Microcystins: An Emerging Biomarker and Toxicity Prediction in Human and Animal

Kailash Prasad Jaiswal[1], A.S. Yadav[2], Ragini Gothalwal[1]
[1]*Department of Biotechnology Bioinformatics Centre, Barkatullah University, Bhopal - 462026 (M.P.) India.* [2]*Department of Higher Education, Govt. of M.P., Vallabh Bhavan, Bhopal-462004, India.*

Abstract:
Microcystins (MCs) are cyanotoxins produced mainly by the Microcystis species and are reported to be hepatotoxic. The toxicity on exposure to MCs was reported worldwide in fish, animals and in humans for over a century. The findings of investigation revealed that about 100 known variants of MCs till date and of them the most toxic and widely distributed MC is Microcystin-LR (MC-LR). Epidemiological studies is linked the exposure to MCs (MC-LR) with high incidence of liver cancer (hepatotoxic) and a potent tumour promoter. The microcystins biomarker for microcystins toxicity prediction in human domestic and wild animal has been reported to detect MCs toxicity. The biomarkers for biochemical alterations in the human and animal cells also suggests tools to be develop, validate existing methods to detect MCs and its biomarkers in body fluids of living beings. The microcystin toxin, biotransformation and oxidative stress parameters have been most extensively investigated. The present review deals with the emerging use of MCs as Biomarkers for toxicity prediction and recent development in this area.

Key word: *Emerging biomarkers, hepatotoxicity,*

Introduction:

In the 21st century there has been apparent increase in the frequency and intensity of toxin producing cyanobacteria in the fresh water of rivers, dams and lakes, toxic metabolites (cyanotoxins) produced by cyanobacterial species which have high impact on ecosystem and public health. Microcystins (MCs) are hepatotoxins produced by cyanobacteria of genera *Microcystis* found in wide range of water bodies. MCs can induce acute and chronic effects on humans and animals, after ingestion/contact with contaminated water. The microcystins are ubiquitous in freshwater systems that are frequently subjected to eutrophication, the fact that they are found globally, the risk of widespread human, animal and wildlife exposure from many sources (air, water and food). Biomarkers are "cellular, biochemical or molecular alterations which are measurable in biological media such as human tissues, cells, or fluids" also use for detecting predicts risk are important for disease prevention. Although there has been significant progress in the technical development of biomarkers, implementation of their use in human populations has progressed much more slowly therefore, needs in the development of biomarkers for fill gaps in our ability to observe steps in the continuum from exposure to disease, the relationships between biomarker responses, the sensitivity, specificity, and variability of biomarkers need to be better validated as predictors of disease risk and public health tools. The identification and subsequent development of methods and tools to measure MCs exposure biomarkers will play a key role in investigating the role of these toxins in liver. Recently, the measurement of circulating miRNAs has been promising in identifying new biomarkers of liver injury. More studies are needed to evaluate the sensitivity and specificity of the emerging biomarkers and it is also important to validate the omics biomarkers to develop tests that are both clinically useful and cost-effective. This review aims to summarise the information published so far regarding most powerful microcystin toxicity biomarker as well as to provide tools discover and validate emerging microcystins biomarkers for toxicity prediction that will facilitate epidemiological studies for toxicity prediction at early stage in animal and human.

Background:

Cyanobacteria, also known as blue-green algae proliferate in water bodies such as ponds, lakes, reservoirs, many cyanobacteria species produce a group of toxins known as microcystins, some of which are toxic. Upon ingestion, toxic microcystins are actively absorbed by fish, birds and mammals, microcystin primarily affects the liver, causing

minor to widespread damage, depending on the amount of toxin absorbed as well as people swimming, waterskiing, or boating in contaminated water can be exposed to microcytins [6]. Microcystins are produced by the cyanobacterial Microcystis spec. cells, when the alga dies, the cell walls burst, releasing the toxin (cyanotoxin) into the water. Microcystins are extremely stable and resist common chemical breakdown such as hydrolysis or oxidation under conditions found in most natural and fresh water. These toxins can break down slowly at high temperature (40 °C or 104 o F) at either very low (<1) or high (>9) pH . The half-life, the time it takes for one-half of the toxin to degrade, at pH 1 and 40 ^{o}C in full sunlight is 3 weeks; at typical ambient conditions half-life is 10 weeks [35,36]. The most frequently reported cyanotoxin is the hepatotoxin microcystin mainly microcystin-LR, RR and YR) which are the most toxic and abundant species [8]. The Microcystin group of cyclic heptapeptides comprises approximately 90 variants, being microcystin-LR (MCLR) the most frequent and toxic variant [18]. Microcystins (MCs) are cyclic heptapeptides, composed of seven amino acids, more than 90 variants are known, many of which are potent hepatotoxins, and it has been demonstrated that MCs have toxic effects to various organs in human and animals [53].

