Clinical Case Reports International

Review Article
Published: 03 May, 2023

Heavy Metal Induced Molecular Alteration Leads to Gallbladder Cancer

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Abstract

Gallbladder cancer is reported very high in northern India. One-fifth of all gallbladder cancer cases are reported only from northern part of India. The cases are more prominent in females than males. There were many risk factors associated with gallbladder cancer, but the heavy metals are play crucial roles in induction of gallbladder cancer. Heavy metal may interfere with molecular mechanism and activate mTOR, MAPK, and transcription factor. Hyper activation of cysteine due to arsenic exposure interfere with the function of the mTOR signaling pathway and taurine pathway that causes activation of c-fos, c-myc, and ELK-1 leading to enhance cell division causing gallbladder cancer. Lead, Nickel, Zinc, and chromium activate cholic acid which causes formation of gallstones through activated taurine pathway leading to inflammatory gallbladder disease and formation of gall stones. Prolonged inflammation and stones formation in gall bladder may cause loss of contact inhibition in gall bladder cells leading to gall bladder cancer. The combined mechanism of heavy metals activates different pathway leading to gallbladder cancer through activating cell cycle and angiogenesis, it also inhibits tumor- suppressing genes. It was evident that gallbladder cancer is associated with high levels of heavy metal exposure. Differential mechanism associated with different heavy metal leads to gall bladder cancer.

Keywords: mTOR; MAPK; Taurine; Gall stone; Inflammation

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Received Date: *17 Apr 2023*
Accepted Date: *29 Apr 2023*
Published Date: *03 May 2023*

Citation:
Manisha K, Geetika S, Munish S, Sunil K, Ranjit K. Heavy Metal Induced Molecular Alteration Leads to Gallbladder Cancer. Clin Case Rep Int. 2023; 7: 1539.

Introduction

GBC (Gallbladder Cancer) is the sixth most frequent cancer among all gastrointestinal tract malignancies [1]. GBC have high mortality rate; it accounts for 20th position for death rate among all type of cancers. Mortality rate are very high for gall bladder cancer. In 2020, 115,949 new cases of GBC were reported worldwide, which was 0.6% of overall cancer incidence rate [2]. Differences in GBC incidence rate have been observed in different geographical areas [3]. Asia represents 70.8% of the total gallbladder cancer incidence followed by Europe 10.8%, Latin America and the Caribbean 8.6%, Africa 4.7%, and Northern America 4.5% from. 10% of the global burden of GBC contributed by only India [4]. The 10% to 22% of India's total GBC burden has been contributed by north-east region [5]. In 2020 because of GBC total of 84,695 deaths were reported worldwide including 73.6% of the total mortality from Asia, 10.3% from Europe, 7.6% from Latin America and 5% from Caribbean, and 3.1% from Northern America [2]. These variations observed due to dissimilarity in genetic propensity and environmental exposure as well as other conditions such as gallstones, obesity, gender, bacterial infection associated with chronic inflammation that increase the potential for carcinogenesis, as known risk factors [6].

Heavy metal and gallbladder cancer incidence

Heavy metal as well as trace metal was observed in bile of gall bladder cancer patients [7]. The amount of some heavy metals like Lead (Pb), Chromium (Cr), and Cadmium (Cd) was found significantly higher in patients diagnosed with GBC [8]. The study conducted in the Gangetic plains of Bihar by Kumar et al., [9] found a remarkably high concentration of arsenic (>15 μgL^{-1}) in the blood of 53.6% of patients with gallbladder cancer. High concentration of Copper (Cu) and Nickel (Ni) were found in serum, Cd and Ni were found in bile and high concentration of Cr was found in gallbladder tissues. While Zinc (Zn) and Selenium (Se) have also been detected in low concentration in gall bladder tissues [10]. Role of heavy metal in induction of biological pathways were shown in Figure 1. It gives detailed idea about tumorigenesis in gall bladder and heavy metal incidence.

