toenail while 0.04µg/L for breast tissue.

GIS analysis:

The data of arsenic concentration in breast tissue, blood, hair and toenail samples of the breast cancer patients were taken as input in ArcGIS 10.3. software for preparation of the spatial distribution map, correlation and understanding the exposure pattern. The synoptic view was visualized through the exposure rate pattern. Concentrations of arsenic in the biological samples of the breast cancer patients were analysed with the help of statistical data depicted through a scaled map. To validate the exposure rate, the arsenic background status map was utilized developing thematic layers on ArcGIS platform. The software used in the map layer generation was ArcGIS Version 10.3.

Statistical Analysis:

The data was analysed using the GraphPad Prism 5·0 software and the values generated as Mean ± SEM. Differences between the groups were analysed statistically through one-way analysis of variance (ANOVA) by using the Dunnett's test. The scatter plots were prepared using the statistical software SPSS-16.0 and the linear regression analysis model earlier by Kumar et al., (2021),[26] and Sanz et al., (2007).[27]

Results

In the present study, histopathologically confirmed (n=55) breast cancer patients along with (n=12) breast benign cases were taken up. The epidemiological information of all the patients with studied variables

such as age, cancer stage, demographic area etc. were recorded. Interpretation of the reports of arsenic concentration in breast tissue, blood, hair and in toenail samples were carried out to derive informationon risk assessment along with the cancer stage. Correlation was made between following datasets: arsenic in blood vs cancer patient age, cancer patient's breast tissue vs cancer patient age, cancer patient's hair vs cancer patient age, cancer patient's nail vs cancer patient age, cancer patient's blood vs cancer patient breast tissue and cancer patient's hair vs cancer patient nail samples respectively. Control cases were also represented accordingly for the statistical correlation. Finally, the spatial distribution map depicting cancer cases was plotted to know the prevalence of the disease on district level.

1. **Breast Cancer patient's vs breast benign control cases (Age wise):** Out of total n=55 female breast cancer patients, the cancer incidences the maximum cases were found in the age group interval 41-60, while amongst the breast benign cases, the maximum age group lied between 31-40 (Fig.1).
2. **Breast cancer patient's vs breast benign control subjects (Blood arsenic concentration).**Out of n= 55 breast cancer patient's blood analysed for arsenic concentration, the maximum arsenic concentration observed was 1856µg/L while the minimum value of arsenic concentration observed was 24·6µg/L. None of the patients had the arsenic concentration between the range 0-20µg/L. In the breast benign control group (n=12), the maximum arsenic concentration observed was 18·76µg/L while the minimum arsenic concentration value was zero or not traceable. It is apparent from the results that the levels of blood arsenic concentration in the control group is within the normal limit range of 0-20µg/L(Fig.2).
3. **Breast cancer patient's vs breast benign control subjects (Breast tissue arsenic concentration):** Out of n= 55 breast cancer patient's breast tissue analysed for arsenic concentration, the maximum arsenic concentration observed was 446·4µg/Kg while, the minimum value of arsenic concentration observed was 11.34µg/Kg. In the breast benign control group (n=12), the maximum arsenic concentration observed was 8.85µg/L while the minimum arsenic concentration value was less than 1µg/Kg(Fig.3).
4. **Breast cancer patient's vs breast benign control subjects (Arsenic concentration in Hair samples):** Out of n= 55 breast cancer patientsanalysed for arsenic concentration in hair samples, the maximum arsenic concentration observed was 1296·9µg/Kg while the minimum value of arsenic concentration observed was 107·1µg/Kg. In the breast benign control group (n=12), the maximum arsenic concentration in hair samples observed was 20.79µg/Kg while the minimum arsenic concentration value was less than 1µg/Kg (Fig.4).
5. **Breast cancer patient vs breast benign control subjects (Arsenic concentration in Toenail samples):** Out of n= 55 breast cancer patients analysed for arsenic concentration in toenail samples, the maximum arsenic concentration observed was 621·83µg/Kg. The minimum value of arsenic concentration observed was 1.64µg/Kg. In the breast benign control group (n=12), the maximum arsenic concentration in the toenail samples observed was 33·07µg/Kg while the minimum arsenic concentration value was less than 1µg/Kg (Fig.5).

6. **Correlation coefficient between the age of the cancer patient vs arsenic concentration in blood and breast tissue samples (Breast cancer patients):**
   The study showed significant increase in the blood arsenic concentration with the increase in the age of the breast cancer patients ($r = 0{\cdot}025$ and $p < 0{\cdot}05$). There was also a significant increase in the arsenic concentration in breast tissue with the increase in the age of the breast cancer patients($r = 0{\cdot}084$ and $p < 0{\cdot}05$) (Fig.6).
7. **Correlation coefficient between the age of the cancer patient vs arsenic concentration in hair and toenail samples (Breast cancer patients):**
   The study showed significant increase in the arsenic concentration in hair samples with the increase in the age of the breast cancer patients ($r = 0{\cdot}003$ and $p < 0{\cdot}05$). There was also significant increase in the arsenic concentration in the toenail samples with the increase in the age of the breast cancer patients ($r = 0{\cdot}028$ and $p < 0{\cdot}05$) (Fig.7).
8. **Correlation coefficient between the blood sample of the breast cancer patient vs arsenic concentration in the breast tissue sample of the breast cancer patients and hair arsenic concentration of the breast cancer patient vs the toenailarsenic concentration of the breast cancer patient:**
   The study showed significant increase in the arsenic concentration in the blood samples with the breast tissue samples of the breast cancer patients ($r = 0{\cdot}203$ and $P < 0{\cdot}05$). There was also significant increase in the arsenic concentration in the toenail samples with the hair samples of the breast cancer patients ($r = 0{\cdot}116$ and $p < 0{\cdot}05$) (Fig.8).
9. **Breast cancer patient's stage wise study:** Out of total n= 55 breast cancer patients, n=2 patients were in the Stage I, n=15 patients were in the Stage II, n=10 patients were in the Stage III and n=28 patients were in the Stage IV ($p<0.05$). This denotes that 51% of the cancer patients were in the highly advanced stage of the disease (Fig.9).
10. **Stage wise distribution of Breast cancer patients with the arsenic contamination in the breast tissue:** In the present study, there was significant rise in the arsenic concentration in the breast tissue of the breast cancer patients (n=55). The average arsenic concentration in the breast tissue in the Stage I was $15{\cdot}19 \pm 3{\cdot}85\mu g/Kg$, in the Stage II was $64{\cdot}2 \pm 8{\cdot}29\mu g/Kg$, in the Stage III was $89{\cdot}80 \pm 16{\cdot}10\mu g/Kg$ and in the Stage IV was $144{\cdot}1 \pm 20{\cdot}62\mu g/Kg$ respectively. This denotes that arsenic exposure is directly proportional to the stage of the patient (Fig.10).
11. **Geospatial distribution of Breast cancer patient's:** The maps show the district wise geospatial distribution of breast cancer patient with the exposure rate in their biological samples such as breast tissue (Fig.11A), blood (Fig.11B), hair (Fig.11C)and toenails (Fig.11D).

