Minireview Article

Enviromental risk factors associated with primary liver cancer in Western Kenya(A mini-Review).

Abstract

Primary Liver Cancer (PLC) is a global health burden, which is poorly addressed in developing countries. It is ranked the third leading cause of cancer-related mortality worldwide, with high incidence rates reported in Asia and Africa. Currently, in Kenya, there is an upward trend of PLC cases reported with no confirmed causes. Furthermore, there is lack of sufficient knowledge on the prognosis mechanisms of PLC, despite some of its known risk factors being established. These additional factors, ranging from lifestyle choices to pre-existing environmental concomitants, could actually play role on the etiology of the disease yet remain unexplored. Due to this, there was a need on evaluating the impacts of exposure to environmental risk factors such as pesticides, food contaminated with aflatoxin, or harmful cyanobacteria algae blooms in regions where PLC is endemic. As a result, this mini-review, aimed at analyzing relevant epidemiological data on primary liver cancer underpinning the mechanism of action of environmental toxin as an emergent risk factor in Western Kenya. This was achieved through meta-synthesis analysis of the previous research findings, with the purpose of integrating their results to inform the present intrinsic case study.Among the many epidemiological studies associating PLC and environmental toxin as an emergent risk factor reviewed in the current study, environmental exposure to microcystin toxin was inferred to constitute a public health hazard due to the continued presence in drinking water sources. Majority of the epidemiological data are in support of the potential association between environmental microcystin toxins and Primary Liver cancer in developing countries. These findings can be used to edify the health and medical professionals at all levels of prevention, including the diagnosis and treatment of liver disease patients, also these findings can act as baseline data that is required for better and informed Lake management water quality.

Key words: *Cyanobacteria; Microcystins (MCs);, Primary Liver Cancer (PLC;), Afflatoxin; ;, Serine-threonine Protein-phosphatase*; *Dichlorodiphenyltrichloroethane (DDT)*;

1.0 Introduction

Primary Liver Cancer (PLC) is a global public health problem which is poorly addressed in developing countries. This malignancy which affects primary liver cells represents the sixth most common neoplasm and the second leading cause of cancer-related mortality worldwide [**Error! Reference source not found.**]. About 8.2% of total cancer-related deaths in 2020 were due to PLC [61].Majority of the incidence rates occurred in regions such as Asia and Africa. Currently, in Kenya, there is an upward trend of primary liver cancer cases with no confirmed causes. According to Burden et al., [**Error! Reference source not found.**]in Kenya, Primary liver cancer ranked 6^{th} in males and 5^{th} in females as per the Nairobi cancer registry 2016. However, its ranking moved to third most common malignancy in males occurring with a peak incidence rate at 40 years of age [49]. The high number of cases (5.2%) was reported in Nyanza region, with countries bordering the lake region reporting the high prevalence rate [49]. The attributable risk was congruent to the global prevalence of 4.6% within the region. Prior studies have associated PLC with risk factors such as exposure to hepatitis B and C virus (HBV, HCV), aflatoxins,

excess alcohol intake, and tobacco [**Error! Reference source not found.**]. Other studies have identified iron overload, obesity, *Diabetes mellitus*, genetic predisposition, and α_1-AT deficiency as risk factors [2]. Nevertheless, 18 % of the PLC cases are of unknown causes [38]. A recent study indicates evidence that environmental toxins such as organochlorine pesticides, aflatoxins, microcystins contribute to liver cancer development [41]. However, their mechanism of action and pathophysiological pathways in pathogenesis of PLC occurrence is understudied.

2.0 Environmental risk factors.

Environmental risk factors are defined as environmental changes that have a huge negative impact on human health and wellbeing [41]. They comprise of external physical, chemical , biological and work-related factors but excluding natural environmental factors that cannot be modified [53].These environmental risk factors threaten the health of the present and future human generation, imposing a great disease burden on health sector. Limited studies have been done on environmental risk factors that may act as pointer to the increased risk of developing primary liver cancer [13]. According to American Society of Clinical Oncology (ASCO) 2021 data, exposure to environmental risk factors such as chemicals such as pesticides or consumption of food contaminated with aflatoxin are associated to liver cancer development with undocumented magnitude (ASCO 2021). Environmental risk factors are said to be cumulative [41], and therefore having exposure to more than one risk factor by an individual increases a person risk's factor of developing liver cancer even more. There is therefore need to identify these environmental risk factors, and ascertain their involvement at physiological and molecular level to PLC presentation. This knowledge will be useful in mitigating PLC, in order to reduce the burden risk in health sector.

This mini-review therefore, intends to shed more light on the linkage between environmental risks factors exposure and primary liver cancer disease presentation in hospitalized patients in one of the Teaching and Referral hospital in Western Kenya, Nyanza region. Specifically epidemiological data on hepatocellular carcinoma, supporting environmental microcystin toxins exposure as an emergent risk factor for occurrence of PLC is discussed.