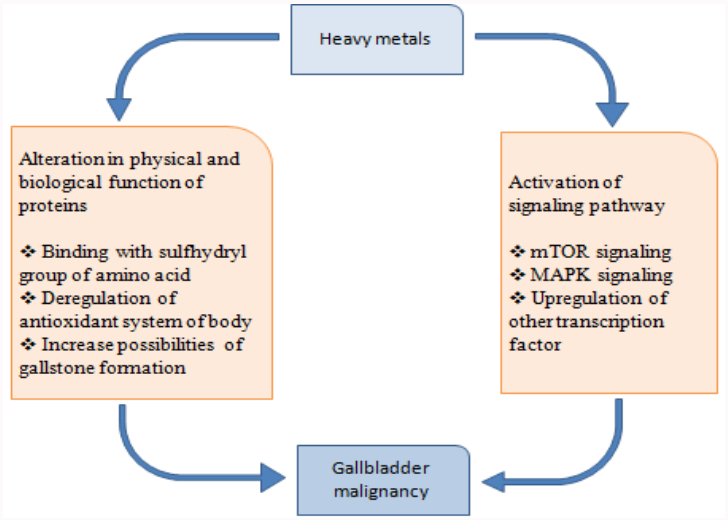

Figure 1: Showing role of heavy metal in gall bladder cancer through different mechanism.

Molecular mechanism involved in gallbladder cancer due to heavy metals

Mondal et al., [11] conducted a study that provides concrete pieces of evidence in support of heavy metals-induced premalignant and malignant development in gallbladder. It has been reported that the deregulation in antioxidant system plays a major role in carcinogenesis [12]. Heavy metals induce cancer through the generation of Reactive Oxygen Species (ROS), which leads to DNA damage through redox imbalance and by interrupting the repair pathway of damaged DNA [13]. The strong affinity of heavy metals for free sulfhydryl group makes it capable to disturb the metabolism and physiological function of several proteins [14]. Heavy metal exposure such as As, Hg, Cd and Pb compromises with cysteine status, which significantly took a part in the inducible endogenous detoxification system of body [6] and heavy metal also associated with alteration in function of enzyme involved in antioxidant system, lead to malignancy by DNA damage [15].

Heavy metal binding with cysteine & its metabolites leading to gallbladder cancer: The semi-essential sulfur-containing amino acid cysteine is carried from extracellular space by a catalytic subunit of the transport system called xCT in exchange for glutamate [16]. In the liver, cysteine decomposed into GSH (glutathione) by hepatic enzyme ϒ-Glutamyl Cysteine Synthetase (GCS) and disintegrates to cysteine sulfate by another hepatic enzyme Cysteine Dioxygenase (CDO), cysteine sulfate further catabolized to hypo taurine by the action of enzyme Cysteine Sulfinate Decarboxylase (CSDC), which later on oxidized to taurine [17]. The free sulfhydryl group of Cysteine binds with heavy metal such as arenite resulting in metabolic alteration and decrease cysteine concentration in intercellular space [18]. Cysteine transportation could also limit by phosphorylation of serine 26 present on the cytosolic N terminus of the cystine-glutamate antiporter xCT by mTOR signaling [19]. Decreased level of cysteine leads to disturbance in its physiological role such as protection against inflammatory response and oxidative stress by deregulating GSH synthesis [20]. According to Lewerenz et al. [21], the SLC7A11 transporter is expressed in the number of malignancies where transporter-assisted glutathione production decreases oxidative damage and protect cancer cell from death (Figure 2).

Role of GSH and its contribution to GBC due to heavy metal exposure: GSH protects the intracellular environment from oxidative stress and detoxifies cells by acting as a scavenger [22]. GSH reduces the oxidative level through binding its thiol group directly with free radicals, it also functions as a cofactor for enzyme peroxidase, which metabolized H_2O_2 and lipid peroxide [23]. The high affinity of heavy metal for GSH leads to a reduction in intracellular concentration of glutathione by suppressing the action of essential enzyme ϒ-glutamyl cysteine synthetase required for GSH synthesis [24]. Decreased levels of glutathione lead to elevation in redox imbalance which increases the frequency of damaged DNA [25] by modifying methylation of DNA or by interfering in the mechanism of DNA repair (Figure 2). Mutated DNA gets accumulated and eventually causes cancer [26]. It has been observed that when ROS generation is high, the antioxidant system loses its ability to maintain equilibrium and results in oxidative stress [27].

