Section: Biochemistry

Original Research Article

ASSESSMENT OF SERUM MAGNESIUM LEVELS IN PATIENTS WITH LIVER CIRRHOSIS AND ITS CORRELATION WITH SEVERITY OF DISEASE

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Abstract

Background: Liver cirrhosis is an escalating public health concern associated with high mortality rates across genders. It is marked by the substitution of healthy liver tissue with scar tissue, impairing liver function and its ability to metabolize many vital micronutrients including magnesium. Given the potential worsening of liver dysfunction by magnesium deficiency, this study aims to assess serum magnesium levels in patients with liver cirrhosis and investigate any potential link with disease severity. **Materials and Methods:** The present study was conducted in the Department of Biochemistry in collaboration with the Department of Medicine at M.M Institute of Medical Sciences and Research, MMDU, Mullana, Ambala on 100 participants divided into two groups: Group I included 50 individuals diagnosed with liver cirrhosis, categorized by disease severity, while Group II constituted 50 healthy controls. Serum magnesium levels were analysed using the Xylidyl blue Method on a fully automated Analyzer. **Result:** The cases exhibited statistically significant lower mean magnesium levels (1.11 ± 0.13mg/dl) in contrast to the control group (1.65 ± 0.27mg/dl) ($p<0.001$). Furthermore, a comparative analysis of levels of serum magnesium across different classes of cirrhosis (Child Pugh's Class A, B, and C) revealed a progressive decline in magnesium levels with the advancing severity of the disease. **Conclusion:** Magnesium is recognized as an essential cofactor in various liver functions. This study found that patients with cirrhosis exhibited lower magnesium levels, and the severity of the disease correlated with the degree of magnesium depletion. Consequently, prompt rectification of magnesium imbalances in these patients could be a promising strategy for averting or ameliorating complications and the onset of hepatic encephalopathy.

INTRODUCTION

The liver is a multifaceted organ crucial for metabolic processes and various biochemical activities, including metabolic regulation, excretion, secretion, storage, and detoxification.[1] Liver cirrhosis represents the last stage of liver fibrosis, marked by developing regenerative nodules surrounded by fibrous septa due to persistent hepatic injury. While alcoholism and hepatitis B or C infections are prominent causative factors, diverse elements such as advanced age (age >50 years), obesity, insulin resistance/type 2 diabetes, gastrointestinal disorders, hypertension, and hyperlipidemia collectively contribute to its pathogenesis. This pathological condition poses a significant global health challenge, capable of inducing portal hypertension, leading to the development of gastroesophageal varices and intestinal edema, thereby compromising the liver's physiological function.[2,3]

Magnesium stands as the second most plentiful intracellular ion and holds the fourth position among cations within the human body. A mere 0.3% of the total body magnesium resides in serum, highlighting its predominantly intracellular distribution. Magnesium exhibits a broad presence across various cellular compartments, including the nucleus, cytoplasm, mitochondria, and endoplasmic reticulum.4 Its significance extends to numerous cellular processes, encompassing DNA replication and repair, ion transportation, intermediary metabolism, cell proliferation, and signal transduction. Functionally, magnesium forms complexes with ATP, serving as a vital cofactor in approximately 300 enzymatic reactions of various metabolisms. It is essential for a healthy immune system, acting on various processes like antibody production, immune cell interaction, destruction of infected cells and responses mediated by T-helper and B-cells.[4]

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Liver diseases, whether acute or chronic, frequently coincide with magnesium deficiency. Under normal circumstances, the liver synthesizes albumin, a vital carrier protein for magnesium in the bloodstream. However, in individuals with cirrhosis, there is a marked decline in serum albumin levels, impeding the transportation and maintenance of magnesium homeostasis. The liver also plays an important role in the inactivation of various hormones. When this function is compromised, elevated levels of hormones such as aldosterone, growth hormone, and glucagon ensue, consequently promoting the urinary excretion of magnesium. Furthermore, the administration of magnesiuric diuretics, such as furosemide, commonly used to manage ascites in cirrhotic patients, exacerbates urinary magnesium loss. Thus, multiple factors are implicated in the development of magnesium deficiency. This deficiency further impairs hepatic function by exacerbating mitochondrial dysfunction, ultimately contributing to additional inflammatory and fibrotic changes in the liver.[5,6]

Magnesium homeostasis exhibits a robust association with hepatocellular function, prompting an investigation into its potential implication in the aetiology of chronic liver diseases. Studies have shed light on the significance of elevated levels of cyclin M4 protein in driving the progression of cirrhosis. This protein is involved in transporting magnesium out of the liver, which may lead to magnesium levels imbalance leading to aggravation of liver diseases. Furthermore, there is growing evidence that magnesium supplementation can improve liver function in certain hepatic diseases. In view of this, the current study was designed to analyze the serum magnesium levels in patients with liver cirrhosis.[7]

MATERIALS AND METHODS

Study Area: The study was conducted in the Department of Biochemistry in collaboration with the Department of Medicine, M.M Institute of Medical Sciences and Research, MMDU, Mullana, Ambala after obtaining the approval from institutional ethical committee.

