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Arsenic Exposure and Epigenetic Modification: An Updated Systematic Review of Epidemiological Findings

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Abstract

Exposure to inorganic arsenic (iAs) is a global public health concern. It is believed that the dysregulation of epigenetic mechanisms contributes and plays a major role in arsenic-associated adverse health effects. The present authors have already conducted a systematic review that summarized the findings of arsenic-induced epigenetic disruptions from *in vitro* and animal studies. In recent years, an increasing number of studies have been conducted with humans becoming available. In order to further clarify the association between arsenic exposure and epigenetic modifications, the authors reviewed the effects of arsenic on epigenetic mechanisms, including DNA methylation, histone post-translational modifications, and altered microRNA (miRNA) levels in studies conducted in human populations. Our systematic review reveals that in humans exposed to arsenic, global DNA methylation appears to increase, which is contrary to *in vitro* and the majority of animal studies. Specific genes that were identified as having their methylation profile altered by exposure to arsenic were associated with various cancer and noncancer diseases. The histone modifications post-arsenic exposure in humans are more diverse and amino acid-specific. Arsenic exposure changes the expression of specific miRNAs in human studies. Overall, it is consistent that exposure to arsenic leads to alterations of epigenetic mechanisms in almost all human studies; however, current studies do not seem to identify conserved epigenetic biomarkers across studies. Further studies are warranted with standardized study design, clearly defined outcomes of health effects, and measurements of arsenic exposure.

Keywords: DNA methylation; epigenetic mechanisms; histone modification; human epidemiologic investigation; inorganic arsenic exposure; microRNA

Introduction

Inorganic arsenic (iAs) is one of the World Health Organization's (WHO) 10 chemicals of major public health concern, affecting more than 140 million people worldwide who are drinking water contaminated with arsenic at levels higher than the WHO standard of 10 μg/L.[1] Most of these 140 million people live in developing countries such as Bangladesh, India, and China. However, the developed countries, such as the United States, are also affected.[2,3] Exposure to iAs usually occurs through ingestion of contaminated drinking water, foods, and soils as well as exposure in industrial settings. In the human body, inorganic pentavalent arsenic (iAs^{V}) is reduced to inorganic trivalent arsenic (iAs^{III}), and further metabolized to monomethylated arsenic (MMA) or dimethylated arsenic (DMA).[4–6]

Exposure to iAs has been found to be associated with an increased risk of various cancers, cardiovascular disease, neurological diseases, type-2 diabetes, respiratory diseases, and reproductive disorders.[6–9] One of the manners by which iAs is believed to lead to disease is through the modification of epigenetic mechanisms, namely DNA methylation levels, histone post-translation modifications, and changes in microRNA (miRNA) levels as already reviewed.[10] Changes in epigenetic mechanisms alter the phenotype of an organism without changing the genotype by modifying the packaging of DNA or interfering with transcription or translation.

Methylation of DNA occurs on a cytosine base that precedes a guanine, called a 5'—C—phosphate—G—3 (CpG) dinucleotide. A methyl group is added to the 5-carbon position of the cytosine by one of the following two methyltransferases: Dnmt3a and Dnmt3b. DNA methylation is maintained as DNA replicates and cells undergo mitosis and meiosis. There are high concentrations of CpG sites within many gene promoter regions, and the methylation of these CpG sites may shut off access of transcription elements to the promoter region, inhibiting gene transcription. These reactions can also occur in reverse, leading to demethylation of these CpG sites, which would open their promoter regions to transcription.

Histones are protein complexes to which DNA is wrapped around to form a unit called the nucleosome. Each histone is composed of

two of of the following four histone proteins: H2A, H2B, H3, and H4. Each of these has an amino-terminal (N-terminal) tail and a body, and the amino acids within these tails can be modified, which thus is called histone post-translational modifications (PTMs). The modification of the groups on these tails can alter chromatin structure and influence the expression of the genes in the DNA that are wrapped around the histones. Histone acetylation and methylation are two most common post-modifications of histones. Acetylation of lysine on histone tails leads to opening up of the chromatin. This is believed to be due to the acetyl group neutralizing the charge of the lysine group. Acetylation of these lysine groups is done by a group of enzymes known as histone acetyltransferase (HAT). HATs transfer the acetyl group from coenzyme A to the lysine on histone tails. Deacetylation may also occur through histone deacetylase (HDAC). Changes to histone acetylation have been indicated to be associated with various disease, including neurological disorders, and cancers.[11,12] For arsenic, *in vitro* and animal studies have consistently linked arsenic exposure and the alterations of histone acetylation level.[10] Similarly, to acetylation, methylation of amino acids (i.e., lysine and arginine) on the histone tail can alter chromatin structure as well. Contrary to acetylation, the methylation of histone N-terminal tails is believed to tighten up chromatin structure and make the genes on the DNA transcriptionally inactive. The amino group on lysine or arginine can be either monomethylated (me1), dimethylated (me2), or trimethylated (me3). This is done by a family of enzymes called histone methyltransferases (HMT), which transfer methyl groups from S-adenosylmethionine (SAMe). The methylated histone tails can also be demethylated by another family of enzymes, called histone demethylases. Similar to acetylation, changes to the methylation status of amino acids on histone tails has been associated with various diseases, such as cancers and developmental disorders.[13–15] As such, any effect of arsenic on histone methylation levels can lead to an increased risk of disease.

