Review Article:

Biomarkers of Liver Injury

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Abbreviations :SGOT: serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase,GGT : gamma-glutamyl transferase ,HPC: hepatic progenitor cells,HSC: hepatic stellate cells,HMGB1: High mobility group box1,Mi RNA:microRNA,RNA: Ribonucleic acid,DNA: Deoxyribonucleic Acid,ACLF: acute on chronic liver failure,MELD: Model End Stage Liver Disease, ALF:Acute Liver failure.

Introduction:

Liver injury in children is common. It can present clinically as either subclinical hepatitis, acute hepatitis, chronic hepatitis, compensated liver chronic disease , decompensated liver cirrhosis , acute liver failure or acute on chronic liver failure. Routine biochemical parameters that are analysed as Liver Function test includes total bilirubin, direct bilirubin, SGOT, SGPT, albumin, pro-thrombin time & GGT

.With recent advances and applications of technology newer biomarkers are now available . These biomarkers are earlier studied in animal models, later in humans. They do have diagnostic and prognostic value. They give an idea about histopathological status of liver. But they have their own limitations. This review of biomarkers of liver injury in children underscores newer possibilities and approaches to diagnosis of liver disease. Studies targeting individual biomarkers from therapeutic point can also be considered in near future.

Liver injury:

To understand the biomarker it's important to understand pathogenesis and pathophysiology of liver disease in general. Detail histopathological marker analysis is beyond the scope of this article.

Liver consists of hepatocytes, cholangiocytes, kupffer cells, hepatic progenitor cells (HPC) hepatic stellate cells (HSC), bone marrow derived macrophages. It has been well studied now that different types of liver injury elicit different responses. Hepatocyte proliferation is seen in acute liver failure. [1] Hepatocyte progenitor cells (HPC) activity is higher in acute on chronic liver failure. HPC proliferation is comparatively more in acute on chronic liver failure as compared to chronic liver disease .More the HPC proliferation ,more is the component of inflammation & higher is the degree of liver necrosis and fibrosis. [1] Corresponding markers of liver pathology are expressed in the serum and liver tissue. This bio- phenomenon helps us to study biomarkers of liver injury.

Hepatic stellate cell (HSC) activation plays an important role in fibrogenesis. Activated HSCs are more in acute on chronic liver failure than in acute hepatitis vs chronic hepatitis. The number of HSC and HPC are proportionate to each other in the liver histopathology. [2] Biomarker that activates HSC and stimulates fibrosis e.g. HMGBI play a crucial role. [3] This can be studied and analysed depending on the grade and stage of liver disease.

HPC are bipotential cells.They are capable of forming both hepatocytes and cholangiocytes. During chronic hepatitis, HPCs are responsible for ductular reactions; increased number of ductules which when uncontrolled leads to hepatic fibrogenesis.[4] HPCs act as source of chemokine like chemokine ligand 2 & CX (3) CL1which stimulates macrophages. These chemokines are studied as biomarkers of liver injury and its response. [5]

Kupffer cells activation dominates as first line response while bone marrow derived macrophages act as emergency response team. [6] [7]. Newer biomarkers like soluble CD163 are studied as response to macrophage activation in different liver diseases. Cell tracking, multi-omics phenotypes and single cell RNA sequencing help in analysis of these biomarkers .[6] [7] Chemokines like CXC and interleukin 1 L- 1B are secreted by activated kupffer cells which induce reactive oxygenated necrosis .[8]. This biomarker's application in clinical scenario needs further validation.

What is biomarker?

A biomarker is an objectively measurable indicator of biological process. It can be physiologic or pathological .It can predict outcome of the disease. [9] Newer biomarkers are now increasingly available from bench side to bedside. Initial enthusiasm is now faded and practical limitations of them are realized. Their proper application in clinical setting can help us in diagnosis and prognosis of children with liver diseases.

Biomarkers for liver injury are either biochemically measured like serum phosphorus, serum fibrinogen, alpha fetoprotein, CD markers of differentiation or genetically oriented like miRNAs. Various biomarkers are direct expression of liver cell injury or some are indirect by-products of cellular –intercellular reactions.