Study Design: Case-Control Study

Selection of Patients: 100 individuals aged 40 years and above, with no differentiation by gender, were enrolled, and were divided into two separate groups:

A. Group I comprised 50 individuals diagnosed with liver cirrhosis, categorised into subclasses A, B, and C, reflecting varying degrees of severity as per the Child-Pugh Scoring system.[8]
B. Group II consisted of 50 healthy controls matched for age with the participants in Group I.

Before their inclusion in the study, all participants provided explicit written consent after receiving comprehensive information regarding the study's objectives and procedures.

Inclusion Criteria

1. Participants meeting the criteria for liver cirrhosis diagnosis were included in the study. Diagnosis confirmation relied on thorough clinical evaluation encompassing detailed patient history, positive clinical findings, pertinent biochemical analyses, and confirmation via ultrasonography imaging.
2. Participants aged 40 years or older, regardless of gender.

Exclusion Criteria

1. Pregnant individuals.
2. Patients diagnosed with any of the following conditions:
   - Chronic kidney disease
   - Type 2 Diabetes Mellitus
   - Gastrointestinal disorders such as celiac disease or chronic diarrhea
   - Viral hepatitis B or C.
3. Patients currently undergoing Mg2+ supplementation.

Blood samples were collected from all study participants in a fasting state. Liver function tests including serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), and bilirubin were performed via kits using an autoanalyzer. Serum Mg2+ levels were quantified utilizing the Xylidyl Blue method (Colorimetric Endpoint Method), on a fully automated Analyzer. In this assay, Mg2+ ions react with Xylidyl Blue to generate a blue-violet complex, with the intensity of color directly correlating with the concentration of Mg2+ in the sample.

Statistical Analysis: Following data collection, all information was entered into a Microsoft Excel spreadsheet. Statistical analysis was then performed using SPSS version 20 software. Quantitative data were expressed as Mean ± standard deviation (SD). To assess differences between the study and control groups, an independent Student's t-test was employed. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. The association between Mg2+ levels and disease severity was evaluated utilizing a one-way analysis of variance (ANOVA) test, followed by post hoc analysis to explore specific group differences. Receiver operating characteristic (ROC) analysis was done which is a graphical plot of sensitivity against one minus specificity for different cut-offs. The optimal cut-off value was determined using Youden Index J, the farthest point on ROC curve from the diagonal line of equality.

RESULTS

The study group was well-matched in age with control group. The average age in the study group was 50.96 ± 8.34 years, and the control group had an average age of 51.94 ± 10.64 years. Statistical analysis confirmed no significant difference in age

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between the groups (p > 0.05), mitigating concerns about age-related bias influencing the study results.

There was a notable discrepancy between the study and control groups regarding Mg2+ levels. The mean Mg2+ level in the study group was 1.19 ± 0.13 mg/dl, significantly lower than the control group's mean of 1.65 ± 0.27 mg/dl (p < 0.001). This statistically significant difference is highlighted in [Figure 1 and Table 1].

All three Child Pugh's groups (A, B, and C) exhibited significantly elevated levels of liver enzymes (AST, ALT, ALP) compared to the control group (p-values < 0.001) as displayed in Table 2. This finding suggests the presence of liver damage or dysfunction in all three groups. Serum total bilirubin levels were significantly higher in groups B and C compared to the control group (p-values 0.006 and 0.014, respectively), while group A showed no significant difference. This suggests that liver injury may be more severe in groups B and C. Direct bilirubin levels were only significantly elevated in group B compared to the control group (p-value = 0.023). This suggests a potential blockage in the bile ducts in group B. Groups B and C displayed the presence of ascites, whereas neither Group A nor the control group did. This finding further supports the notion of more severe liver dysfunction in Groups B and C.

Furthermore, when analysing Mg2+ levels alongside other variables across various classes of liver cirrhosis, a significant variation was observed with disease severity, as depicted in [Table 2]. This underscores the dynamic relationship between Mg2+ levels and the progression of liver cirrhosis.