miRNAs are non-coding RNAs, about 21–24 nucleotides in length, which modify gene expression post-transcriptionally by binding to messanger RNA (mRNA) at the 3' untranslated regions (UTR) by either inhibiting translation or targeting the mRNA for digestion by dicer protein. Additionally, a single mRNA may be regulated by different miRNAs. Along with histones and DNA methylation, miRNAs are epigenetic signatures, having potential to serve as important biomarkers of disease. An increase in miRNAs usually decreases expression of the genes that they bind to, and a decrease in miRNAs usually does the opposite. However, recent studies have demonstrated that this is not always the case. Increase in miRNAs has been found to upregulate expression of some genes, although presently the mechanism behind these associations is not clear.[16]

The present authors and others have already investigated and reviewed the impact of iAs exposure on global methylation levels, gene-specific methylation levels, histone modifications, and changes in miRNA levels, particularly *in vitro* and animals studies.[10,17,18] Although the relation of iAs exposure and epigenetic changes is complex, the epigenetic changes, particularly DNA methylation changes, are largely consistent and comparable within *in vitro* or animal studies. In recent years, several human studies have been published on iAs exposure and epigenetic modifications. In the present study, the authors have aimed to systematically review the current literature on human studies and explore these epigenetic effects compared to the results of *in vitro* and animals studies. In addition, this study aimed to identify research gaps and promote discussion in the field.

Methodology

Search methods

The present search was not limited by language or date of publication. The authors employed two online databases, PubMed and Google Scholar, available through the University at Buffalo NY. From June 2019 to February 2020, the authors searched for articles involving exposure to arsenic and modifications to the epigenetic mechanisms: DNA methylation, histone PTM, or altered miRNA levels in human populations. Each combination of search terms, including the exposure term (i.e., arsenic), and one outcome term, including epigenetic, epigenomic, epigenome, DNA methylation, DNA hypomethylation, DNA hypermethylation, histone, histone acetylation, histone methylation, histone phosphorylation, and microRNA or miRNA, separated by the Boolean operation "AND," included only literature that contained both the exposure and the outcome of interest. In addition, names of countries where arsenic exposure is endemic were added in some searches to find more papers specific to human populations in those countries, such as Mexico, Argentina, China, Bangladesh, and India. The present authors also searched for several key review papers relevant to the topic. These review papers were hand-searched for original articles, including information on arsenic and epigenetics in reference sections.

Selection Criteria

The present authors included studies only if: (1) the report has been published in peer-reviewed journals and contained original data from human studies; and (2) there was either an internal or an external measure of arsenic exposure. To determine eligibility, at least two student reviewers examined the title and abstract from each reference obtained from the literature and added to a Microsoft word document containing each paper's title, author(s), year, and one to two sentences summarizing the abstract. Studies that did not meet these selection criteria were excluded, and relevant papers were collected and stored in a shared folder. The remaining articles were uploaded to a shared folder and explored in more detail. The data extracted included author and date, the country in which the study was conducted, the type of epidemiological study, the sample size, age, and gender, arsenic exposure sample and dose range, epigenetic measure, sample type and method, measure of health effects, and key results such as associations, odds/risk ratios, confidence intervals, p-values, and limitations of study. The information was organized in Excel sheets; separate sheets were created in the file for studies pertaining to global DNA methylation, gene-specific DNA methylation, histone PTMs, and miRNAs.

Results

Studies included

The present authors retrieved 363 references from our literature search, 52 of which met inclusion criteria based on selection criteria

of full-text screening (Figure 1). Among these, 30 studies were found to assess arsenic exposure and DNA methylation, either global or gene-specific methylation, and 12 studies assessed arsenic exposure and histone modifications. Most of the studies on histone modification focused on the alteration of methylation and acetylation status of amino-terminal tails, so the present search included only those two histone modifications in this review. There were 11 reports that examined changes in miRNA expression because of arsenic exposure. Studies were published between 2006 and 2020. All studies included in this review were either case-control or cross-sectional studies. Blood samples were used consistently in all studies for measuring epigenetic markers.

DNA methylation

Sufficient evidence suggests that exposure to environmental toxicants, such as arsenic, can lead to changes to DNA methylation, both globally and in specific genes. If so, changes to DNA methylation may result in changes to gene expression, which may lead to an altered risk of certain diseases associated with those genes.

Global DNA Methylation

Table 1 summarizes the results of selected studies related to iAs exposure and global DNA methylation in human populations. In all, 16 studies matched the selection criteria.[19–34] Among the studies, which examined the association in both genders, eight of the 12 studies reported significant global hypermethylation of DNA. Two studies reported changes in global methylation that were gender-specific, with DNA methylation hypomethylated in females and hypermethylated in males.[29,31] Of the three studies only conducted in female subjects, two of the studies reported hypomethylation.[21,24] One study, conducted by Alegria-Torres et al, used two transposable elements in the DNA, LINE1 and Alu, as measures of global DNA methylation.[19] In this study, exposure to iAs was positively associated with Alu methylation and negatively associated with LINE1 methylation.

Gene-Specific DNA Methylation

Table 2 summarizes the results of 14 papers, with most papers being case-control or cross-sectional studies. All included studies matched the selection criteria and examined the impact of iAs exposure on gene-specific DNA methylation in human populations.[21,35–47] Across these studies, a large number of different genes were reported to have been altered significantly with iAs exposure (*note*: only top genes were included in the table).