The microcystins inhibit serine-threonine protein phosphatises 1 and 2A in animals including humans, thereby causing damage to the liver as well as nephro- and neuro-toxicity [53]. The Epidemiologic studies performed to detect Microcystins intoxication with sub-lethal and prolonged exposure to cyanobacteria failed to describe a quantitative relationship between microcystin concentration in plasma and injury extension, liver cancer associated to prolonged ingestion of MCs contaminated water and death induced by progressive liver failure (Fig-1) associated with chronic intake of dietary supplements of MC-LR, [15, 36,29] as well as the potential impacts of microcystins (MC) on the immune system of the freshwater zebra mussel. Mussels were fed three toxic cyanobacterial strains, with different toxin profiles (presence of MC-LR and MC-LF), the two most toxic strains a potential short-term inflammatory response to MC. MC appears to have an potential sublethal effect of MC on freshwater organisms and illustrates the relative toxicities of the different MC variants, MC-LF being potentially more toxic than MC-LR [24]. Molly fish were used as animal models to detect the toxicity of microcystins consumed along with the diet intake, signs of toxicity exhibited by Molly fish indicated that the bloom samples were predominantly hepatotoxic in nature [21, 43]. The World Health Organization (WHO) adopted a provisional guideline value of 1 μg^{-L} for a maximum concentration of MC-LR in drinking water [49], the recently updated International cyanotoxin drinking water guidelines shown in Table-1.

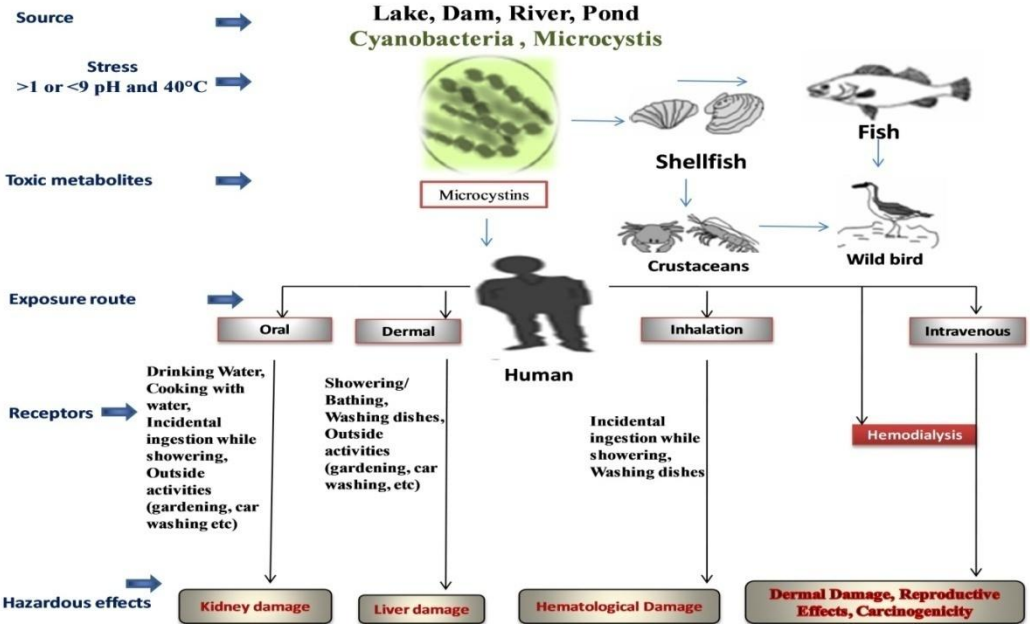

Fig-1: Hypothesized diagram for microcytins exposure and their toxic effects in Human, domestic and wild animal and wild bird.

Table-1: International cyanotoxin drinking water guidelines [49,13].