Geological Aspect: The high anomalous concentration in toenail samples, tissue, hair and nail samples clearly correspond with the endemic provinces in the state of Bihar. These are the districts either lying along the oscillation zone of Ganga river or in the districts of North Bihar. These as a result of geological studies have been established as regions with high contamination in groundwater and other media. The present study is also to do with samples of patients having domicile in these districts and it is likely that

they have been exposed to sustained arsenic contamination for a prolonged period. On the contrary, the lesser values are mostly forming a constellation in districts of south Bihar which have lesser or no incidences of arsenic with a geogenic control. The districts shown with white colour are the ones which have not been sampled for this study.Only in case of blood samples of patients from Gaya and Rohtas that values exceeding >400 ppb have been recorded. There is a possibility that these patients may have migrated from the arsenic prone districts or may have been exposed to localised problems of arsenic as observed around Amjhor Pyrite mines in Rohtas district, Bihar.Therefore, the spatial distribution map is also indicative of the morpho-stratigraphic controls for arsenic which is heavily tilted towards districts of North Bihar and the riparian districts along the oscillation zone of Ganga river, deriving sediments primarily from Extra peninsular source. This however calls for detailed understanding of the domicile of the sampled population with confirmed cancer incidences.

Discussion

Population inhabiting in the arsenic affected districts are to the ill effects of the element for a sustained period leading to severe health manifestations as a result of changes in the metabolic function of the body system. The arsenic through drinking water reaches the human metabolic system where it is metabolised into the least toxic form i.e. dimethyl arsenic acid (DMA) from either arsenic (III) or arsenic (V). However, arsenic (III) is more toxic to the cells in comparison to the arsenic (V).[28–32] The arsenic from the liver is mostly eliminated through the kidney via urine. But, it has the affinity to bind with the sulfhydryl groups disrupting the metabolic functions of the body. Hormones are also influenced due to arsenic toxicity. Female reproductive hormones such as oestrogen, progesterone, luteinising and oxytocin hormones are disturbed due to the xenoestrogenic nature of arsenic. It not only disrupts the functions of oestrogen hormone but other associated hormones as well leading to hormonal imbalance which is the major risk factor for the cause of carcinogenesis of breast in female subjects.[33,34]

There are various markers for breast cancer which speculates the disease confirmation but not the etiology. Most of the studied aetiologies are in the form of speculations. But the present study, confirms the relationship of arsenic exposure with the breast cancer. This study is the maiden attempt to ascertain the arsenic concentration levels in breast cancer, breast tissue, blood, hair and toenail samples. In the present study, there was significantly very high arsenic concentration in the female breast tissue, blood, hair and toenail samples of the (n=55) breast cancer patients. Moreover, the study also compared these levels with the female breast benign cases as the control group (n=12). There has been no benchmark range set up for the arsenic concentration in the breast tissue by any of the environmental agencies. The findings also indicate a significantly high arsenic concentration level in the breast tissue (446·4µg/Kg) while the lowest level determined was 11·34µg/Kg. Moreover, n=53 (96·36%) patients had relatively much higher arsenic concentration in comparison to the control group (n=12) where the maximum arsenic concentration was recorded as 8·85µg/Kg. Similarly, in the blood, the highest arsenic concentration was 1856µg/L in comparison to the control group 18·76µg/L. Previously, very high arsenic concentration in the blood samples of the cancer patients was reported by.[26,35] The hair and the toenail samples relatively

had very high arsenic concentration in hair samples (1296·9µg/Kg) while in toenail samples, as high as 621·83µg/Kg was recorded. This was significantly higher than the control samples. The normal levels of arsenic contamination in the unexposed human population ranges between 20-200 µg/Kg in hair and 20-500 µg/Kg in nail.[36]

The correlation coefficient study between the age of the breast cancer patient with the arsenic concentration in blood and breast is highly significant (r = 0·025 & 0·084 respectively). It denotes that with increase in the age of the breast cancer patient, the arsenic concentration in blood and breast tissue significantly increases. This indicates a strongly positive correlation. Further, when the blood arsenic concentration was compared with the breast tissue, a significant correlation (r=0·003) was found. Finally, the correlation coefficient study between the hair and toenail samples indicated a significant association (r=0·028), indicating that the sustained exposure to arsenic has a strong impact on the blood, breast tissue, hair and nails of the body.

Moreover, arsenic has the affinity to bind with the sulfhydryl groups and keratin protein of the body. The hair and nail are the examples of the keratin tissue. Hence, contamination in them denotes the severity of the exposure level and the deposition of arsenic in them may have triggered the incidence of breast cancer.[19,36–42]

The stage wise distribution of the breast cancer patients reveals that the majority of the patients were in the advance stage of the disease i.e. III and IV. However, in the breast benign cases the arsenic concentration was non-significant as their disease presentation was in pre-cancerous stage. The most significant part of the study is that there is significant ($p<0·05$) increase in the arsenic concentration in the breast tissue of the breast cancer patients with the cancer patients' stage. Moreover, the stage III and the stage IV which are the advance stage of the disease accounted the maximum arsenic contamination in the breast tissue which validates this novel finding.

Thus, the exposure to arsenic could significantly increase the risk of the disease several folds. The spatial distribution of the breast cancer patients with increased arsenic concentration in their biological samples helps to infer that the disease burden is very high in the riparian districts along the Gangetic plains of Bihar. These primarily include Buxar, Bhojpur, Patna, Vaishali, Saran, Begusarai and Khagaria districts with anomalous values of >300µg/L, while other districts away from the regime of Ganga river (Gopalganj, Sitamarhi, Muzaffarpur, Nalanda, Gaya, Saharsa etc) have value ranges < 300 µg/L.

Recently, similar, study to establish the etiology of blood arsenic with cancer has been extensively carried out.[26] Arsenic since prehistoric times, is a known poison but IARC and WHO has recognised it as a Category-I carcinogen causing cancer of skin, lung, bladder, kidney and colorectum in recent times in the exposed population.[43–45] Arsenic exposure may also enhance the risk of breast cancer many folds.[21] However, there may be various signalling pathways involved, which might be disrupted due to this metal toxicity. Usually due to regular exposure of arsenic the up-regulation signalling pathways in breast carcinogenesis such as BCL-2, MMP-2, MMP-9, VIM, Snail twist, MT, MLH and in downregulation of Casp-

3, PTEN, E-CAD and BAX are transformed. Moreover, the KRAS, p53, TGF-β, WNT, NRF2 and AKT are mostly activated.[46] At the genetic level, mutations in CHEK2, PALB2, NBN, BRCA1 and other susceptible genes have been found to be mutated due to metal toxicity.[47–49]

Marciniak et al., (2020),[35] have evaluated the arsenic exposure with 13 folds risk of breast cancer in the exposed population. The study also correlates the blood arsenic levels with the breast cancer risk in women. Moreover, the other groups have also identified the arsenic relationship with toenail, hair, blood or urinary arsenic species in the cancer and controls.[50–53] These studies are in line with the present study - Blaurock-Busch et al., (2014);[54] Benderli et al., (2011);[55] Garland et al., (1996);[56] Altaise et al., (2010);[57] Gamboa- Loira et al., (2017);[58] Joo et al., (2009).[59]

Geological Investigations are constantly indicating a probable extra peninsular source to the possible geogenic cause of Arsenic in the foredeep basin. The drainages emanating from the Himalayas are supposedly carrying more Arsenic laden sediments into the gangetic foredeep which due to secondary processes and conditions is undergoing secondary enrichment. With increased tube well revolution the shallower aquifers are being vociferously targeted for drinking, domestic and agricultural needs thus exposing a huge population to the deleterious effects of Arsenic. The research to work out the exact source which appears to be more than one is progressing on an encouraging pace and is likely to bring out the source conditions responsible for the endemicity.