2.1 Aflatoxin

Aflatoxin are a group of mycotoxin produced by the common *Aspergillus flavus* and *Aspergillus parasiticus* which are common and widespread in nature. They are known to contaminate a large fraction of world's food such as cereals, peanuts, cassava among others [4]. Greatest health risks of about 4.5 to 5.5 billion people are affected due to consumption of food contaminated with aflatoxin[38]. This is reported from developing countries where the populations rely on these commodities (maize, peanuts and cereal grains) as their staple food [61]. However, the contribution of afflatoxin exposure to primary liver cancer incidences is still unknown in these countries and hence a challenge in patient management. This is because the food measurement survey used to estimate aflatoxin exposure provides crude measurements that do not tally with the risk assessment statistics and may thus fail to account for secular trends in exposure or individual variations in exposure [23].There are four types of aflatoxins (AFB1, AFB2, AFG1, and AFG2), that are known to be carcinogenic to both human and animals at various tolerance levels[32]. AFB1 is a major risk factor linked to human liver cancer, and several cases of PLC with high prevalence (4.6-28.2%) have been reported in regions such as China, Sub-Saharan Africa and South Asia [46]. It is hypothesized that afflatoxin induce activation of proto-

oncogenes and mutations in the tumor suppressor gene p53 whereby there occurs a G-to-T transversion at codon 249[20,38] .AFB1 is metabolized by cytochrome-P450 enzymes to the reactive intermediate AFB1-8, 9 epoxide (AFBO) which binds to liver cell DNA, resulting in DNA adducts. DNA adducts interact with the guanine bases of liver cell DNA and cause a mutational effect in the *P53* tumor suppressor gene at the codon 249 hotspot in exon 7, which may lead to HCC[17] . According to (Kimanya et al) [28], 28.2% of the annual liver cancer cases, globally, are linked to aflatoxin exposure. In this regard, Obade et al., [46] reported that aflatoxins exposure levels in Kisumu one of the Lake region counties range between 0-34.5ng/g, which is above the Kenyan regulatory limit of 10ng/g. This acts as a pointer to one of the environmental factors that would be contributing to PLC currently observed in the region and need a comprehensive and elaborate follow-up [51] in order to understand their molecular mechanisms and association with PLC.

2.2 Pesticides

Pesticides are chemicals that are used to control and manage pests, affecting humans, agricultural and horticulture crops [58]. These chemicals are exposed to human beings through ingestion, inhalation and dermal contact [41].This occurs when the household pesticides fails to degrade due to lack of moisture, microorganism and sunlight thus facilitating human exposure [57]. Experimental studies have demonstrated that exposure to pesticides such as dichlorodiphenyltrichloroethane(DDT) and dichlorodiphenylethylene (DDE),(Fig 1a:1b) in rodents led to development of liver tumors and hepatocellular carcinoma [7].Whereas in animals(rats/monkeys), studies have confirm that the primary target organ of toxicity for DDT and the related compounds is the liver [**Error! Reference source not found.**].The level of impact of toxicity is directly proportional to dose-exposure to an animal studies, with chronic exposure leading to initiation of liver tumors in animals [65] .For instance, experimental studies reports that high dose administration of PB in rodent liver cells results into increase in liver size and smooth endoplasmic reticulum[**Error! Reference source not found.**,**Error! Reference source not found.**]. However, according to Lake et al., [**Error! Reference source not found.**], this was not the case in human hepatocytes cultured cells, whereby PB-induction did not increase replicative DNA synthesis, unlike in cultured rodents hepatocytes which produced a stimulation of replicative DNA synthesis as well as after an in vivo administration [52]. Limited studies have been done to explore the impact of pesticides exposure to human in regards to hepatocellular carcinoma development. As a results, no accurate data has been provided to support the significant association of DDT and its related compounds with development of hepatocelluar carcinoma [55] . For instance , one study in USA reported that farmers were at an increased risk for HCC development compared to non-farmers [3] while other studies report a non-significant increased risk for HCC among farmers[58] .In addition, according to Harada et al., [19] ,in an experimental study to examine DDT serum/urine-levels and serum/urine markers of liver damage found no significant associations with serum enzymes indicative of liver dysfunctions e.g increased AST,ALT or bilirubin,among the subjects in Brazil. Contrastingly, a cohort of 52 children age 4 years old from Ribera D'Ebre Spain, reported a significant association between DDE/DDT levels in blood samples and urine levels of total porphyrins,coproporphyrin I and coproporphyrin III;(indicators of damaged hepatic heme synthesis in the liver)[58].Over the last few years, a case-control study was carried out using geographical information system to portray the association between organochlorine pesticides and number of people at risk of PLC basing on gender. The results showed a significant association between organochlorine pesticides and

increased PLC risk in males (OR 2.76 95% CI 1.58–4,82), but not in females (OR 0.83 95% CI 0.35–1.93)[65]. Collectively, epidemiological studies in China and the USA provide evidence, though not consistent that pesticide exposure in agriculturally concentrated areas increases PLC risk in farmers. There is therefore a need to unravel the impending dangers that could result from long-term exposure to pesticides especially in regards to promotion of liver cancer prognosis.