Cytokeratin – 18 measured by M30 antibody & M65 antibody. It is released after cellular hepatocyte injury. Hepatocyte mitochondrial protein biomarker molecule like carbamoyl phosphate synthase 1is studied and noted to be more specific than SGPT.[10] Other Mitochondrial damage markers of hepatocytes are mtDNA, glutamate dehydrogenase (GDM) , glycodeoxycholate . [11]

Leucocyte cell derived chemokine2 (Lect 2) is secreted into the serum by the liver. It plays the critical role in stimulation of liver regeneration. Lower the serum level of Lect 2 better is the prognosis. [11] Serum Lect 2 is inversely proportionate to SGPT and SGOT in acute liver failure. [12] The peripheral neutrophil lymphocyte ratio (NLR) is also proposed as biomarker. It is calculated from complete blood count .NLR > 5.7 correlates with poor prognosis. [13]

Right application of biomarker is important in any given clinical setting. Temporal association and rise - fall of biomarker should be analyzed and correlated with the

stage and pattern of liver injury. Blind application of these biomarkers may prove counterproductive. Clinically important biomarkers are discussed here.

1. Cytokeratin 18 (K18).

Cytokeratin 18 is an intermediate filament protein highly expressed in epithelial cells. [14] When released into extracellular space, K18 can be used as biomarker. Apoptosis and necrosis are the two major approaches to hepatocyte cellular death. Caspase cleaved keratin 18 (CCK18) is considered to represent hepatocyte apoptosis measured by M30 antibody ELISA .Keratin 18 (cleaved & un-cleaved) represents necrosis measured by M65 ELISA antibody. M30: M65 ratio is proposed to differentiate apoptotic and necrotic cell death.

M65 Epideath is a modified version of total cell death indicator. It is predictive for mortality independent of age, gender, MELD Score, Child Pugh score and presence of complications of cirrhosis and infections. [15] Cleaved K18 is also studied marker in non alcoholic fatty liver disease. [16] In acute on chronic liver failure non apoptotic pattern of cell death is predominant .It is confirmed by cleaved K18: K18 ratio reduction. [17] M65 levels are higher in ALF as compare to ACLF. M30 / M65 ratio is noted to be useful in ACLF. [18] Mixed pattern of response sometimes do make picture difficult to analyse. Acute liver failure study group (ALFSG) demonstrated that M30 rather than M65 is an effective tool to predict patient that would require liver transplantation. A new ALFSG index is now used to prognosticate [19] these patients.

The limitation of this biomarker is the presence of cytokeratin in non hepatic epithelium. Extra hepatic epitheliolysis can also raise the biomarker levels. [20] In clinical settings, combined and mixed pattern of injury, association with sepsis make it complex scenario. Mixed patterns of cell deaths in which apoptosis and necrosis both take part in cellular injury by different molecular mechanisms can confabulate the interpretation of the biomarker. [21]

2. Liver specific micro RNAs(Mi RNA):

Mi RNAs are small RNA (Ribonucleic acid) molecules that are transcribed from genomic DNA. They do not code for protein. They regulate the transcript. In liver injury, circulated MiRNA are studied and are noted to be useful biomarker for determining the extent of liver damage.

MiRNA - 122 is the most abundant micro RNA in hepatocyte which is released into serum after liver injury. [22] In chronic liver disease, MiRNA - 122 correlate inversely with severity of liver fibrosis. [23] MiRNA - 122, MiRNA - 130, MiRNA - 183 , MiRNA - 196, MiRNA - 209 and MiRNA - 96 are the potential markers of liver injury. Blocking or stimulating these may unveil the therapeutic strategies in management of liver injury in future. [24]

Higher serum level or MiRNA-122, MiRNA-21, MiRNA - 221 are reported in the survivors of acute liver failure. [25] In patient experiencing acute rejection post transplant, serum MiRNA-122 levels are increased.[26] The release of MiRNA - 122 - 5P; MiRNA - 192 - 5P and MiRNA - 1224 - 5P from hepatocytes is generally attributed to oxidative stress .[27] MiRNA – 122 is proposed to predict graft outcome after liver transplant. [28]. MiRNA – 122 inhibitors like Miravirsen and RG – 101 are studied in hepatitis C opening the therapeutic dimension for the future. [29] [30].

Unfortunately all these biomarkers take time for results by conventional quantitative RT - PCR or microarray. This time -lag to determine the outcome in condition like acute liver failure or post transplant rejection is an important limitation. Technological advances may reduce the time required for analysis.