The increasing population residing in endemic provinces are ignorantly getting exposed to this metalloid and in turn its sustained toxicity on the human physiological and metabolic system. The continuous arsenic exposure (which is a xenoestrogen) to women causes hormonal imbalances, which intensifies the hormone levels many folds. The estrogen hormone which controls over the FSH, LH, prolactin and oxytocin hormones causes cellular changes in the breast tissue such as ductal proliferation or alveolar differentiation (proposed pathway Fig.12). The women lacking basic health facilities, supplemented by their ignorance of the symptoms of the disease such as lumps of the breast are eventually getting diagnosed at an advanced stage where survival gets even more difficult. From the start of the exposure till the initiation of the breast cancer disease, it usually takes 10-15 years. The exhibition of the breast cancer disease symptoms in the third or fourth stage is still a mystery. Moreover, there are studies which speculate that downregulation of p16 gene ($p16^{INK4a)}$ and p53 (guardian of the genome) tumour suppressor genes especially the methylation of the genes could lead to the silencing of the disease symptoms in breast cancer as well as other cancer types.[60–68] Various other studies have also established the biological and molecular modifications caused by arsenic in the development of breast cancer.[21,69,70]

Previously, a study in the year 2018 speculated that ground water arsenic contamination in the Ganga basin could be the major health danger to the exposed population. The study also emphasizes that arsenic catastrophe in the Indo-Gangetic plains could elevate the magnitude of the disease burden many folds. Hence, the exposed subjects would be more vulnerable or at high risk to the disease.[71-73]

This study draws through its findings that significant changes do occur at the subcellular levels due to sustained exposure to arsenic leading to increased risk to cancer. What is even more disturbing that the control subjects are equally vulnerable to the risk to disease in future. The presence of high arsenic concentration in the breast tissue does validate the correlation of arsenic and the disease etiology. The finding of this study is the first of its kind globally, establishing a direct correlation of arsenic and breast cancer. Moreover, the arsenic contamination in the breast tissue of the cancer patients was directly proportional to the stage of the patients, hence the major chunk of the patients were in the advance stage of the disease with maximum arsenic accumulation which validates this novel finding. Focussed and collaborative studies in the field of medicine and geology is thus required to validate the increasing breast cancer cases, gradually gaining rounds in the Gangetic plains of Bihar.

Conclusion

The present study demonstrates arsenic contamination in the breast tissue, blood, hair and nail samples of the breast cancer patients in comparison to the breast benign control group. Arsenic poisoning affecting around 10 million people in the state is a matter of concern as the numbers are increasing briskly with the passage of time. The women population is showing increased breast cancer etiological accentuated by poor health standards, malnutrition and lack of awareness.

The arsenic accumulation in the breast tissue is a guiding evidence which validates the correlation between the breast disease and the exposure to arsenic. However, focussed studies are required to validate these findings especially the molecular and signalling pathways. Furthermore, the etiology of subdued symptoms of disease in the exposed subjects is a matter of research, and a prudent effort in that direction could lead to better management helping in the early diagnosis of the disease.

Declarations

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Author contributions:

A.K and A.K.G. conceptualized the entire work. A.K. is the principal author and had the major contributions in writing the manuscript but support was also provided by A.K.G., A.B., D.K., P.J. G.P., R.R, M.S. and A.R., literature search was done by V.A, V.K and A.P., experimental work and data analysis were done by P.K.N, V.A, V.K, M.S.R, geospatial mapping figures were designed by S.S., A.B. and S.K., final data interpretation was done by A.K., M.A, R.K, D.K., R.R, M.S. and A.B. All authors read and approved the final manuscript.

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Data availability

The data that support the findings of this study is available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing interests.

Statement: This is to confirm that all methods were carried out in accordance with relevant guidelines and regulations.

Consent to publish: Written informed consent was obtained from the patients for the publication of this study and accompanying clinicopathological data.

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Figures

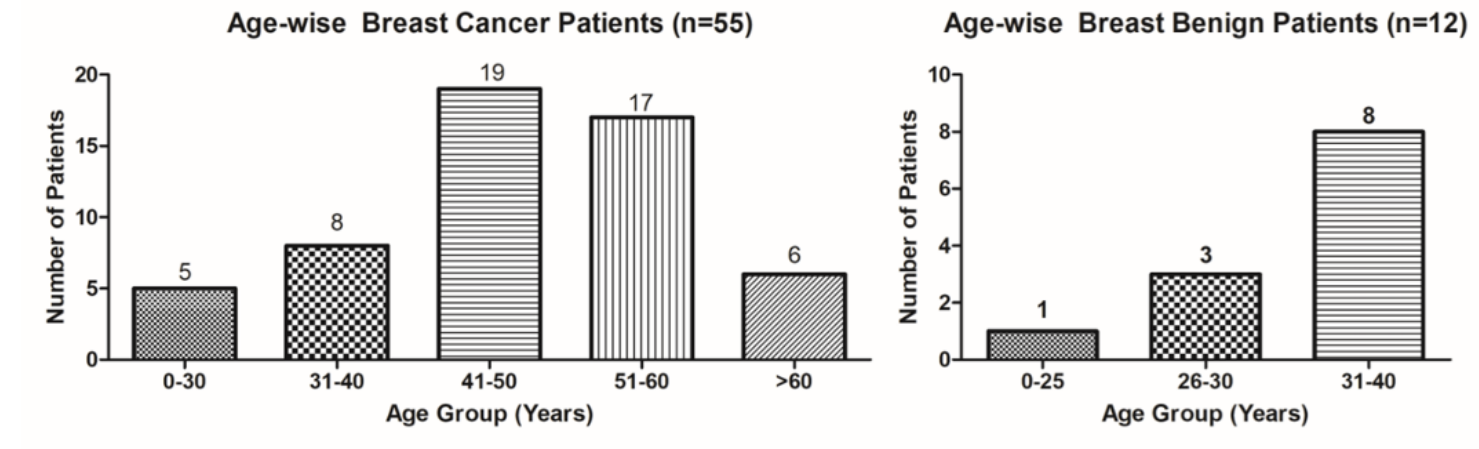

Figure 1

Graph figure represents the age wise distribution of female breast cancer patient's vs female breast benign control subjects. (ANOVA-Dunnett's Test, $p<0.05$).