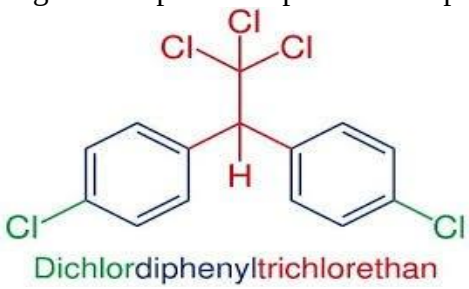

Fig. 1a, Chemical Structure of DDT; Courtesy of Rachel et al.,[55].

Fig. 1b, Chemical structure of DDE

According to IARC 2018, chronic exposure to DDT has been considered carcinogenic to animal liver arising during metabolism. Once the pesticides DDT has been ingested and transported in blood into the Liver, DDT is primarily converted into a less toxic component DDD, with smaller amount being dehydrochlorinated to DDE [65] DDD readily degrades through several intermediates to form DDA, which is readily released in urine, however DDE is poorly eliminated and therefore bio-accumulates in lipid-rich tissues hence toxicity arises[1] (Figure 2.).

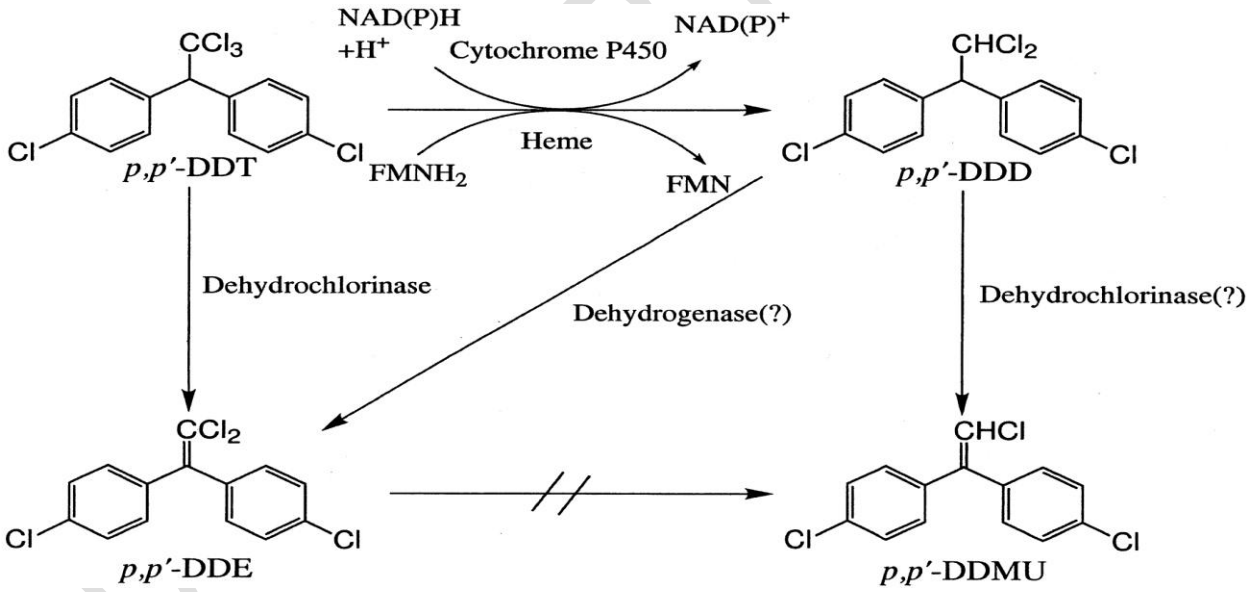

Fig. 2 . Metabolism pathway of DDT

Experimental studies in animals postulated that, the main mechanism of action of DDT to initiate hepatocellular carcinoma is via initial inductions of microsomal liver xenobiotic metabolizing enzymes(CYP monooxygenases),interference in DNA synthesis and cell proliferation [19].In regards to cell proliferation, it has been hypothesized that this initiates the production of eosinophilic abnormal hepatic foci(AHF),whose quantity correlates with dose and time of duration of exposure[65].Moreover, an increase in 8-OHdG and LPO is observed as an indication of hepatic oxidative stress and damage to DNA, which may be an added contributing