3. Exosomes and Microparticles

Exosomes are endosomal derived small vesicles. They carry variety of cargos like MiRNAs , micro RNAs , non coding RNA s. All these convey cellular information and intercellular communications which enables them to be studied as biomarkers. Hepatocyte releases exosomes to neighbouring cells and communicate signal to regenerate, repair or necrose. CTGF exosome carrying fibrosis signal is now well studied in humans [31] [32] Exosomal MiRNA - 192 from damaged hepatocytes represents potential biomarker in NASH. It represents progression of liver histopathology from steatosis to hepatitis. MiRNA - 192 exosome is increased in patient with NAFLD advanced stage as compared to early stage. [33] Upon liver injury damaged hepatocyte produces exosomes which bind to toll like receptor - 3 (TLR - 3) and activates HSC .This interaction enhances production of chemokines (C - C Motif) ,ligand 20 (CCL20) and interleukin - 17A (IL - 17A) resultant in increase in liver fibrosis. [34] Mesenchymal stromal cells dervived exosome have been studied to have therapeutic value. MSC containing MiRNA 125 b reduces

hepatic fibrosis by inhibiting hepatic stellate cell activation. [35] Further studies are needed for their clinical application.

4 .Bile acid Conjugates:

Bile acid concentrations are increased in liver injury. The ratio of tauro conjugates to glyco-conjugates is increased in hepatic injury suggesting tauro - conjugates are more sensitive measures of liver injury. [36] The high values of conjugated bile acid like glycocholic acid , glycochenodeoxycholic acid are associated with disease likes paracetamol poisoning, chronic hepatitis and other primary liver disorders .Secondary bile acid conjugates like deoxycholic acid are increased in hepatic dysfunction secondary to cardio-vascular disease. [36] This can help in differentiation in co-morbid hepatocellular and cardiovascular pathologies.

In Obstructive cholestasis liver injury, studies done earlier reported hepatocellular death secondary to apoptosis. [37] Recent studies on humans have confirmed increase in M65 antibody secondary to acetylated HMIGB1 indicative of necrosis pattern in these patients.[38] High mobility group box1 (HMGB1) is passively released from necrotic cells and after hyper-acetylation is secreted from inflammatory cells.[39] HMGB1 is thus an important biomarkers of necrotic cell death pattern .

5 .Other Biomarkers:

APAP - protein adducts (related to paracetamol poisoning). GLDH, mt DNA, acylcarnitines , CPS - 1 , are shown to be increased in plasma after paracetamol hepato cellular failure. [40] Argininosuccinate synthetase levels can be detected even before rise in SGPT. [41]

Hypophosphatemia in liver failure is suggestive of good prognosis.Hyperphosphatemia is predictive of poor recovery. Hypophosphatemia may be the consequence of liver regeneration serum phosphorus level > 2. 9 mg / dl suggests massive hepatocyte necrosis and lack of liver regeneration. [42] [43]

Serum fibrinogen is also studied. Plasma fibrinogen level is significantly low in non survivor of acute on chronic liver failure patient compared to Survivor of acute on chronic liver failure; chronic hepatitis B and control patients. [44]

Serum alphafetoprotein is studied biomarker of liver failure. The alpha-fetoprotein ratio defined as day 3 alphafetoprotein level divided by that observed on day 1.

The rise in alphafetoprotein value from day 1 to day 3 indicated better prognosis. [45] It reflects hepatic regeneration. [46] Collagen IV is proposed to be biomarker of liver fibrosis. Hyaluronic acid is also synthesized by fibroblast and is studied as a biomarker.

Collagens - 1, CK - 7, SMA are associated with increased mortality and need for liver transplant in cases of biliary atresia. [47] Increased expression of SHH (sonic hedgehog pathway) Gli- 2, collagen - 1, CK - 7, SMA, CD 34, TLR 317 and decreased expression of PXR and CAR (pregnone and receptor and constitutive androstane receptor) serve as markers of poor transplant free survival in billiary atresia patients. [48] Fibrotic gene signature than inflammatory gene signature is co-related to have lower transplant free survival [49].

Conclusion:

Biomarkers in pediatric liver disease require further validation. They vary depending on inflammatory, angiogenetic or fibrotic stage. The expression of biomarker needs to be co-related with grading and staging of liver injury. Early grades biomarker may not stay true for end stage liver disease. Inflammatory biomarkers tend to predominate depending on necrotic, cholestasis, drug induced or apoptotic liver injury pattern. Fibrotic biomarkers at end stage liver disease have different representation. Methodologically robust studies are need of hour. Therapeutic options targeting specific level of response / injury representative of biomarker is the logical next step in research. Understanding the pathogenesis and pathophysiology of liver injury by clinical biomarker signature would expand our vision in diagnostic, prognostic and therapeutic domains in liver Injury.

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