Overall, studies reported changes in the methylation status of various genes. Only three of these genes were altered in multiple studies, the apoptosis regulator death-associated protein kinase (*DAPK*), the cell cycle inhibitor *p16* gene, and the tumor suppressor *p53* gene. They all retain the same direction of association in all the studies they appear in, with *DAPK* being reported to have increased methylation in two studies,[35,38] *p16* being reported to have increased methylation in four studies,[35,37,38,46] and *p53* being reported to have increased methylation in two studies.[37,44]

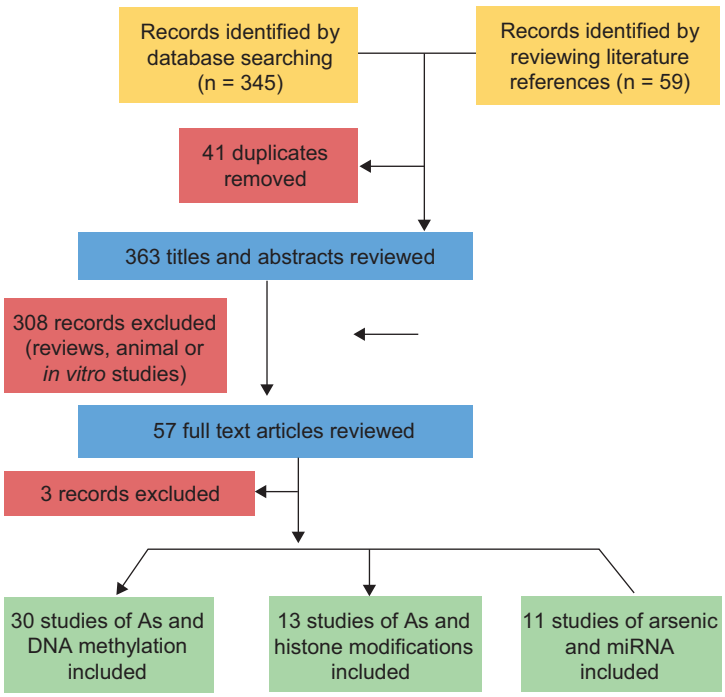

Figure 1. Flow diagram of the study selection process.

Table 1. Global DNA methylation and arsenic exposure.

As Measurement*	Dose (μg/L)#	Global Methylation	Measure	References
uAs	170 ± 171	Hyper	PBL 3H methylation	Pilsner et al.[30]
uiAs	110	Hyper	>14,000 genes	Smeester et al.[34]
uAs	127.6 (232.9)	Hyper (both) Hyper (males) Hypo (females)	WBC 3H methylation, LINE1	Pilsner et al.[31]
uMMA	0.6–6.1	Hypo (females)	>25,000 promoter sites	Bailey et al.[21]
As	12 (1–510)	Hyper	473,844 CpG sites	Kile et al.[26]
uAs	4.1 (1.8–6.6)	Hyper	385,249 CpG sites	Koestler et al.[27]
uAs	167 (10–548)	Hyper	PBMC 3H methylation	Niedzwiecki et al.[28]
uAs	66 (20–457)	Hypo	CpG sites	Broberg et al.[22]
As	0.5–499	Hyper	369,064 CpG sites	Seow et al.[33]
uAs	121 (12–544)	Hyper (males) Hypo (females)	Global %5mC and %5hmC	Niedzwiecki et al.[29]
uAs	73.87 (6.2–319.7)	Hypo	>18,000 genes	Rojas et al.[32]
uAsC	26.44 (1.93–139.55)	Changed	LINE1 hypo/alu hyper	Alegria-Torres et al.[19]
uAs	182 (10.1–1251)	Hyper (females)	436,949 CpG sites	Ameer et al.[20]
As	73.09 (6.52)	Hypo (females)	LINE1 methylation	Hossain et al.[24]
uAs	23.2 (0.34–137.5)	Hyper	385,183 CpG sites	Kaushal et al.[25]
uAs	266.35 ± 182.40	Hypo	5%-mC	Guo et al.[23]

*uAs: urinary total arsenic (μg/L); UiAs: urinary inorganic arsenic; As: inorganic arsenic in drinking water; uAsC: urinary arsenic adjusted by urinary creatinine (unit, μg/g).
#Arsenic level (unit, μg/L), unless otherwise included.

Table 2. Arsenic exposure and gene-specific DNA methylation.

As Measurement*	Dose (μg/L)#	Hyper	Hypo	References
As	0–1000	p16, p53		Chanda et al.[37]
As	NA	DAPK		Chen et al.[38]
uAs	230 (10.1–1251)	p16, MLH1		Hossain et al.[43]
As	87.36 (1–1,475)	p53		Intarasunanont et al.[44]
uMMA	0.6–6.1	PRKCD	GAD1, INNPL1, INS, IRF1, MAP3K1, PDX1, TNFRSF1B, TRAF6, SOCS6, and VAMP2	Bailey et al.[21]
uAs	500	p16, DAPK		Banerjee et al.[35]
tnAs	>0.1442	BSG, SNRNP200, FAM176A, MAG, ADCK1, MICA, CCDC46, AP3D1, NFIX, LOC100101938, BTNL2, CDC7, NAA30, PAK2, ANK3, and WDFY3	SUPT6H/SDF2, FRYL, EGLN1, BSDC1, and ELL	Liu et al.[45]
As	>500	p16		Lu et al.[46]
As	3.76		LYRM2	Green et al.[42]
uAs	191.5 ± 165.5	MMP9		Gonzalez-Cortez et al.[41]
uAs	77	IGFBP3a	IGFBP3a	Gliga et al.[40]
uAs	114.1355 ± 134.1683	CDH1, GSTP1	ERCC2, EREG, and MGMT	Zhang et al.[47]
uAs	199.0	NBR1, ATP1B3, SOCS3, UBTD1, BMF, TPK1, FOXL2NB, SNHG12, CDH23, USP14, and FUOM	ABR, SEMA4G, MAPRE2, GBAP1, NSMF, ENTHD2, SNX25, KIAA1755, MYEOV, UNKL, NELF, EML2, USHBP1, ANKRD13B, SMOC2, MRRF, MSI2, SLC16A7, and C19	Demanelis et al.[39]
nAs	1.71 ± 3.2	DNMT3A		Bozack et al.[36]

*iAs: inorganic arsenic in drinking water; uAs: urinary total arsenic; uMMA: urinary monomethylated arsenic; tnAs: toenail arsenic (unit, μg/g).
#Arsenic level (unit, μg/L), unless otherwise included.