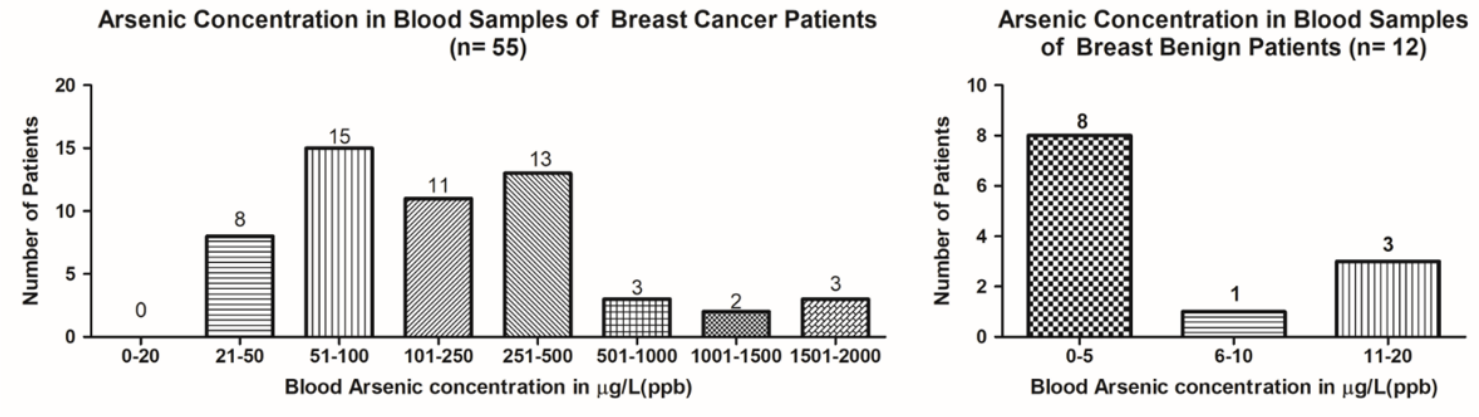

Figure 2

Graph figure represents the distribution of blood arsenic concentration in female breast cancer patient's vs female breast benign control subjects. (ANOVA-Dunnett's Test, $p<0.05$).

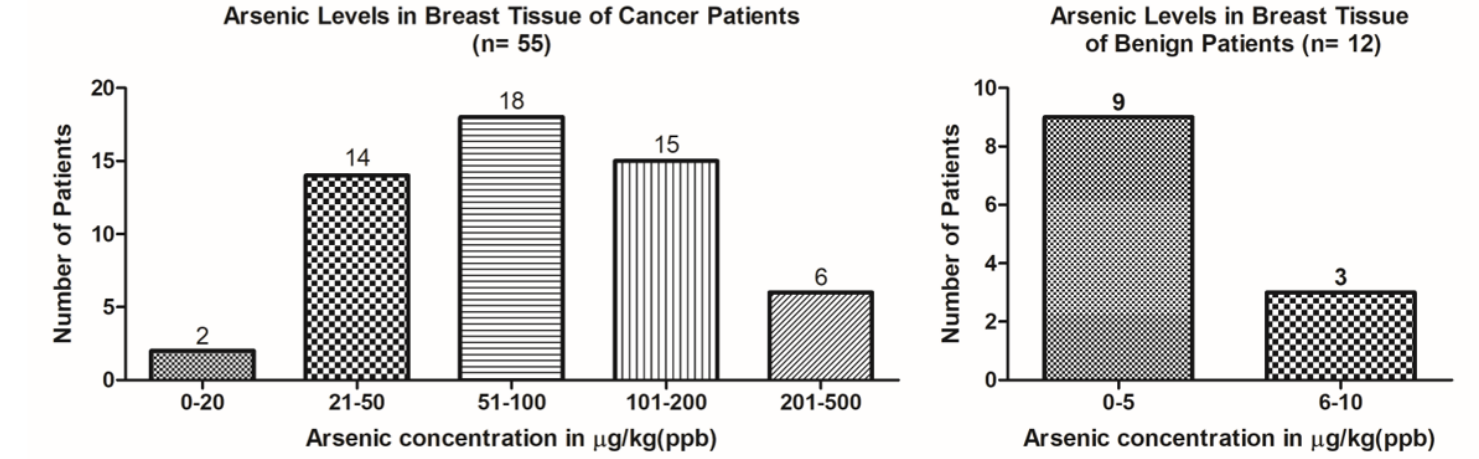

Figure 3

Graph figure represents the distribution of arsenic concentration in breast tissue of female breast cancer patient's vs female breast benign control subjects. (ANOVA-Dunnett's Test, p<0.05).

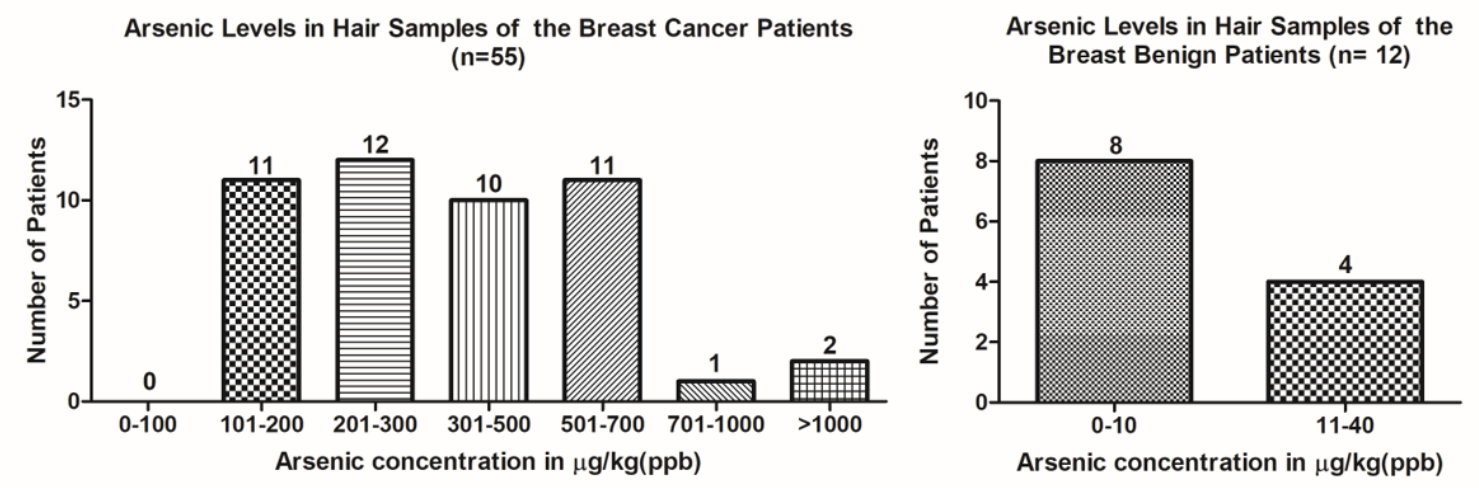

Figure 4

Graph figure represents the distribution of arsenic concentration in hair samples of female breast cancer patient's vs female breast benign control subjects. (ANOVA-Dunnett's Test, p<0.05)