Authority/Country/State	Microcystin Value (lifetime)
World Health Organization (WHO), 2002	1 µg/L MC-LR
Health Canada, 2002	1.5 µg/L MC-LR
Brazil, 2005	1 µg/LMC-LR
Australia, 2009	1.3 µg/L MC-LR TE
New Zealand, 2009	1 µg/L MC-LR TE
Singapore, Poland, Norway, Netherlands, Korea, Japan, Italy, Germany, France, Finland, Denmark, Czech Republic, China, 2015.	1 µg/L MC-LR

Mechanism of cellular intoxication and biomarker prediction:

Amoung the microcystin congests the MC-LR is one of the first detected microcystin studies on its toxic effects have since been conducted both in vitro, observed that microcystins leads to disruption of liver cells, loss of sinusoidal structure, intrahepatic hemorrhage, hemodynamic shock, and finally heart failure and death of mice [13,52,32] and in vivo [19,9] and microcystins poisoning in mammals is characterized by disruption of hepatic architecture, leading to massive intrahepatic hemorrhage and death in a few hours [34] and chronic exposure studies reveal a wide range of hepatocyte damage like cytosolic vacuolation, single-cell necrosis, fibrosis, apoptosis and tumor promotion, the liver failure (Caruaru syndrome) and gastrointestinal syndromes , skin damage, respiratory problems and liver cancer is also associated to microcystin-LR (MCLR) intoxications [14, 20, 23, 21]. Despite studies on cellular intoxication of MC-LR that increases other proteins in pathways leading to apoptosis by which microcystins destroy livers [18] and the study of cytotoxicity of microcystin 16 types of variants on primary culture rat hepatocytes, MC variants containing D-Asp3 and Dha7 and/or Dhb7 residues were found to exhibit stronger cytotoxic activities than their corresponding normal MC variants, the Z-Dhb7 residue of the MC variants is important for their cytotoxic potential [42]. MC-LR exposure in the presence of FK506, supporting the hypothesis that MC-LR appeared to cause neuronal toxicity by activation of CaN and the CaN-mediated mitochondrial apoptotic pathway [13] and toxin extract revealed severe changes in liver histology and displayed apparent signs of degenerative hepatic structure and cytoplasmic vacuolation and parenchyma exhibit hepatocytes degeneration and the effect of the extract on blood contents and liver function of white mice was investigated, the result showed signs of acute cellular and physiological damage involving oligocythemia, leucoytosis, marked increase in serum urea, cholesterol, triglycerides, creatinine, Aspartate Aminotransferase (AST), alanine aminotransferase (ALT), hematocrit (HCT) and mean cell hemoglobin concentration (MCHC), while blood platelets showed abnormal increase suggesting an inhibitory action on haemopiesis [1]. MC-LR variants comparatively MC-LR and MC-RR induced cytotoxicity in human intestinal cells, a major difference in IL-8 production was observed [23] and microcystin LR are also used for MC-LA, RR and YR, MC-LR induced greater effects on cytotoxicity and IL-8 secretion when compared to MC-RR, although no differences in intracellular ROS production were observed between these two MCs are highly dependent on the MC variants [45] as well as MC-LR toxicity induced production of reactive oxygen species (ROS) in Chinese hamster ovary (CHO) cells and human bronchial epithelial (HBE) cells, and ROS production involved in the procession of reproductive toxicity and respiratory toxicity induced by MC-LR and intracellular oxidative stress reactions induced by ROS lead to apoptosis [30]. MC-LR exposure induced Tau and VASP hyperphosphorylation during cytoskeletal reorganization in HL7702 cells, increasingly phosphorylated Tau was distributed in the cytosolic fraction, and disassociated from microtubules [44].The pathways of MCs toxic action in human and animal cells are represented in Figure-3. Recently [30] found that in the liver cell line Tau phosphorylation may be regulated by the PP2A mediated activation of the p38 MAPK signal pathway, although VASP phosphorylation

increased markedly in response to MC-LR treatment, the phosphorylation of VASP seemed to regulate its intracellular location but not its cytoskeletal regulation activities, and it was not a target of PP2A. The Tau is likely a key factor in the destabilization of the cytoskeleton induced by MC-LR, which may be involved in the subsequent MC-LR hepatotoxicity, while MC-LR-induced VASP alteration [21]. MC-LR also induces vascular inflammatory process in cultured human umbilical vein endothelial cells (HUVECs) and enhanced apoptosis and induced intracellular reactive oxygen species formation (ROS) in HUVECs [41]. Biomarkers are biological entities or characteristics that can be used to indicate the status percentage of healthy or diseased cells, tissues, or individuals, mostly molecular makers, such as genes, proteins, metabolites, glycans, and other molecules, that can be used for disease diagnosis, prognosis, prediction of therapeutic responses, as well as therapeutic development [5,46,17,22] some biomarker and their mechenism of action summerised in Table-1.