In the study conducted by Bailey et al.,[21] all but one of the cases in their study population was either diabetic or pre-diabetic; therefore, the researchers investigated changes to the methylation status of genes known to be involved in the development of diabetes mellitus; 11 genes were found to be significantly associated with iAs exposure, and 10 of these genes were hypomethylated. In the study conducted by Liu et al.,[45] the authors tested the association between iAs exposure and methylation at CpG sites using the Illumina 450k assay. The genes altered were associated with different diseases, including various cancers, type-1 diabetes, neurological disorders, and diseases of the digestive system. Bozack et al.,[36] examined the methylation of *DNMT3A* gene in cord blood, which was involved in *de novo* DNA methylation, after exposure to arsenic in utero. The authors found a positive association between DNA methylation of *DNMT3A* and maternal arsenic exposure during pregnancy, which mediates the association between in utero arsenic exposure and birth outcomes. This study also reported a trend of increasing DNA methylation with greater arsenic exposure. Gliga et al.[40] examined arsenic exposure during pregnancy and DNA methylation of the insulin-like growth factor binding protein gene *IGFBP3* in children aged 9 years. This gene is important in circulating insulin-like growth factor throughout the body as well as being involved in development and progression of tumor by regulating cell growth and apoptosis. The study analyzed 39 CpG sites on the *IGFBP3* gene and found both hyper- and hypo-methylation depending on the specific location on genome. Zhang et al.[47] investigated promoter DNA methylation and urinary arsenic (uAs) levels in adults. Five genes were examined, including *CDH1*, *EREG*, *ERCC2*, *GSTP1*, and *MGMT*. Aberrant methylation of the 5' gene promoter regions of these genes is considered common in many cancer types. The state study found hypermethylation of *CDH1* and *GSTP1* and hypomethylation in *ERCC2*, *EREG*, and *MGMT*, although none of these was significantly associated with total uAs levels.

Histone Modifications

There were 12 papers that fit the selection criteria.[48–59] In order to compare the data to the results of *in vitro* and *in vivo* studies, the PTMs relevant to this review were compared to information from another review that focused on iAs and PTMs in *in vitro* and *in vivo* studies.[12]

Histone Acetylation

Changes in histone acetylation and methylation in human populations exposed to iAs are summarized in Table 3 and Figure 2.

H3K9ac is a PTM that was found to be modified by chronic iAs exposure in different studies. Chervona et al. and Arita et al. reported that global H3K9ac levels were found to have an overall negative association regardless of gender.[48,53] In Cantone et al., H3K9ac levels were found to have a positive association in an all-male study population.[52] This observed different result of H3K9ac could be attributed to the different iAs exposure sources and the varied measurements of iAs levels. Studies conducted by Chervona et al. and Arita et al.

Table 3. Arsenic exposure and histone modifications.

As Measurements*	Dose (μg/L)#	Histone modification		References
		Increased	Decreased	
As	0.1 (0.01–0.31)	H3K4me2, H3K9ac		Cantone et al.[52]
As	0.1–960	H3K9me2	H3K9ac	Arita et al.[48]
uAs	91.5	H3K9me2	H3K9ac	Chervona et al.[53]
		H3K4me3, H3K27me3 (females)	H3K4me3, H3K27me3 (males)	
		H3K18ac (males)	H318ac (females)	
uAs	128 (33–604)		H3K9me3	Pournara et al.[58]
uAs	121 (11–1770)	H3K36me2 (males)	H3K36me2 (females)	Howe et al.[55]
uAs, hAs	34.19 ± 14.5, 0.33 ± 0.27	H3K36me3	H3K9me2, H4K20me2	Li et al.[56]
uAsC, hAs	37.43 (18.23–100.7) 0.27 (0.03-0.86)	H3K36me2	H3K18ac, H3K9me2	Ma et al.[57]
nAs	2.19 (0.01–27.7)		H3K27me3	Tauheed et al.[59]
uAs	(60.9–434.1)	H3K79me1	H3K36me3	Bhattacharjee et al.[49]
uAs	WOSL^: 232.73 ± 180.78 WSL&: 256.43 ± 198.87		H3K36me3	Bhattacharjee et al.[50]
As	165	H3K18ac	H4K8ac	Ge et al.[54]
uAs	218.86 ± 45.67		H4K20me3	Bhattacharjee et al.[51]

*iAs: inorganic arsenic in drinking water; uAs: urinary total arsenic; uAsC: urinary arsenic adjusted by urinary creatinine (unit, μg/g); hAs: hair arsenic (unit μg/g); bAs: blood arsenic.
^WOSL: without arsenic-induced skin lesions.
&WSL: with arsenic-induced skin lesions.
#Arsenic level (unit, μg/L), unless otherwise included.