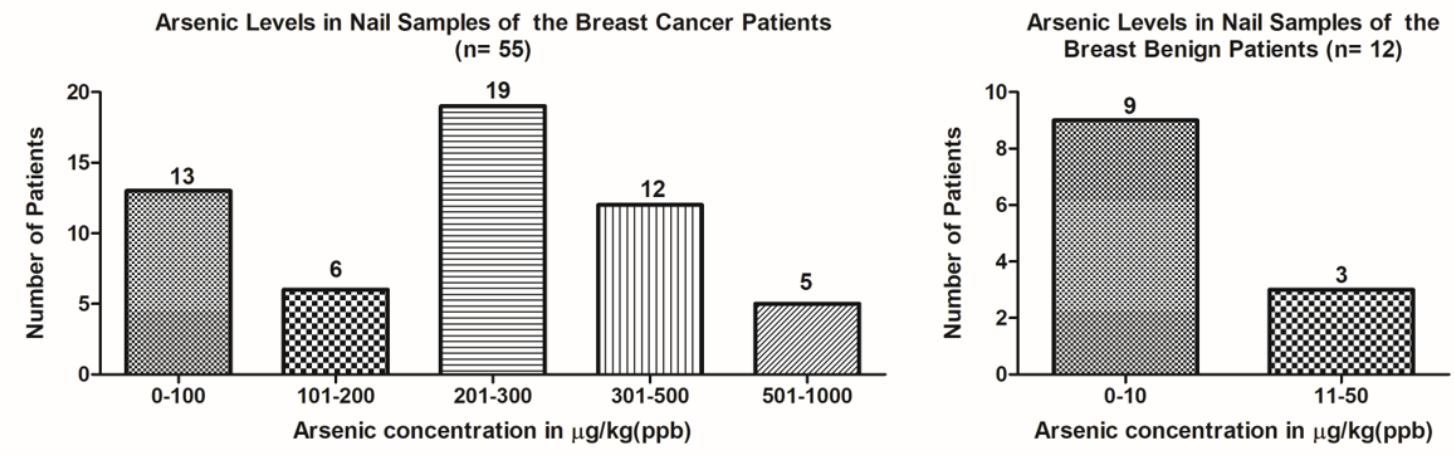

Figure 5

Graph figure represents the distribution of arsenic concentration in toenail samples of female breast cancer patient's vs female breast benign control subjects. (ANOVA-Dunnett's Test, p<0.05).

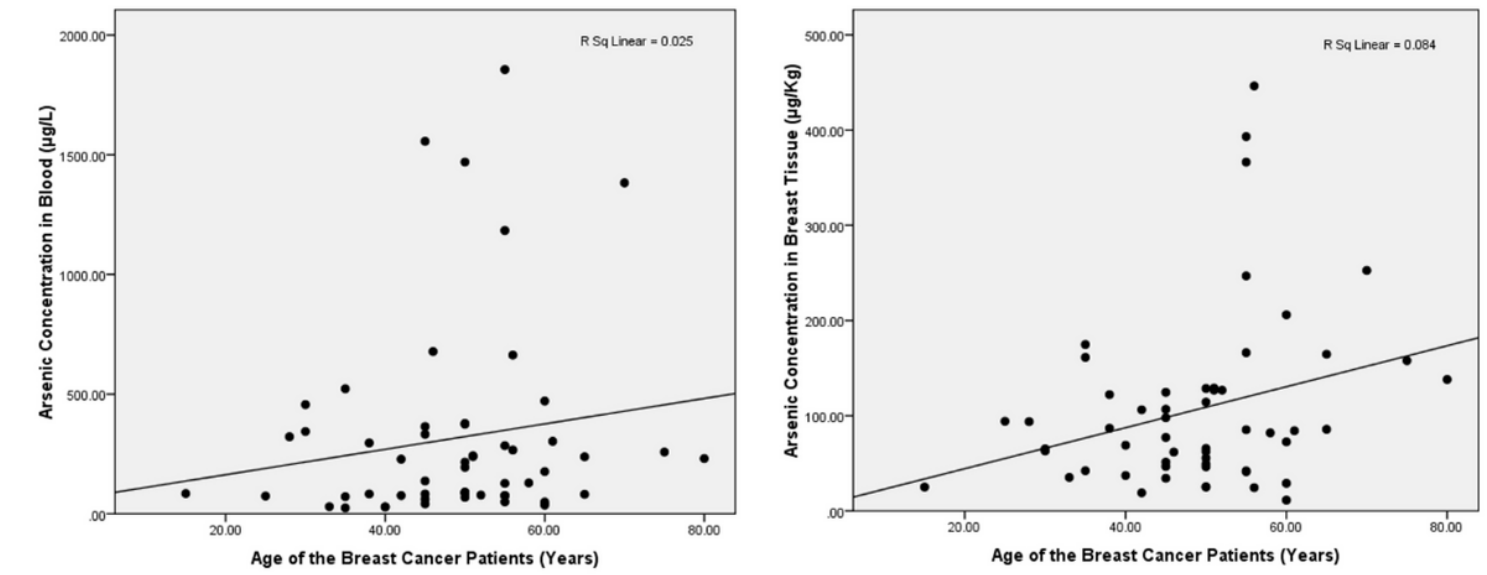

Figure 6

Correlation coefficient between the age of the breast cancer patients with the arsenic concentration in blood and breast tissue of the breast cancer patients ($p < 0.05$).