Effect of heavy metal induced oxidative tension on DNA: High oxidative stress could induce either through mitochondrial dysfunction or malfunctioning of the ROS elimination system, heavy metal limits the function of MRCC1 and MRCC2 mitochondrial respiratory chain complexes which enhance the oxidative stress [28]. Free radicals also produced either by stimulation of H_2O_2 production through heavy metal toxicity or feedback inhibition of amino-levulinic acid synthase, which leads to an elevated level of heme precursor Amino-Levulinic Acid (ALA), enolization of ALA followed by autoxidation generated free radicals [29]. Increased level of free radical by inhibition of antioxidant system has been observed in fishes when exposed to metal toxicity by Zheng et al. [30]. Effects of increased oxidative stress on DNA impairment is complex and pleomorphic. Increased oxidative stress caused by heavy metal leads to DNA damage and it can initiate cancer processes as well as participate in its progression [31]. Oxidative DNA damage has also been identified in the lymphocytes of the person exposed to the heavy metal environment [32]. Chromosomal aberration and DNA strand break has been well demonstrated in cultured mammalian cells even at the minute deposition of heavy metal [33]. Simultaneously ROS causes DNA damage by oxidizing nucleoside bases such as 8-oxoguanine, which if left unpaired, it may cause G-T and G-A transversion [34]. Moreover, heavy metal exposure can produce