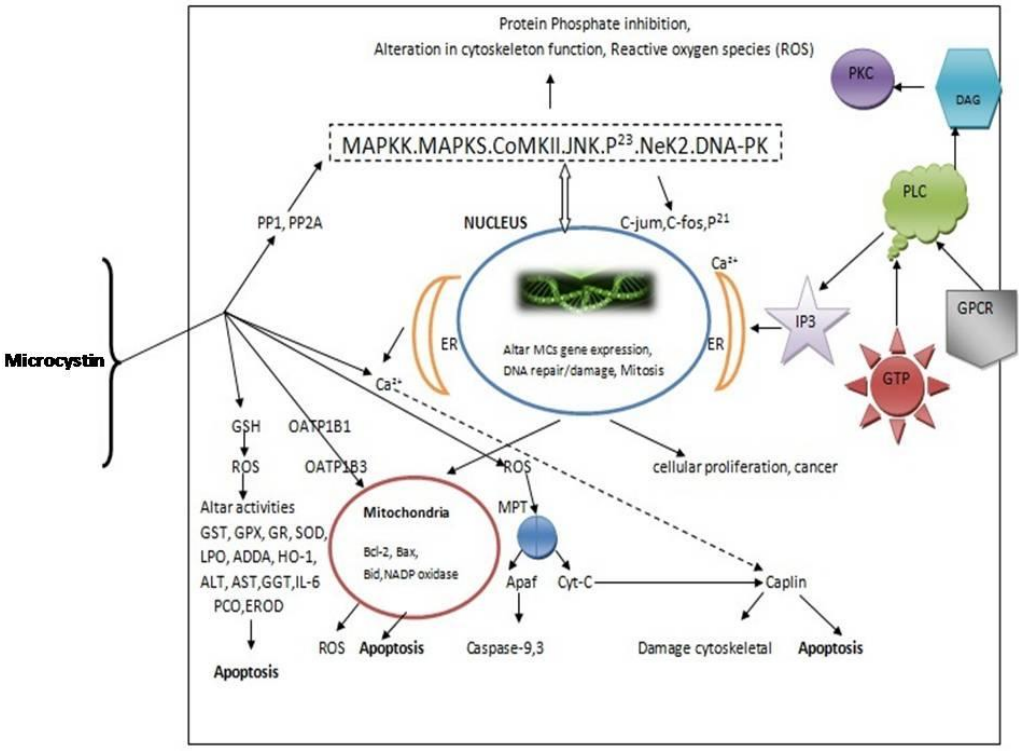

Fig-3: Suggested pathways of MCs toxic action in human and animal cells [7].

Table-2: Types of biomarker based on application and use in disease management:

Biomarker application	Mechanism of action	Disease managements	Reference
Stratification biomarker	Stratification analysis is used to identify predictive biomarkers.	select beast treatment for each patient	[39]
Efficacy biomarker	Biomarker as early killer or as approved surrogate marker	improve patient compliance in the absence of early clinical improvement	[39]
Differentiation biomarker	Differentiate efficiency or safety of drug within the same class.	select beast treatment for each patient	[39]
Toxicity biomarker	Side effects attributable to the mechanism of action of molecular targeted agents thus represent “on-target” modulation in normal tissues. These mechanism-based toxicities can be pharmacodynamic effects of pathway inhibition and, in tumors depending on the inhibited pathway for	monitor and avoid potential toxic effects	[38]

	proliferation, might be biomarkers of efficacy.		
Screening biomarker	This is contribution of phenotypic screening to the discovery of first-in-class small-molecule drugs exceeded that of target-based approaches.	Early disease detection and early treatment	[39]
Prognostic biomarker	prognostic markers contribute to increase knowledge on the drug and the underlying disease mechanisms.	predict likely course of disease	[11]

Microcystin used as emerging Biomarker for exposure and cellular damage and toxicity prediction:

The using microcystins biomarker aspects of MCs toxicity prediction, molecular mechanisms of microcystins toxicity in the knowledge of the activity at cellular and molecular level as well as molecular basis of MCs exposure, intoxication, and biotransformation is being elucidated [3]. Glutathione Transferases (GSTs) are phase II detoxification enzymes known to be involved in the molecular response against induced toxicity, study by Bruno et al., [4] the time-dependent changes on gene expression of several GST isoforms (pi, mu, sigma 1, sigma 2) in parallel with enzymatic activity of total GST were investigated in gills and hepatopancreas of the bivalve *Ruditapes philippinarum* exposed to pure MC-LR (10 and 100 μg^{-L}). MC-LR affected in gills and hepatopancreas and GST transcriptional changes in gills promoted by MC-LR were characterized by an early (12 h) induction of mu and sigma 1 transcripts. The different transcription patterns obtained for the tested GST isoforms, the potential divergent physiological roles played by these isoenzymes during the detoxification of MC-LR [4]. Many studies on prediction of cyanotoxins biomarkers positive correlation between the concentration of MCs in serum with liver damage markers such as the pyruvic- and glutamic-transaminases (AST, ALT) and the gammaglutamyl- transpeptidase (g-GT) [10] , the biomarkers of prolonged exposure to microcystin-LR in mice found ALT, ALP, Bb, MetHb, ROOHs, glutathione, a-tocopherol levels, SOD activity and plasma lipid profile alterated, these parameters determined in plasma, for the detection of MC-LR in plasma or urine could be used as biomarkers [40]. The exposure to microcystin-LR, the relative expression of plasma levels of 4 miRNAs miR-122-5p and let- 7c-5p, the liver-enriched microRNAs, miR-148a- 3p which promotes the hepatocarcinogenic specific phenotype in mammals, and miR-92a-3p, a cell proliferation and angiogenesis promoter, potentially hepatocarcinogenic[16] also tumor promoter rather than a tumor initiator, [13,33]. The molecular mechanism of microcystin toxicity has been studied at cellular and molecular level and the molecular microcystin biomarkers in human and animal and their role in MC mediated cell injury is presented in the Table-3.

Table- 3: Molecular Biomarker of Microcystins and their toxic effects.

Microcystins	Biomarkers	MCs Toxic effects	Reference
MC-LR	Glutathione Peroxidase(GPX) Glutathione Reductase (GR) Superoxide Dismutase(SOD) Lipid Peroxidase(LPO)	Oxygen mediated toxicity in Liver and Kidney	[25]
	Glutathione –S Transferase(GST)	Sphingolipid inhibition and alter cell signaling pathaway,toxicity in liver and kidney	[37]
	PP1(Protein Phosphate-1) PP2A(Protein phosphate 2A)	Protein Phosphate inhibition, alter cell signaling pathway	

	ADDA (3-amino-9 methoxy-2,6,8-trimethyl 10Phenyledeca,4,6-dienoic acid)	target cellular proteasome and selectively inhibit proteasome trypsin-like (TL) activity	[32;45]
			[54;20]
	Alanine transferase (ALT) Aspartate Transaminase(AST) γ-Glutamyl Transferase(GGT)	Hepatotoxicosis	
	Bax,bid	Decrease protein/gene expression	[48]
	DNA-PK	Decrease activity of DNA repair synthesis in animal cell	
	CaMKII	Increase activity of hepatocyte, alter cell signaling pathway	[50]
	NeK2	alter cell signaling pathway ,increase activity of Nek2	[26]
	P^{53}	Increase protein expression, alter gene expression in FL cell, hepatocyte.	[31]
			[50;52]
	MAPKs	Alter cell proliferation, signal transduction, increase gene expression	
			[25]
	IL-8	Increase expression, alter cell signaling pathway	[47]
	P-glycoprotein	Increase protein expression and gene activity in cell	
	miR-122-5p	Hepatotoxicity	[27;28]
			[2]
			[16]

Conclusion and future perspectives:

Among all cyanotoxins the microcystins (MCs) is most potential toxin have 100 variants; their prolonged and sub-acute intoxications are sub-diagnosed. The international agency for research on cancer reported that microcystin-LR is a tumor promoter rather than a tumor initiator and many researchers also found carcinogenicity studies of purified microcystin-LR in human and animal cells. The biomarkers studied in human, animal and wildlife exposed to microcystin toxin, intracellular intoxication and oxidative stress parameters have been most extensively investigated. However, the individual role of the several GST isoforms in the MC detoxification process is still unknown in the animal cells. Therefore, it is necessary to develop tools to discover, develop and validate biomarker that allow their effective detection and that can be transferred to health care systems in affected areas. In the future molecular studies are needed to discover identification and validation of potential emerging biomarkers which provide a clear view about the microcystin toxin prediction. The role of the cyanotoxins may also be understood by the study of mutant genes of cyanobacterial strains, such mutants, gene (s) for a known cyanopeptide are knocked out, but still the internal cell regulation compensates for the missing peptide need to study and develop, validate biomarker toxin peptide functions for the cells of human and animal.

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Conflict of interest:
Authors declare that there are no conflicts of interest.

Reference:

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