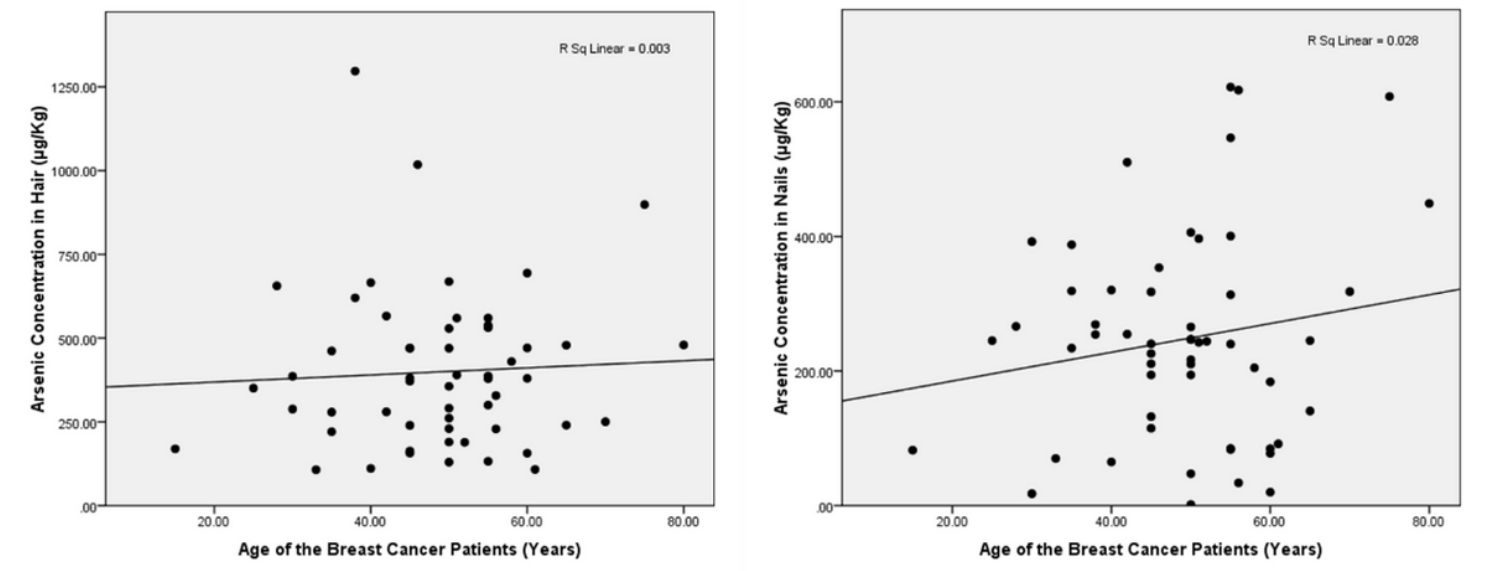

Figure 7

Correlation coefficient between the age of the breast cancer patients with the arsenic concentration in hair and toenail samples of the breast cancer patients ($p < 0{\cdot}05$).

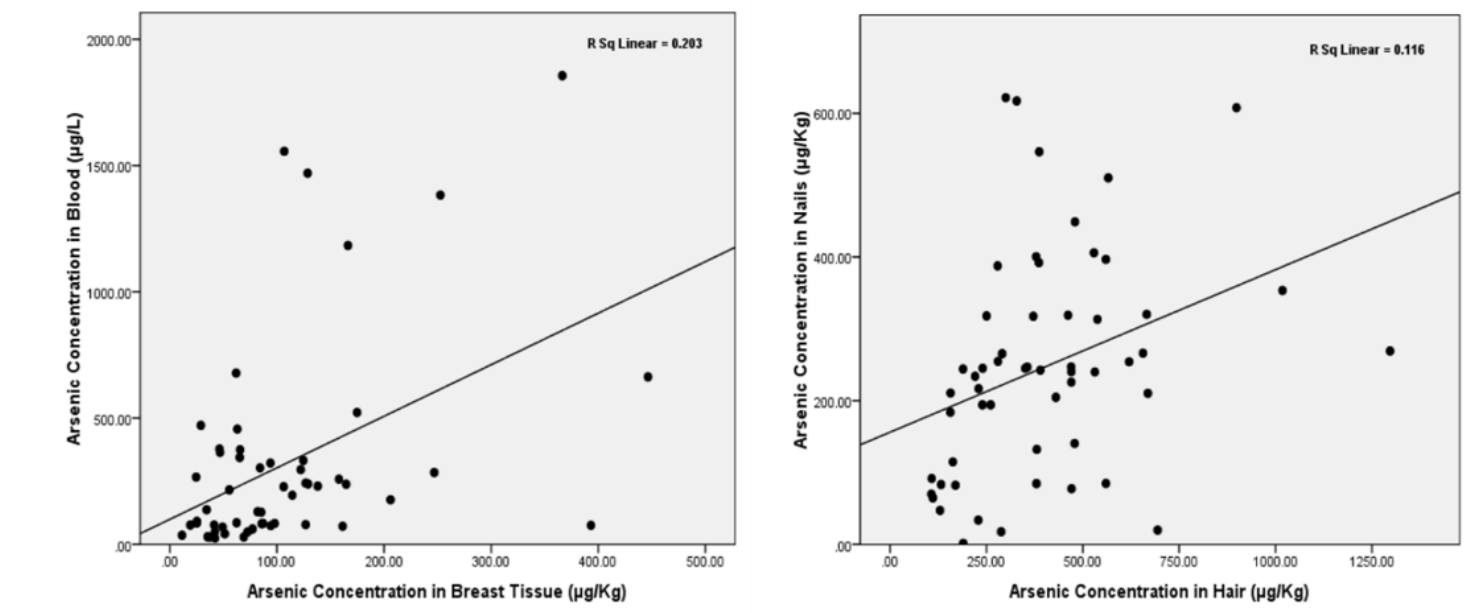

Figure 8

Correlation coefficient between the blood sample of the breast cancer patient vs arsenic concentration in the breast tissue sample of the breast cancer patients and hair sample of the breast cancer patient vs arsenic concentration in the toenail sample of the breast cancer patients ($p < 0.05$).