factor to liver non-neoplastic changes which eventually may results to hepatic tumor formation [68]. In an vitro studies, increased oxidative stress and reactive oxygen species (ROS) due to DDT exposure in Hep2 cells is thought to activate the Jak/STAT3 pathway, ultimately resulting in impaired expression of E-cadherin, which is known to be associated with hepatocellular carcinogenesis and poor prognosis in humans [65]. A number of hepatic health effects has been observed in both in-vivo and in-vitro studies using phenobarbitone (PB) chemical in rodents and humans hepatocytes cells [41] .For instance PB-induction in both rodent and human liver led to activation of CAR, altered gene expression specific to CAR activator, increased cell proliferation[16] , induction of CYP2B and other CYP enzymes and liver hypertrophy. In regards to liver hypertrophy, experimental studies reports that high dose administration of PB in human liver cultured cells results into increase in liver size and smooth endoplasmic reticulum[58] .Contrastingly, according to Asia et al.,[3]PB-induction in cultured rodents hepatocytes produces a stimulation of replicative DNA synthesis as well as after in-vivo administration, however in cultured human hepatocytes PB-induction does not increase replicative DNA synthesis[58].On the other hand, Asia et al.,[3]reports an ability of xenobiotic agents to induce CYP3A4 & CYP2B6 using stable hepatoma cell line expressing human pregnane X-receptor(hPXR) and cytochrome P4503A4, CYP3A4 distal and proximal promoter plus the luciferase reporter gene. Their results showed that persistent exposure to pesticides led to expression of hPXR and cytochrome genes,which responded directly proportional to dose response exposure and time. These suggest that human-chronic exposure to pesticides may results to a huge human risk negative potential to develop liver cancer. However according to (IARC, 2018) data, no human studies have been done to unravel the mechanistic danger of continuous exposure to pesticides, that has been hypothesized to initiate hepatocellular carcinoma. In this regard future studies are recommended in this area.

2.3.0 Cyanobacteria Harmful algal blooms

Hepatocellular Carcinoma cases are highly reported in regions experiencing cyanobacterial harmful algal blooms [15,64] . The cyanoHAB comes as a result of photosynthetic cyanobacteria multiplying within freshwater systems given favorable environmental factors, such as light intensity, nutrients, pH, short-wavelength radiations, and temperature [41]. Cyanobacteria thrive in both freshwater lakes and hyperoligotrophic open oceans. They can also be found living in psychrotrophic conditions near 0°C, in hot springs at 73°C, and in areas with between this two extreme temperature[38]. Some genera such as: *Anabaena, Microcystis, Oscillatoria, Planktothrix and Nostoc* [50] are able to produce harmful secondary metabolites known as cyanotoxins; (Microcystin (hepatotoxin) , Neurotoxin ,cytotoxin); However, the microcystins constitute an important and prevalent cyanotoxin in surface waters globally, posing environmental and health hazards[31].

Following exposure to cyanotoxins, acute symptoms such as abdominal pain, vomiting, diarrhea, skin irritation, weakness, sore throat, pale mucous membrane, and muscle tremors to chronic diseases such as those related to liver cancer and colorectal malignancies have been observed in animals and humans[15] . Several epidemiological studies have identified potential linkages between microcystin exposure and liver cancer disease [15,42,64] . However in Kenya, health risk to MC exposure in the Nyanza gulf of Lake Victoria remains unknown. Lake Victoria (LV), being one of the fresh water lake body in Africa, is reported to have an increase bloom forming cyanobacteria throughout the year with the ability to produce hazardous cyanotoxins [59].

Around Lake Victoria region there are undocumented claims of cyanotoxin poisoning as a result of cyanobacteria that need to be ascertained [47]. This observation is based on studies by Mchau et al.,[39] on Lake Victoria waters who reported that the concentration of cyanobacteria cells were beyond the (WHO) recommended limits; for instance species of *Microcystis aeruginosa* ranged from 90,361.63 to 3,032.031.65 cells/mL and *Anabaena spp.* range from 13,310.00 to 4,814,702 cells/mL . These visible blooms in the Lake were further associated with Gastrointestinal illness (GI), vomiting and skin irritation as compared to water which had no visible bloom ($P< 0.001$). e.g, well water and pipe treated water. Their results suggested that GI was significantly elevated among lake water users as compared to piped and well water users ($P< 0.001$). The concentration of cyanobacteria blooms poses greater risks especially to the riparian communities. Furthermore, Simiyu et al.,[59] reports of fish within the Lake, are contaminated by the cyanotoxin (microcystin, MC) as a results consumptions of these fish as source of protein dietary is hypothesized to be of human health risk. For instance, Over their period of study, samples of fish from Kisumu bay were reported to have a higher concentration of MC ranging from; 190 ± 51 to 543 ± 26 ng MC/g as compared to those from Rusinga channel which had MC levels of 56 ± 56 ng MC/g, as determined by ELISA. These were reported to be statistically significant when measured using ANOVA on ranks; ($p = 0.034$, Tukey test $p < 0.05$) as shown in fig.3 below. This is in further support by Moreira et al., [43],who reports the health status of communities within Kisumu area whose health could have been threatened by the consumption and use of cyanoHab contaminated Lake water. All these taken together with, Onyango et al.,48] study, confirms further on the implication of microcystin on the Lake Victoria fishery and, ultimately, on the human population who depend on the Lake water as source of domestic use. Going by the above observations, the communities living around Lake Victoria who draw cyanotoxin polluted water for their domestic and animal use are exposed to high level of toxins thus the need for surveillance and determination of risk factors under current conditions.