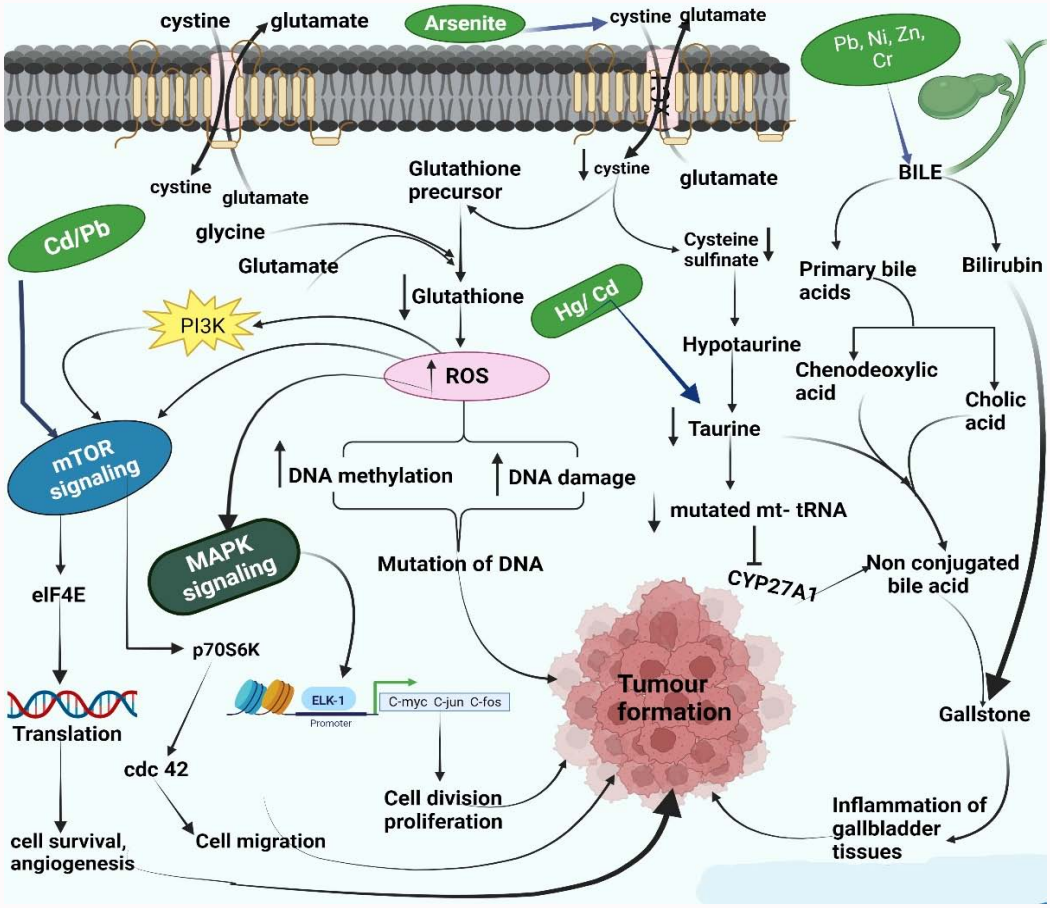

Figure 2: Showing molecular mechanism altered by heavy metal leads to gallbladder cancer through different pathways; 1) Arsenite (trivalent arsenic) binds to cysteine and decreases the level of the precursor of glutathione, consequence into accelerate oxidative tension which leads to modification in DNA through methylation and damage result into the accumulation of mutation which finally leads to cancer. 2) Metabolite of cysteine, cysteine sulfinate converts into taurine in multistep through enzymatic actions. Association of heavy metals like Mercury and Cadmium with taurine leads to the depletion of taurine which prevents the conjugation of primary bile acid by suppressing the expression of CYP27A1 gene encoding for sterol-27-hydroxylase due to modified taurine component of mt-tRNAs. Later on, it leads to gallstone formation by the accumulation of non-conjugated bile acid and bilirubin secretion enhanced by chromium, nickel, zinc, and lead. Prolonged gallstones deposition causes inflammation, which culminates in carcinoma of the gallbladder over time. 3) Hypoxia, DNA damage, and oxidative stress induced through heavy metal toxicity up-regulate mTOR signaling & MAPK signaling pathways, which stimulate downstream signaling of different proteins including transcription factors and regulate target gene expression. mTOR signaling up-regulates translational factors such as eIF4E and phosphokinase p70S6K, which encoded for angiogenesis and cell migration respectively. MAPK signaling activate ELK-1 transcription factor via regulating different downstream proteins which further expressed AP-1 transcription element site present at DNA which leads to proliferation.

intrastrand bifunctional adducts through covalent binding with the N-atom of guanine and adenine present at 7th position of DNA residue leading to the destabilization of the DNA helix [35].

Suppression of DNA repair mechanism and genotoxicity due to oxidative stress caused by heavy metal: Giaginis et al. [36] has reported about suppression of mismatch repair, nucleotide excision, and base excision DNA repairs mechanisms due to heavy metal toxicity, either by binding to residues of negatively charged surface on DNA repair protein or by replacing Zn-atom in their Zn finger motif. Inhibition of repair mechanism result into conformational changes and disturbance in DNA protein interaction required for DNA repair process and preservation of genomic integrity. Substantiation of aberration in chromosome, micronuclei, and high sister chromatids exchange due to heavy metal has observed by Valverde et al., [37] in person exposed to heavy metal environment. Heavy metal compounds alter expression of tumor suppressor gene and oncogene through deregulation in DNA methylation pattern [38]. Which may be cause hypomethylation leading to oncogene activation through suppression of DNA methyl transferase [39] or transcriptional inactivation due to hypermethylation of the p53 gene at the promoter region by heavy metal [40]. Heavy metal also reduces the levels of S-Adenosyl-L-Methionine (SAM) and methyltransferase gene which encode for enzyme that transfer a methyl group to nucleotides residue of DNA. Dysregulation in methylation processes due to heavy metal exposure has also observed in rats' model [41]. Methylation at promoter region of tumors suppressor gene family CDH1, CDKN2A, p16 and REpRIMO is responsible for apoptosis and inhibition of angiogenesis in gallbladder malignancy. Methylation of p73, MGMT, DCL1 were also observed in cell of gallbladder malignancy [42]. Modification in promoter region and remodeling in process of methylation of DNA have a tumor-encouraging effect and enhance the up regulation of the proto-oncogene pool out-turn into mutation and accumulated mutation with time results in tumor formation.