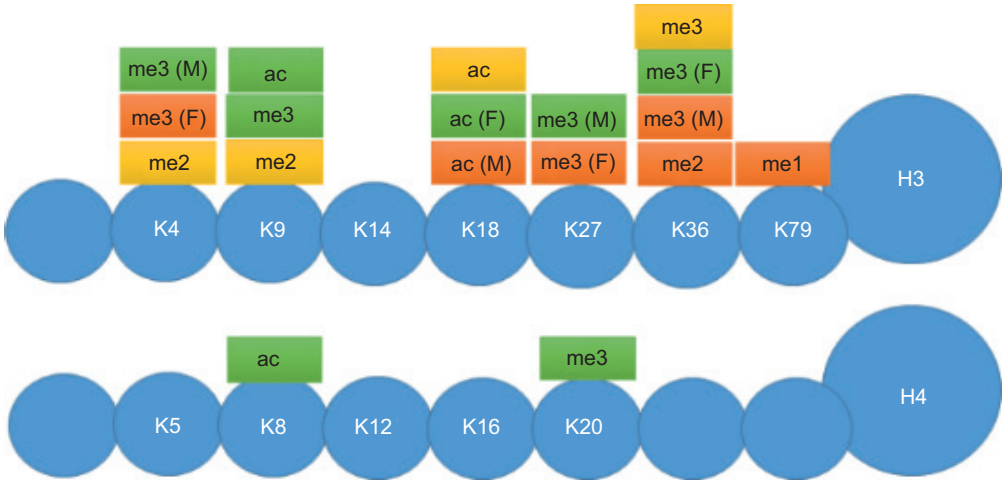

Figure 2. Histone acetylation and methylation associated with iAs exposure in human populations. Orange indicates a positive association; green shows a negative association, and yellow represents an inconsistent association. Gender-specific results are marked with (M) for male and (F) for female.

used an internal measure, uAs and blood arsenic (bAs), respectively, and iAs contamination in drinking water was the main exposure source.[48,53] In comparison, Cantone et al. used the amount of iAs present in particulate matter for analysis, as the breathed air was the main source of exposure for factory workers in this study.[52]

In Chervona et al.'s study,[53] H3K18ac was found to be modified differently depending on gender, a positive association was seen in males and a negative association in females. In two other studies, Ma et al. and Ge et al. reported contradictory results of H3K18ac.[54,57] H3K18ac level was negatively associated with iAs exposure in Ma et al.'s study and positively associated in Ge et al.'s study.[54,57] Exposure measurements were not the same across these three studies. Chervona et al.[53] and Ma et al.[57] used internal measures of arsenic exposure, such as uAs, while Ge et al.[54] reported using iAs in drinking water as the measure of exposure.

H4K8ac was reported to be changed in only one study, that is, Ge et al.[54] This study reported a decrease in H4K8ac in a Chinese population who was exposed to iAs through drinking water. However, one *in vitro* study reported that no change occurred to H4K8ac.[12]

Histone Methylation

H3K4me2 and H3K4me3 were reported to be positively associated with arsenic exposure in Cantone et al.[52] and Chervona et al.[53], respectively. However, H3K4me3 was only positively associated in females and was negatively associated in males. Interestingly, a study conducted in mice contradicts the results in humans from Chervona et al.'s[53] study and reported a positive association in males and a negative association in females.[60]

H3K9me2 was reported as altered in four human studies. In comparison with control population, arsenic exposure led to a higher level of H3K9me2 in Chervona et al.[53] and Arita et al.,[48] and a lower level in Ma et al.[57] and Li et al.[56] All these studies except Arita et al.[48] reported using uAs as their exposure measure. Li et al.[67] also used hair arsenic (hAs) and Arita et al.[48] used bAs as mentioned above. Therefore, in studies that showed increased H3K9me2, the exposure measures were different, and in studies that showed decreased H3K9me2, they all used the same exposure measure, uAs. H3K9me3 was reported to have changed in only one study in humans. In Pournara et al.,[58] iAs exposure was analyzed in females and was found to be negatively associated with H3K9me3.

H3K27me3 was another PTM where gender-specific results were reported by Chervona et al.[53] iAs exposure was positively associated with H3K27me3 in females and negatively in males. In a study conducted by Tauheed et al.,[59] the association was only negative. While the measure of exposure for both studies was an internal measure of arsenic, the medium was different. Chervona et al.[53] used urine to measure iAs exposure, while Tauheed et al.[59] used a toenail sample.

H3K36me2 was reported to be altered gender-specifically in Howe et al.[55] Arsenic exposure was positively associated with H3K36me2 in males and negatively in females. It was also positively associated with arsenic exposure in Ma et al.[57] H3K36me3 showed conflicting results between multiple studies. In two studies conducted by Bhattacharjee et al.,[49,50] H3K36me3 showed negative associations, and in Li et al.'s study, there were positive associations.[56] All studies used uAs for arsenic exposure; however, Li et al.[56] studied villagers in China who used arsenic-enriched coal to cook and were exposed to arsenic through polluted air and food. Bhattacharjee et al. focused on arsenic exposure through water.[49,50]

Bhattacharjee et al. was the only study to have results on H3K79me1.[49,50] They observed upregulation in multiple histone methylations, including H3K27me1, H3K79me2, and H3K9me1, but found the most significant one to be H3K79me1. They observed no gender-specific difference but found a three-fold increase in H3K79me1 levels in the group within their study population that had arsenic-induced skin lesions compared to the group without arsenic-induced skin lesions. Another important finding was that

H3K79me1 levels increased linearly with the increasing dose of arsenic.[49]

Li et al. reported lower levels of H4K20me2 in participants that had arsenicosis compared to controls.[56] The decrease was only significant in the severe arsenicosis group. Overall, their results showed that as H4K20me2 decreases, along with other histone modifications, genetic damage increases. A a study conducted in 2020, Bhattacharjee et al. observed a significant decrease of H4K20me3 in arsenic-induced tumor tissue compared to nontumor tissue.[51] This study had the main objective of understanding epigenetic regulations on telomere length in arsenic-induced skin cancer patients and observed that longer telomere length was correlated negatively in a significant manner with H4K20me3.