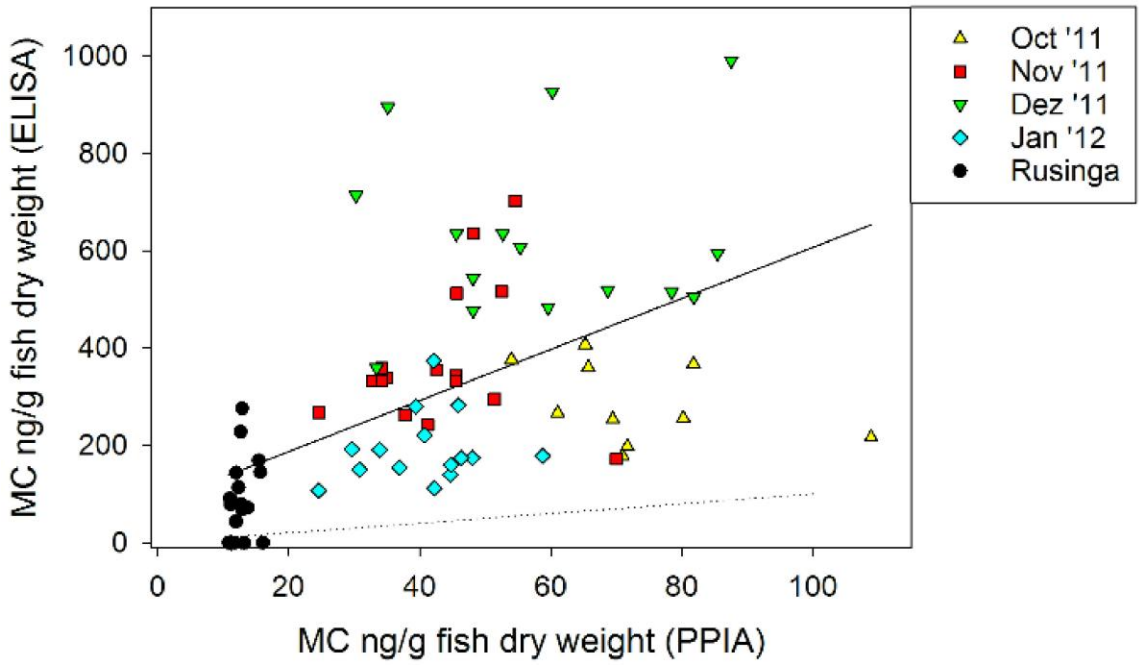

Figure 3. Correlation of microcystin contents in small fish (ng/g in dry weight) as determined by ELISA vs. microcystin contents as determined by the PPIA ($R2 = 0.29$, $p < 0.0001$). The PPIA

results were on average 8 (±0.5)-fold lower than those obtained by ELISA. The one-to-one relationship is indicated by the dotted line, Simiyu et al., [59].

2.3.1 Microcystin

Microcystins (MC) are the most prevalent and significant of the freshwater cyanotoxins[39].They are produced by cyanobacterium *Microcystis aeruginosa*, *Anabaena and Plankthorix* [50].These toxins are mainly stored inside the cyanobacterial cell and are released into the water during cell lysis [22], potentially leading to high toxin concentrations in water bodies[31]. The toxins thus pose a substantial health risk to livestock, humans and aquatic animal species who rely on such water for drinking, sanitization or as food source [31] .Various environmental parameters affects MCs production within freshwater ecosystems, including pH, nitrogen, phosphorus and water temperature [21]. MCs contain a unique molecular substructure, 3-amino9-methoxy-2, 6, 8-trimethyl-10-phenyl-deca-4,6-dienoic acid (Adda)[**Error! Reference source not found.**](Fig.4). Two variable sites (2 and 4) within the heptapeptide diff erentiates individual congeners. These variations in the amino acid composition, as well as in the presence of methyl groups, causes differences in the toxicity[54].Over 250 congeners have been identified to date, with MC-LR (leucine, arginine) being the most studied and toxic variant of MCs [**Error! Reference source not found.**].