Role of heavy metals on the physiological role of taurine responsible for gallbladder tumorigenesis: Cysteine derived Taurine (2-aminoethane sulphonic acid) freely present in intracellular space play a key role in the reduction of oxidative tension induced by heavy metal toxicity [43]. It has been observed that taurine reduces

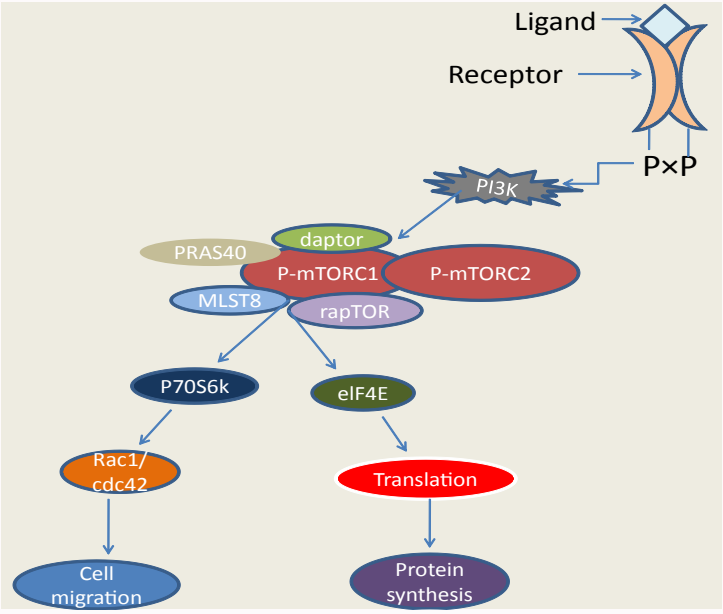

Figure 3: mTOR component comprises two separate mTORC1 and mTORC2 multi protein complexes, which regulate complicated signaling, involved in cell growth and proliferation by influencing a variety of key cellular functions like cell migration and protein synthesis. mTORC1 interacts with four additional proteins: mammalian lethal with SEC 13 protein eight (mLST8), rapTOR (mTOR regulatory protein), PRAS40 (proline-rich AKT substrate), and DEP-domain containing mTOR-interacting protein. S6Kinase (S6K) and 4E-BPs (eukaryotic translation initiation factor-binding proteins) are substrates for mTORC1. 4E-BPs governs translation initiation by binding eukaryotic translation initiation factor eIF4E required for protein synthesis, P70S6K activate Rac1/cdc42, which help in cell migration.

superoxide synthesis by elevating electron transport chain activity of mitochondria [44]. Toxicity of heavy metal leads to a consequential reduction in taurine accumulation and falloff of the membrane protected by taurine [45]. Taurine is critical for gallstone formation, which induces inflammation in inner lining of gallbladder tissues and eventually turns into a malignant mass of cells through mutation [1]. Taurine also has been spotted in mt- tRNAs as a modified component of 5-taurine methyluridine and 5-taurine methyl-2-thiouridine [46]. Increased ROS level due to reduction in GSH leads to enlargement and malfunctioning of mitochondria [47]. Mitochondrial malfunctioning and high susceptibility of mt-DNA to ROS leads to alteration in mt-tRNA [48], and mutated mt-tRNAs leads to several metabolic diseases as well as cancer [49]. Disintegration of the inner membrane of mitochondria by modified mt-tRNA results in a remarkable reduction in CYP27A1 protein expression in taurine-depleted liver [50], which further inhibits bile acid formation in addition to deterioration in the flow of bile, results in reduction of conjugation of taurine with bile acid [51]. Accumulation of un-conjugated bile acid leads to the formation of gallstones, which cause irritation and turn into cancer in the long run. Modification due to heavy metals in MDR3, a gene involved in bile formation, and CYP27A regulates cholesterol metabolism in the liver, play critical role in gallstone formation [52].