miRNA modifications

Exposure to arsenic has been associated with altered miRNA expression in *in vitro* and *in vivo* animal and human studies.[10,61] Alteration of miRNAs through iAs may dysregulate the genes they are associated with and lead to disease. To date, 11 human studies have matched the selection criteria related to iAs exposure and miRNA levels in human populations (Table 4).[62–72] One study using samples from the National Children's Study (NCS) found no changes in miRNA in human placenta associated with arsenic exposure.[67]

miRNA upregulation

Among 29 miRNAs altered in response to iAs exposure, 25 miRNAs were upregulated. Among those, only one miRNA, miR-21, was reported to have been associated with iAs exposure in multiple studies.[62,66,72] These studies reported that exposure to arsenic was positively associated with levels of miR-21, all studies used urinary levels of arsenic. Banerjee et al.,[62] also evaluated the levels of proteins known to be regulated by miR-21, and found that miR-21 levels were inversely associated with protein levels. Another *in vitro* study also reported similar findings.[73]

Rager et al. conducted a study comprising 40 mother–child pairs, where they measured the total arsenic in the mother's urine before birth, and measured miRNA levels in cord blood at child's birth.[69] They found 12 miRNAs that were positively associated with iAs exposure. Of these 12 miRNAs, ten were reported to be involved in pathways associated with cancers, six were involved in pathways associated with diabetes mellitus, and six were involved in pathways associated with respiratory diseases.

Chatterjee et al. conducted a case-control study in West Bengal, India, examining the effect of higher than normal levels of arsenic in drinking water in subjects with peripheral neuropathy and controls.[64] They reported all the six senescence related miRNAs, miR-34a, miR-29a, miR-126, miR-141, and miR-424, to be upregulated in the iAs-exposed group compared to the unexposed group (control).

Beck et al. conducted a study comprising 82 individuals from the Zimapan and Lagunera cohorts in Mexico, which were exposed to iAs in drinking water. The study findings showed an increase in circulating plasma miRNA, miR-30c-15p, in the subjects exposed to higher levels of iAs.[63]

Rahman et al. conducted a study in a Bangladeshi birth cohort, examining the effects of iAs exposure on gestational age and birthweight among pregnant women and infants at birth.[70] They reported that a low birth weight was significantly associated ($P = 0.003$) with exposure to high levels of arsenic, specifically with the presence of an elevated miR-1290.[70] Ruiz-Vera et al. conducted a cross-sectional study that examined association between iAs exposure and

Table 4. Arsenic exposure and miRNA levels.

As Measurments*	Dose (μg/L)#	miRNA		References
		Increased	Decreased	
As	55 (55–137)	miR-21, miR-221		Kong et al.[66]
uAs	64.5 (6.2–319.7)	let-7a, miR-107, miR-126, miR-16, miR-17, miR-195, miR-20a, miR-20b, miR-26b, miR-454, miR-96, and miR-98		Rager et al.[69]
pAs	3.9 (2.4–25.5)	None	None	Li et al.[67]
As	190	miR-21		Banerjee et al.[62]
uAsC	30.5 ± 25.5		miR-126	Perez-Vazquez et al.[68]
uMMA, uDMA	300 1200		miR-548c-3p	Cheng et al.[65]
uAs	190.15 ± 83.42	miR-29a, miR-34a, miR-141, and miR-424		Chatterjee et al.[64]
As	10.3–215.2	miR-423-5p, miR-142-5p-2, miR-423-5p, miR-320c-1, miR-320c-2, miR-454-5p, and miR-30c-15p		Beck et al.[63]
As	0.70 (0.09–6.0)	miR-129		Rahman et al.[70]
uAs	25.3	miR-155	miR-126	Ruiz-Vera et al.[71]
uAs	Case: 27.85 (18.69–38.85) control: 21.36 (15.70–28.91)	miR-21, miR-145, miR-155, and miR-191		Zeng et al.[72]

*iAs: inorganic arsenic in drinking water; uAs: urinary total arsenic; uAsC: urinary arsenic adjusted by urinary creatinine (unit, μg/g); pAs: placenta arsenic (unit pg/g); uMMA: urinary monomethylated arsenic; uDMA: urinary demethylated arsenic.
#Arsenic level (unit, μg/L), unless otherwise included.

cardiovascular effects related to miRNAs in 105 adult women living in an iAs-exposed region in Mexico.[71] The study reported that there was an increase in serum miR-155 levels in subjects exposed to iAs compared to unexposed groups,[71] similar to the results reported by Zeng et al.[72]

miRNA down-regulation

Perez-Vazquez et al. conducted a study comprising 73 children aged 6–12 years, residing in Mexico, who were exposed to iAs in their drinking water.[68] Exposure was assessed using creatinine levels in urine, and miRNA levels were measured using quantitative polymerase chain reaction (qPCR) technique. They selected two miRNAs for analysis, miR-155 and miR-126, because of their relationship to cardiotoxicity. They found no association between iAs exposure and miR-155 but observed that miR-126 was negatively associated with iAs exposure.[68] Ruiz-Vera et al. also reported miR-126 to be negatively associated with iAs exposure.[71] However, when compared with the results of *in vitro* studies, the results of conducted studies were found to be contradictory where arsenic exposure resulted in an increase in miR-126.[74]

Cheng et al. conducted a study comprising 152 factory workers who were frequently exposed to arsenic.[65] Three miRNAs, which were selected because of their suspected role in regulating AS3MT enzyme, were measured using qPCR. A negative association was found between miR-548c-3p level and urinary MMA and DMA concentration.[65]