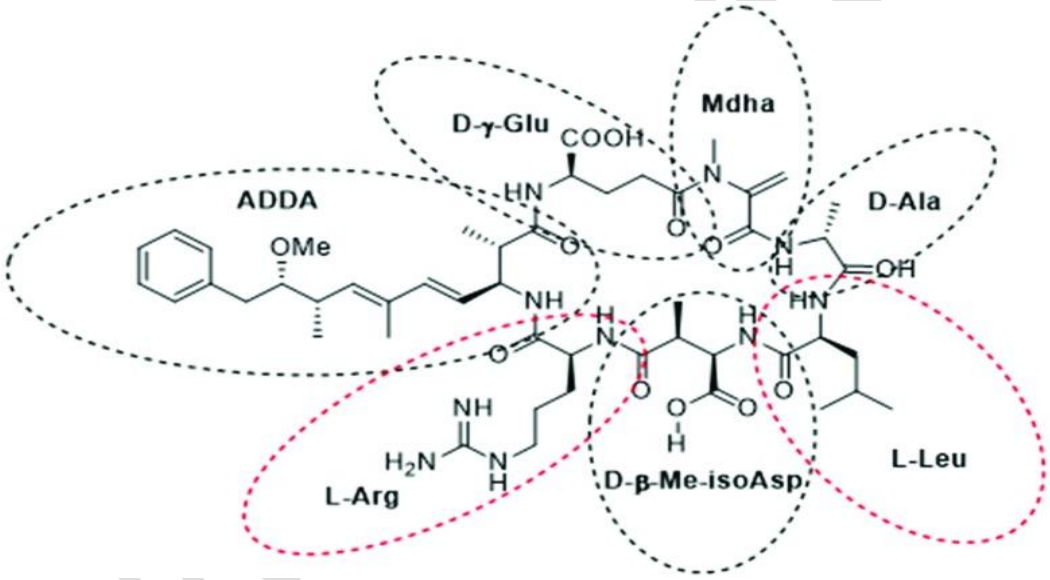

Fig.4 Chemical structure of MC-LR highlighting the seven amino acid residues; adapted from Campos et al., [Error! Reference source not found.]

Microcystin are synthesized in cyanobacterial cells by *microcystin synthetases* enzyme encoded by *mcy E* gene. The Microcystin toxin biosynthetic gene cluster consists of genes encoding peptide synthetase, polyketide synthase, or modifying enzymes and mapped to a 55 kb cluster. Genes in the 55 kb cluster include *mcyA* through *mcyJ [***Error! Reference source not found.**]. Of the many potentially usable *mcy* genes, *mcyE* gene encodes a mixed polyketide synthase/peptide synthetase involved in synthesis of Adda and the activation and addition of D-glutamate into

microcystin molecule [**Error! Reference source not found.**]. These two amino acids are crucial to toxicity and vary less than do the other amino acids of the molecule [54]. Taken together, the *mcyE* gene region is believed to be a reliable molecular marker for the detection of microcystin producers. Microcystins are extremely stable and resist common chemical breakdown)[**Error! Reference source not found.**] . In spite of the chemical stability of the MCs, specific bacteria that co-exist with cyanobacteria within same aquatic environment are able to degrade these toxins, using an enzyme known as microcystinase which is encoded by microcystinmethylopeptidase (*mlr*A) gene [29]. Only few studies have reported MC degradation capacities by other microorganisms, such as fungi or ochrophyta (Poterioochromonassp),(Zhang et al., 2008) [**Error! Reference source not found.**], thus conferring to bacteria the major biological MC degradation in nature.

The *mlr*A gene encoding methylopeptidase (*mlr*A enzyme) catalyzes the first step of bacterial degradation of cyanobacterial hepatotoxin associated with hydrolysis and ring opening of microcystin molecule at the Adda-Arg peptide-bond formation site [29].In order to provide safe drinking water, biological degradation is considered the most successful solution for MCs removal in the natural environment[34]. However, there is still scarce information about the degrading bacteria species. It is therefore important to unravel the correlation between microcystin producing bacteria and microcystin degrading bacteria in Lake Victoria in order to provide solution of eradicating MCs toxicity to human/animals depending on the Lake as a source of water for domestic use. Nevertheless, toxin quota per cell directly reflects the safety of the water body and it is also therefore important to clarify the correlation between the gene transcript levels of *mcyE* genes, MC concentration and effect on human serine-threonine protein phosphatase serum levels for a holistic understanding of the correlation. Even though it is hypothesized that the correlation of the MC concentration and *mcyE* gene abundance is very strong, the number of gene copies merely reveals the potential to produce the toxin, and does not indicate if the genes of interest are actively expressed and toxins produced [44] hence the need for quantification of the *mcy*E and *mlr*A genes in cyanobacteria.