Signaling expressed in gallbladder carcinoma

Cell signaling is necessary for sustaining critical cellular functions like development, differentiation, migration, and cell growth. Activation of ERK/MAPK [53] and mTOR signaling pathways [54] due to heavy metal toxicity has been reported in gallbladder cell lines, which incite circumstances that fuel cancer progression (Figure 2).

MAPK signaling pathway activation in gall bladder cancer due to heavy metal exposure: Activation of the MAPK signaling pathway in presence of excess heavy metal through generation of ROS was extensively studied [55]. Nanomolar concentration of Cd also activates MAPK signaling [56]. MAP kinase activates different downstream substrate such as transcription factors Elk-1, c-jun, c-fos, c-myc, stress-activated protein kinase (MSK) and Ribosomal S6 Kinase 1 (RSK1) in gallbladder carcinoma [57]. Which stimulate cancer regulatory processes: Angiogenesis, cell proliferation [58], cell differentiation [59], cell cycle progression [60], growth, metastasis [61], invasiveness [62] in GBC cell lines [63].

Role of heavy metals in mTOR activation leading to gallbladder cancer: mTOR activation occurs early in the development of GBC [64]. Activation of PI3K kinase, which stimulate mTOR pathway and phosphorylation of mTOR, S6K1 and 4E-BP1 by heavy metal has reported in GBC [48]. The PI3K/AKT signaling pathway activate mTOR by phosphorylate Ser 2448, which further activates the eukaryotic Translation Factor 4E (eIF4E) by inactivating its inhibitor 4E-BP1.35 [65]. An element PI3K of the mTOR signaling pathway stimulates different tumorigenic cellular processes such as angiogenesis, cell survival, cell proliferation, adhesion, and motility [66]. Riener et al. [67] found a missense mutation in PI3KCA, which contributes to gallbladder carcinogenesis (Figure 3). Outcomes of immunohistochemical analysis of phosphorylated p70S6K has been shown a high prevalence (>80%) of up-regulated p70S6K in gallbladder cell lines [64], which have the potential to rearrange the actin cytoskeleton through its actin filament cross-linking Rac1/cdc42 and leads to cell migration [68].

Other transcription factors up-regulated by heavy metal in gall bladder cancer: Besides mTOR and MAPK signaling hyper activation of other transcription factors MTF1 (Metal Regulatory Transcriptional Factor 1), USF (Upstream Stimulator Factor), Nuclear Factor κB (NF-κB) and NF-E2 Related Factor (NRF2) has also been observed in gallbladder carcinoma which is stimulated by heavy metals as well [69]. Cd-induced tumorigenesis demonstrated through differential display analysis of transformed BALB/C-3T3 cells due to CdCl2 exposure shows over expression of translation

initiation factor-3 and translation elimination factor 1 delta [70]. All these factors induced cell proliferation and cell survival, which is a key step in tumor formation. Nuclear Factor NF-κB regulate downstream target, modulate viability, and boost cell division of gallbladder cancer cells during chemotherapy [71]. The result of an experimental study by Shibata et al. [72], supports the regulation of NrF2 activity by alters the inhibitory function of Keap1gene in gallbladder cancer cell lines [39].

Conclusion

It was evident that gall bladder cancer is multistep process induced by activation of cascades of genes. Heavy metal enhances formation of gall stones which may be converted into gall bladder cancer with time. Taurine-cysteine pathway regulates formation of gall stones on activation due to increased concentration of heavy metal. MAPK, mTOR and transcription factor activation through different heavy metal finally leads to gall bladder malignancy through different pathways. NF kB, Rac1, elF4E and Cdc42 play critical role in cell migration and excessive protein synthesis required for proliferating gall bladder cells. Heavy metal toxicity causes increased chances of gall stone formation and gall bladder cancer through alteration of regulatory genes.

Acknowledgment

Authors are thankful to the Central University of Himachal Pradesh for providing infrastructural support during their study. Authors are also thankful to all staff members who has co-operated during the study.

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