Summary and Discussion

DNA methylation and arsenic exposure

Among the studies that investigated the association between iAs exposure and global DNA methylation, nine of the 16 studies suggested that exposure to iAs is associated with global DNA hypermethylation. Studies also suggested that there may be gender-specific differences to this association. Four of the five studies, which presented female-specific results, reported that iAs exposure was associated with global DNA hypomethylation, and both studies that presented male-specific results reported that iAs exposure was associated with global DNA hypermethylation. The exact reason for this gender-dependent effect is not known, but the varied physiological status and biotransformation ability to iAs probably played a role. In our previous review that investigated global DNA methylation in *in vitro* and animal studies, including three human cells, two animal cells, and six animal studies, all of these studies demonstrated that exposure to iAs resulted in global DNA hypomethylation.[10] It is possible that the reason for contradictions between the results of *in vitro*, animal, and human studies is due to the complexity of human response to iAs exposure. Humans are often exposed to arsenic through multiple sources, and in chronic and life-time exposure. In addition, inter-species differences may also partially be responsible for the contradictory results between studies conducted in animals and humans.

DAPK, *p16*, and *p53* are important players in regulating tumor growth and proliferation, and their dysregulation may result in an increased risk of cancers. Studies conducted *in vitro*, animals, and humans reported a rather consistent result, in which the methylation levels of these genes were increased. This was supported by the results of some of the studies where authors investigated whether the expression of these genes was associated with iAs exposure and an increase in methylation of the genes. In Hossain et al.,[43] iAs exposure was negatively associated with p16 expression ($r = -0.20$) but this finding was only of borderline significance ($P = 0.066$). Also, in Banerjee et al.,[35] expression of *p16* was found to have decreased by 2.2-fold in patients compared to their controls, and *DAPK* expression was reduced by 3.4-fold. Chen et al.[38] also reported decreased *DAPK* expression in 75% of methylated *DAPK* genes compared to 41% of regularly methylated *DAPK* genes ($P = 0.037$).

Histone modifications and arsenic exposure

Although exposure to iAs alters the levels of histone PTMs, the effect of iAs on PTMs is inconsistent across studies. Very few histone lysines' acetylation and methylation altered in arsenic-exposed populations were in one consistent direction in multiple human studies. For example, three PTMs, H3K18ac, H3K9me2, and H3K4me3, had an even number of results between positive and negative. For all three PTMs, contrasting results could be partially due to the different pathways of exposure. Chervona et al. and Ge et al. focused on drinking water whereas Li et al. and Ma et al. studied exposure through air pollution and food from arsenic-enriched coal used for cooking.[53–57] Specifically for H3K9me2, Li et al. found that levels changed early in the process of arsenic poisoning; however, the level of change with increasing levels of arsenic poisoning was not found in their study.[56] This may suggest that this PTM only changes with the incidence and not with the severity of arsenic poisoning. These results also suggested that H3K9me2 and H3K36me3 changes could be important epigenetic biomarkers for arsenic poisoning.[56]

Other than pathway of exposure and type of exposure measurement used, sample sizes and study design could also play a role in why the results were so different between studies, even when analyzing the same histone modification(s). In this review, eight of the 12 studies related to histone modifications were cross-sectional and three were case-control studies. The other was a systematic review. The type of study design does not seem to be related to the results found in the study. In fact, two of the case-control studies found opposite results for H3K36me3. It was positively associated with iAs exposure in Li et al. and negatively associated in Bhattacharjee et al.[49,50,56] The sample sizes also varied, with 21 being the lowest and 326 the highest.[50,54] The locations of the studies were primarily in India, China, and Bangledesh, while one was conducted in Italy and another in Argentina. Populations also varied; some studies were conducted only on females, some comprised only males, and others comprised participants 1-year old or younger. Considering that these studies had such different criteria and conflicting results, it is difficult to determine how arsenic exposure influences each histone modification. It is also unclear whether a certain pathway of exposure influences levels of specific histone PTMs. More studies with consistent designs, sample sizes, populations, pathways of exposure, and type of exposure measurements are required to understand how iAs exposure alters histone PTM levels.

In vitro studies were generally consistent with the results of human studies on the same histone modifications. A positive association between H3K9ac levels in male mice with iAs exposure was consistent with the results found in Cantone et al., where male factory workers experienced an increase in H3K9ac levels of histone modification associated with iAs exposure.[52,60] Both Ma et al. and Ge et al. performed an *in vitro* study of iAs exposure and the effects on PTMs in different cell lines alongside their human studies.[54,57] In Ma et al.,[57] the authors found that human embryonic kidney (HEK) cells exposed to sodium arsenite showed a negative association in H3K18ac,[57] while in Ge et al., the authors found that urothelium cell line (UROtsa) exposed to MMA showed a positive association with H3K18ac.[54] Both these studies were consistent with their own human populations but not with each other. Two *in vitro* results from Howe and Gamble's[12] review on H3K4me2 were consistent with the results of Cantone et al.[52] Both *in vitro* studies reported a positive association between iAs exposure and H3K4me2 in iAs-exposed A549 human cells.[75,76] Chervona et al.[53] reported gender-specific associations for H3K4me3, with a positive association in females and a negative association in males. However, a study[60] conducted in mice contradicts the results in humans from Chervona et al.'s study.[53] They reported a positive association in male mice and a negative association in female mice.[60] The results of *in vitro* studies on H3K9me2 were consistent with those of Chervona et al.[53] and Arita et al.,[48] with two studies reporting increase in H3K9me2 levels of histone modification following exposure to arsenic. Both these studies were conducted in human cell lines and were exposed to As^{III}. One of the *in vitro* studies reported that there was initially a negative association with H3K9me2 after one dose, but after multiple treatments the association was positive.[57,75] An *in vitro* study as well as Tauheed et al.[59] reported a negative association with H3K27me3, while Chervona et al. reported the same negative association in males, but the opposite in females.[53]