2.3.1.1 Human microcystin exposure

MCs are usually contained within the cyanobacterial cells and are abundantly released to the environment during cell lysis. These toxins are soluble in water and are impermeable in the cell membranes of animals, plants or bacterial cells; thus unable to be absorbed using membrane transport system [**Error! Reference source not found.**]. Biological evidence of exposure can greatly improve health studies of human exposure episodes to microcystins by increasing the specificity of any observed associations. Therefore, their primary route of exposure to animals is via oral consumption of contaminated water, vegetables and aquatic organism such as (fish, crustaceans and mollusks) [**Error! Reference source not found.**]. For instance, it is evident that MCs primarily accumulate in fish livers, but can also be detected in muscles and viscera [48]. According to Drobac et al.,[**Error! Reference source not found.**] ,toxin accumulation in fish tissues of the commercial species Liza sp. was found to be high in the liver, intestines, gills and muscles. In Kenya, these possess a great danger to health of the populations that feeds on the fish and therefore creates a health risk for communities living along Lake Victoria. Furthermore, the community use fish harvested from the lake for protein. Thus, chanced exposure to high levels of microcystin is possible since majority of microcystins are maintained [**Error! Reference source not**

found.] and their release into water makes them much more difficult to remove from drinking water by conventional water treatment processes [39].

2.3.1.2 Toxic mechanism of microystin in humans

Microcystin primary target organ in animals is the Liver. This is because their transportation and uptake are facilitated by bile acid transport system known as organic anion-transporting polypeptides (OATPS) which are mainly expressed in hepatocytes cells [18]. However, OATPs are known to be expressed in other organs such as the stomach, small and large intestines, kidney and brain, intimating that MCs could also target other organs in the body[40].Their mechanism of toxicity is based on the inhibition of the protein phosphatases 1 (PP1) and 2A (PP2A) of eukaryotic cells, enzymes that catalyze the dephosphorylation (removal of a phosphate group) of the serine/threonine amino acids from proteins and are important for many signal transduction pathways in the cell (cytoskeletal re-arrangement, cell movement, apoptosis, etc[37]. The different amount of phosphatases and kinases (enzymes that incorporates a phosphate group to the proteins) in the cell may seriously affect the cellular structure and function by even causing uncontrolled cell proliferation and cancer development [**Error! Reference source not found.**,**Error! Reference source not found.**]. As a result, MCs have also been described as possible tumor promoters [40] that needs to be ascertained. Thus MCs are classified as hepatotoxin and human exposure health effects has been of great concern in regions with high endemicity of liver cancer (**Table 1).**Microcystin increases the positive foci of the placental form of glutathione S-transferase (GST-P) in rat liver, which was initiated with diethylnitrosamine (DEN) and/or aflatoxin[18]. Nodularin, a hepatotoxic cyclic pentapeptide stimulates GST-P positive foci in rat liver more effectively than MCLR, and without initiation with DEN[67] . Blood-based tumor genotyping indicate that patients with cancer have markedly higher concentrations of circulating cell-free DNA (cfDNA) than healthy individuals[**Error! Reference source not found.**] . In patients with metastatic cancer, plasma derived cfDNA has been shown to be a reliable surrogate for genomic alterations in tumor tissue[56]. In this regard, the physiological effects and implications of microcystins association to human and animals needs to be address based on its ability to cause cancer through acute doses and potentially promote it through chronic exposure to low MC concentrations in drinking water as reported from previous studies [64]. Thus biochemical characterization evidence for human exposure to microcystins in order to identify uncertainties associated with the interpretation of results and toxicokenetics of microcystins is significant in managing its effects in human.

Table 1. Summary of epidemiological investigations of MCs In Liver cancer endemic regions .

Authors	Country	Study Design	Toxin	Findings
Ueno et al.,[64]	China	Epidemiological survey	Microcystin	MC detection in drinking water sources correlates with a high PLC incidence
Li et al.,[35]	USA	Pilot ecological study	Microcystin	Residential proximity to surface water drinking sources increases HCC risk
Chen et al.,[35]	China	Longitudinal	Microcystin	Concurrent detection of serum MCs and

				liver enzymes indicate hepatocellular damage in fishermen
Svirceve et al.,[62]	Serbia	Descriptive	Microcystin	Significant and persistent blooms correlate with PLC mortality and incidence
Zheng et al.,[42]	China	Case-control	Microcystin	Serum MC detection in patients link to HCC risk