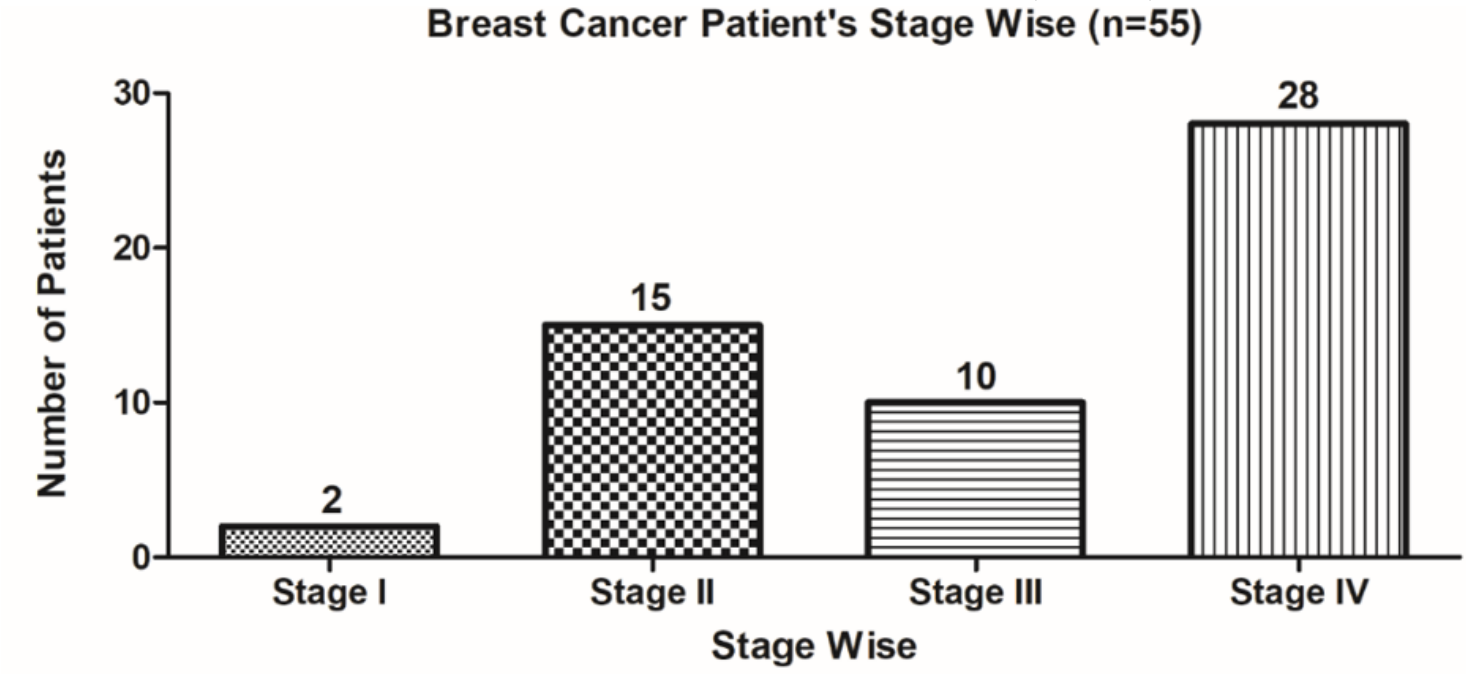

Figure 9

Stage wise distribution of the breast cancer patients ($p < 0{\cdot}05$).

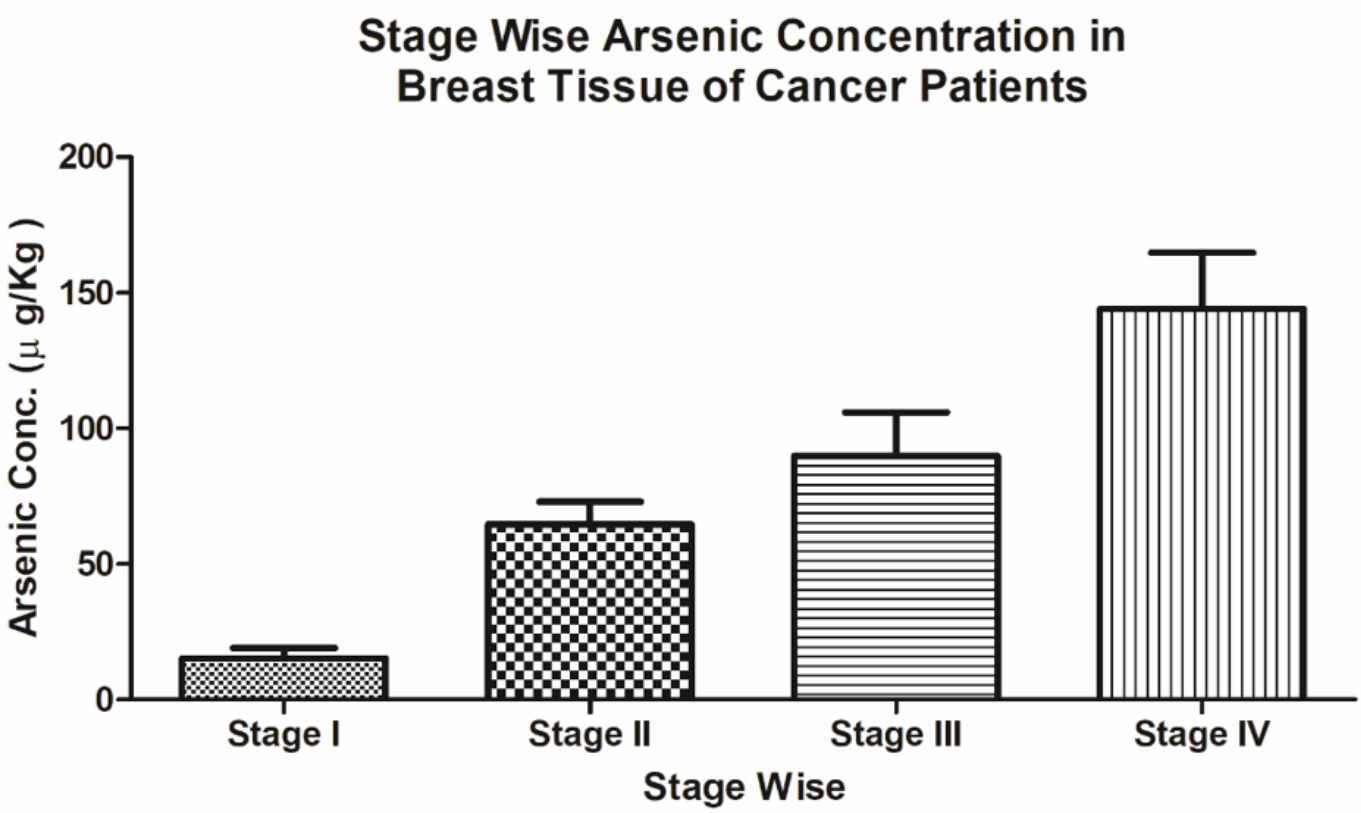

Figure 10

Stage wise distribution of the breast cancer patients with arsenic concentration in the breast tissue ($p < 0.05$).