The results from *in vitro* and mouse studies were also not always inconsistent.[12] For example, two studies conducted in HepG2 and UROtsa cell lines reported increased H3K9ac, while another reported a decrease in Jurkat cells. Two mouse studies reported changes to H3K9ac as well.[12] One reported a decrease, while the other reported gender-specific changes, an increase in males and a decrease in females. This increase in H3K9ac in male mice was consistent with results found in Cantone et al., where male factory workers experienced an increase in H3K9ac associated with iAs exposure.[52] The results of the studies conducted in cell lines were also inconsistent. In both Ge et al.[54] and Ma et al.,[57] the authors also performed an *in vitro* study of iAs exposure and the effects on PTMs in different cell lines. In Ma et al., they found that HEK cells exposed to sodium arsenite showed a decrease in H3K18ac,[57] while in Ge et al., they found that UROtsa cells exposed to MMA showed an increase in H3K18ac.[54] Both of these studies were consistent with their own human populations but not with each other.

Many factors contribute to the complexity involved in elucidating the association between iAs exposure and histone PTMs. The differences between individual's abilities to metabolize arsenic, differences between genders, measure of iAs used, size of the study population, or the kind of exposure pathway may lead to inconsistent results in human studies. When comparing human studies with *in vitro* and *in vivo* studies, more factors are involved contributing to the complexity, such as differences between humans and other species, cell lines being different or different types of cells than the blood cells usually used in human studies, different arsenic compounds used or exposed to, and the duration of exposure (acute or sub-chronic exposure in animal studies versus chronic exposure in human population studies). These all could be the factors that need to be better considered or analyzed in the future studies examining this association.

miRNA levels and arsenic exposure

The present authors have already established that iAs exposure disrupts the genome-wide expression of miRNAs in treated mice before any symptoms are identified.[61] From the available human population studies, several common miRNAs were identified and altered in a similar fashion in different populations. For example, miR21 and miR155 were reported to be upregulated. In contrast, the down-regulation of miR126 was observed in two studies with different populations. Two groups went further and examined whether the findings from human population studies could be repeated in *in vitro* settings. Li et al. reported that six of their miRNAs identified in human studies—miR-16, miR-17, miR20a, miR-20b, miR-96, and miR-107—were consistently found in their *in vitro* study.[73] Similarly, another group reported results consistent with the following seven identified miRNAs: let-7a, miR-16, miR-17, miR-20b, miR-96, miR-98, and miR-126.[74] In these studies, four miRNAs, miR-16, miR-17, miR-20b, and miR-96, were consistently found as positively associated with iAs exposure. Considering the simplicity of miRNA measurement, miRNA has potential of being applied as biomarkers for the early identification of at-risk individuals.

Conclusion

Overall, all evidence supports that exposure to iAs results in changes to levels of epigenetic markers, DNA methylation, histone modifications (i.e., acetylation and methylation), and miRNA in humans. However, the review of these available human studies is unable to generate consistent patterns of epigenetic changes, and thus it is impossible at this time to determine a poll of epigenetic markers that could be applied in the future epidemiologic studies and the public health prevention and intervention. In order to better understand the association between iAs exposure, epigenetic modifications, and health outcomes, the authors believe that some factors must be considered while designing the future studies.

Many of the inconsistencies observed across the human studies could be due to the complex relationship between iAs exposure and epigenetic mechanisms in humans. Notably, there are wide differences in the designs, populations, exposure dose, duration, and measurement of iAs exposure of different studies. One specific area that should be taken into consideration in the future study is the measurement of arsenic exposure. Although all included human epidemiologic studies examined the epigenetic effects of iAs exposure mainly through drinking water, the measurement of arsenic levels varied in these studies. Some measured total arsenic levels in urine or blood, while others used toenail arsenic level. Variation of the measurement could be a key factor contributing to the observed

inconsistencies of results from different studies. In addition, the majority of studies examined the impact of iAs exposure on epigenetic mechanisms, and studies that investigated their associations with arsenic-induced diseases and health outcomes were lacking. Thus, it makes even more difficult to determine the meanings of epigenetic changes caused by iAs exposure, and hence limit the potential application of the information. In addition, all studies depicted epigenomic differences at one time point across the entire period of arsenic exposure. However, considering the long latency (decades) of disease development following arsenic exposure, this one-time picture of epigenomic status may not represent the key changes associated with the etiology of arsenic-induced disease development and progression. Moreover, considering the profiling approach in the study design and multiple comparisons applied in data analysis, it is often difficult to completely prevent false positive association. Therefore, the interpretation of positive findings must be exercised with caution.

When analyzing associations between iAs exposure and epigenetic modifications, almost all the studies used blood cells to measure changes in epigenetic mechanisms. This could obscure true associations between epigenetic modifications and disease. First, blood cells may not be the representative of all other cell types in the body. The diseases that are found to be associated with these epigenetic modifications are involved in the body's all organ systems and tissues, and it is not known whether the epigenetic modifications induced by iAs are the same in the cells of these organs and tissues as they are in the blood. Second, it is known that iAs causes shifts in the counts of different blood cells in a whole blood cell sample. Like the differences between blood cells and cells of other tissues in the body, arsenic might affect epigenetic mechanisms differently between blood cell types. It is thus critical to address differences between target cells and organs of arsenic toxicity and non-targeted peripheral blood cells in the future studies.

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Competing financial interests

All authors have no competing financial interests to declare.

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