Several epidemiological studies have documented report on animal poisoning and death due to drinking microcystin contaminated water. Microcystin in drinking water could be a risk factor for primary liver cancer (PLC) [35], however there is paucity of information regarding this situation. It is hypothesized that the development of PLC results from cirrhosis and chronic viral hepatitis (HBV HCV) though this is not conclusive [62]. According to Li et al., [35], consumption of drinking water contaminated by MCs has a direct correlation to human PLC that needs to be ascertained. Exposure to MCs has also been linked to increased incidence of Primary Liver Cancer, for instance, studies done in Serbia, United states and China reported a positive correlation between chronic exposure to MCs in the drinking water and incidence of PLC [50]. For instance a study carried out among the fishermen at Lake Chaohu confirmed the presence of MCs in human serum for the first time[11]. The levels of serum-MCs detected in 35 samples had a mean of 0.389ng/mL. The range of MCs levels estimated in serum samples of the fishermen was from 2.2-3.9µg/person, which showed to be above the WHO's recommended daily intake (2.0-3.0µg/person) for lifetime daily exposure. Suggesting the high level of health risk to t fishermen and people depending on MCs-contaminated water . From their multivariable analysis, a significant positive correlation between serum liver enzymes and MCs levels was determined, hence hypothesizing the association between Liver damage in fishermen and the continuous exposure to MCs. These results are in support with the study done in Southwest China, whereby a significant positive correlation was observed from serum-MCs and development of PLC [42]. In this study all the known risk factors, such as HBV, alcohol and afflatoxin were statistically controlled. As a result the odd ratio for PLC risk was elevated by 2.3(95% CI 1.5–5.5) as an increase in levels of serum MCs was detected in patients. There was a positive relationship determined with alcohol using Binary logistic regression (synergism index = 4.0 95% CI 1.7–9.5) and HBV (synergism index = 3.0 95% CI 2.0–4.5), but a negative interplay with aflatoxin (synergism index = 0.4 95% CI 0.3–0.7). Their results established serum-MCs as a standalone risk factor for PLC development. Moreover, in China it was observed that PLC cases decreased after cyanotoxin-free drinking water was introduced in the affected regions. [35].However, one limitation to these epidemiological studies is that MCs toxicity were associated with PLC by estimating its presence in drinking water and not serum, thus providing inadequate data on direct linkage between occurrence of Human PLC. There is therefore a need of on extensive research on epidemiological data to determine human-serum microcystin presence and its association with occurrence of PLC within communities living around Lake Victoria, Kenya.

Although few laboratory studies have concluded that chronic exposure to microcystin can cause liver cell damage and ultimately lead to a significant increase in serum liver enzyme levels using animal models [37,56] , limited information have been acquired from effects on human exposure

to microsytin. For instance, Li et al.,[35]linked toxic algae in a water supply reservoir to serum enzyme elevations in children exposed to microcystin. Human serum samples may be easily analyzed for the presence of free microcystins using a screening assay such as the ELISA; however, quantification of free serum microcystins does not accurately represent total toxin concentration. This is an important risk assessment outcome that can be used to measure serum concentration of microcystins- in order to estimate the dose and timing of human microcystin exposure. Quantification of free and bound toxin using a method such as 2-methyl-4-phenylbutyric acid(MMPB technique) may provide a better estimation of absorbed dose [56] . In Caruaru, Brazil, 76 patients died because of dialysate contamination by microcystin [10]. Chen et al.,[11] identified microcystin in the serum of highly exposed Brazilian fishermen as well as indication of liver damage. However, scarce information has been reported using animal models on microcystin effects on protein phosphatases 1 (PP1) and 2A (PP2A) of eukaryotic cells, enzymes that catalyze the dephosphorylation of the serine/threonine amino acids from proteins, which is reported as the main action of toxicity leading to PLC development. Therefore, additional studies on effects of microcystin in human serum levels of serine-threonine protein phosphates among liver cancer patients to determine the association with occurrence of the tumor is paramount.

3.0 Conclusion

Primary Liver disease is a multifactorial disease of identified known risk factors, including alcohol consumption, cigarette smoking, and hepatitis B and C virus infections. Despite these known risk factors being managed medically in the developing countries, PLC cases continue to be on rise. A number of epidemiological investigations hypothesized the association between environmental toxin exposure and hepatocellular carcinoma development in endemic regions. Among the many epidemiological studies associating PLC and environmental toxin as an emergent risk factor reviewed herein, environmental exposure of microcystin toxin is inferred to be one of the main contributor to PLC hence a public health hazard due to its continued presence in drinking water sources. Majority of the epidemiological data are in support of the potential association between environmental microcystin toxins and hepatocellular cancer in developing countries. However, the limitation of these studies is that MCs toxicity were associated with PLC by estimating its presence in drinking water not serum ,thus providing inadequate data on direct linkage between occurrence of Human PLC and Microcystin. There is therefore a need of an extensive research on molecular epidemiological data on determining human-serum microcystin presence and its association with occurrence of reported increase PLC cases, among population living along Nyanza gulf whose microcystin concentration levels is reported to be beyond WHO"s recommended merit of 1.0µg L-1, for quality water safe for consumption. This calls for concerted effort and laboratory based studies in order to unravel and mitigate the pathophysiological risks of MCs. These findings can be used to edify the health and medical professionals at all levels of prevention, including the diagnosis and treatment of liver disease patients

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