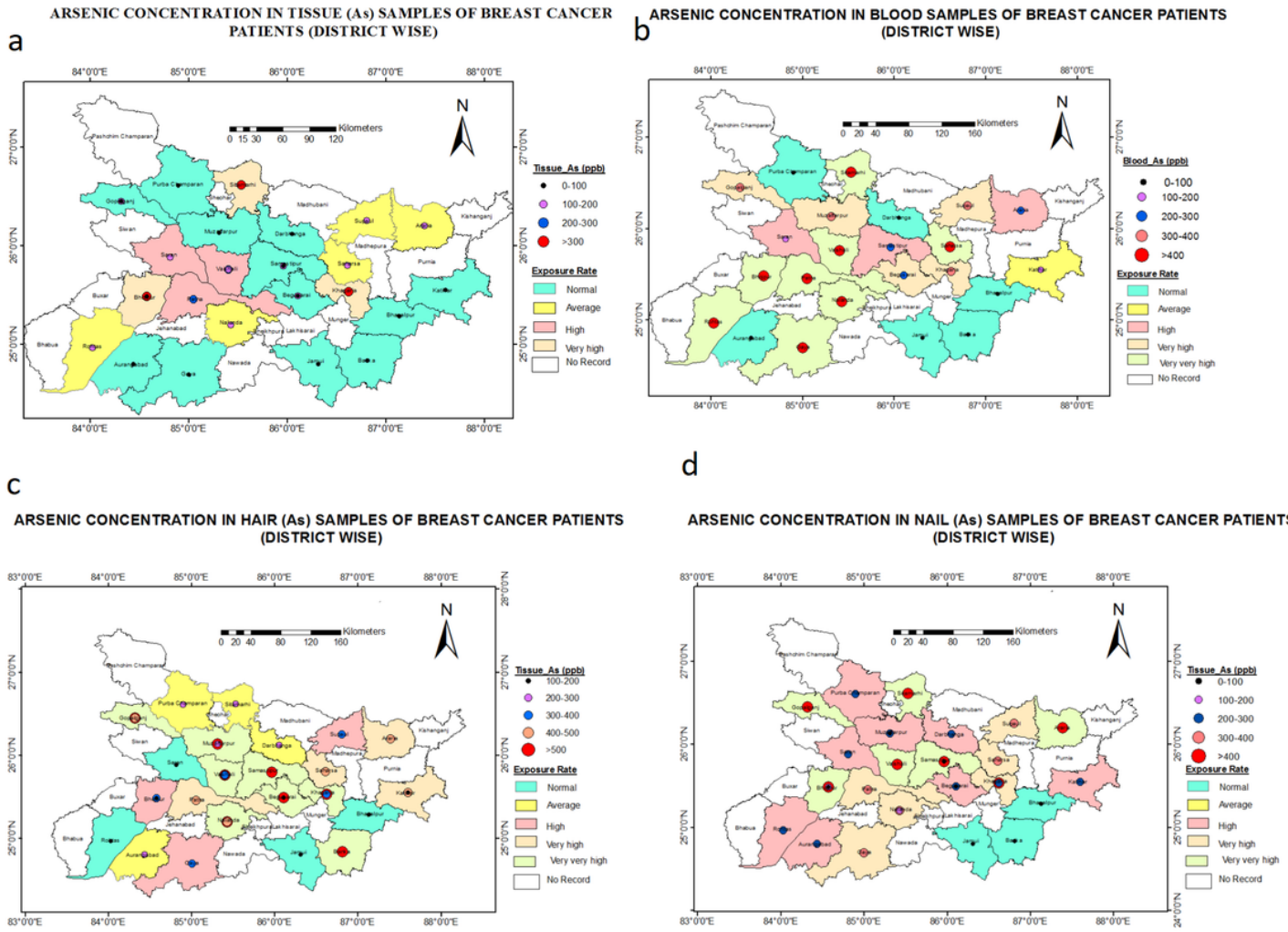

Figure 11

A: Geospatial distribution of breast cancer patients with average arsenic concentration in their breast tissue. (Base map extracted using ArcGIS 10.3). B: Geospatial distribution of breast cancer patients with average arsenic concentration in their blood samples. (Base map extracted using ArcGIS 10.3). C: Geospatial distribution of breast cancer patients with average arsenic concentration in their hair samples. (Base map extracted using ArcGIS 10.3). D: Geospatial distribution of breast cancer patients with average arsenic concentration in their toenail samples. (Base map extracted using ArcGIS 10.3).

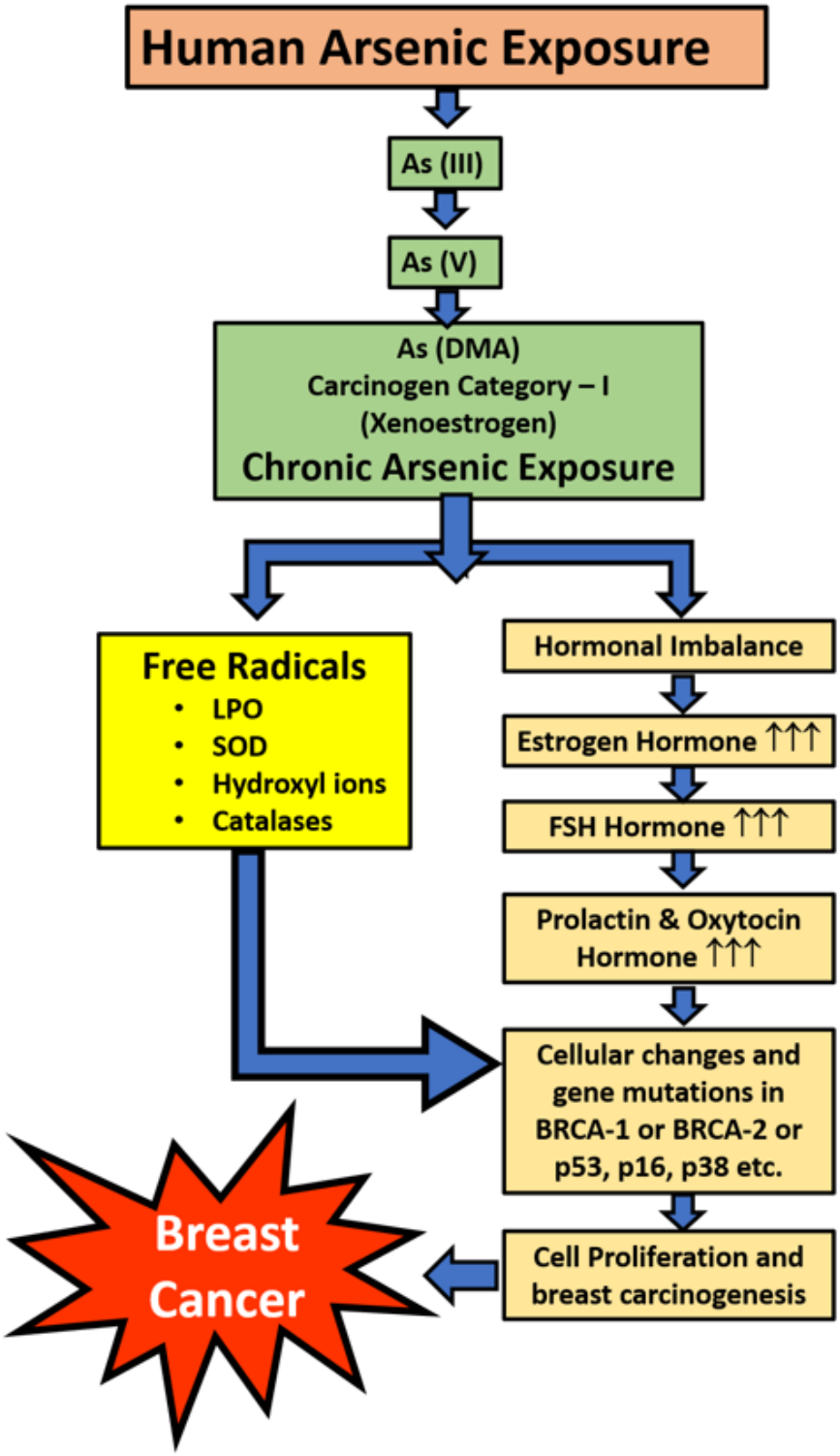

Figure 12

Proposed pathway for breast carcinogenesis due to chronic arsenic exposure