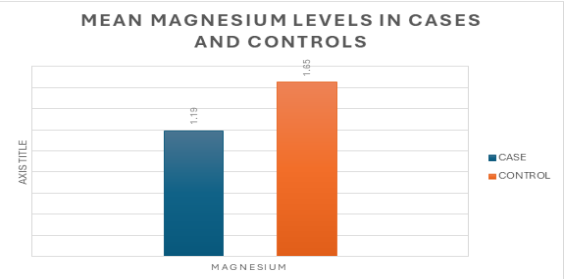

Figure 1. Mean magnesium levels in cases and controls

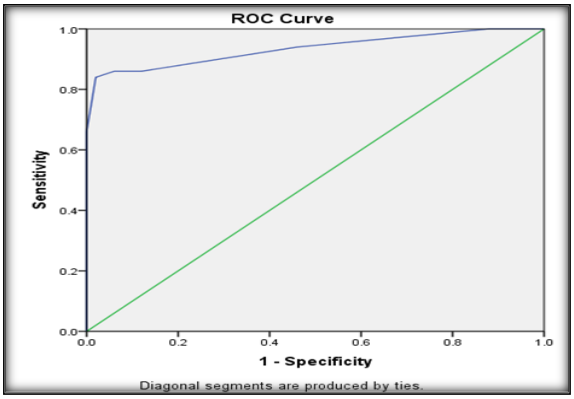

Figure 2: ROC Curve between cases and control to predict diagnostic accuracy of decreased magnesium levels among cirrhotic patients

Table 1: Mean Magnesium Levels In Cases And Controls

	Cases	Control	P value
	Mean ± SD	**Mean ± SD**	
Magnesium	1.19 ± 0.13 mg/dl	1.65 ± 0.27 mg/dl	p<0.001

*significant values P less than 0.05
** highly significant when p less than 0.001

Table 2: Association of various parameters with different Classes of Liver Cirrhosis

Parameters	Child A (n=9) Mean ± SD	Child B (n=16) Mean ± SD	Child C (n=25) Mean ± SD	Control (n=50) Mean ± SD	P value A vs control	P value B vs Control	P value C vs Control
Magnesium (mg/dl)	1.31±0.11	1.18 ±0.03	1.01 ±0.05	1.65 ± 0.27	<0.001*	<0.001*	<0.001*
AST (IU/L)	52.00±4.18	88.40±21.32	282.04±56.84	30.96 ±8.57	0.153	<0.001*	<0.001*
ALT (IU/L)	51.67±7.98	100.73±21.99	278.54±48.83	34.14±9.44	0.010*	<0.001*	<0.001*
ALP (IU/L)	109.67±10.43	147.0±20.98	192.96±38.56	93.56±19.65	0.018*	<0.001*	<0.001*
Billirubin Total (mg/dl)	1.24±0.25	2.38±0.20	5.97±5.09	0.61±0.21	1.000	0.006*	0.014*
Billirubin Direct (mg/dl)	0.62±0.25	1.49±0.27	3.64±3.85	0.20±0.07	1.000	0.023*	0.068
Ascites	Not Seen	++	+++	Absent			

*Significant values P less than 0.05. ALT= alanine transaminase; AST=aspartate transaminase, ALP= alkaline phosphatase

As revealed in [Table 2], patients across all three classes (A, B, and C) displayed significantly lower magnesium (Mg^{2+}) levels compared to the control group (p-values < 0.001). This suggests a possible link between low Mg2+ levels and the severity of liver disease.

[Figure 2] shows Receiver operating characteristic (ROC) curve for Mg2+ used to identify cirrhotic patients. It shows a good diagnostic ability, starting from the bottom left corner and rising steeply towards the top left corner. The best cut off point of serum Mg2+ to differentiate between liver cirrhosis and controls was 1.35 mg/dl with 94% sensitivity, 86 % specificity, 87% PPV, 93% NPV and 90 % accuracy. The area under the curve (AUC) is 0.934 (p-value < 0.001), which is considered a high value and further

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supports the good diagnostic performance of decreased levels of magnesium in identifying patients with cirrhosis.

DISCUSSION

In our study, mean serum Mg2+ levels were markedly lower among cirrhotic patients as compared to the control group, with the most pronounced decrease noted in individuals classified under Child Pugh's class C Cirrhosis. There was statistically significant difference in Mg2+ levels within the 3 classes of study group ($p<0.001$). These findings were in accordance with the research conducted by Koivisto et al, 6 Rahelic et al,[9] Bemeur et al,[10] Saxena et al,[2] and Ahad et al,[11] which similarly documented diminished Mg2+ levels in patients with liver cirrhosis.

Bémeur et al observed diminished serum Mg2+ levels in individuals with liver cirrhosis and noted that Mg2+ therapy resulted in improved hepatic enzyme levels.[10] Similarly, Ahad et al attributed decreased serum Mg2+ levels to factors such as reduced dietary intake of the mineral, heightened Mg2+ excretion due to diminished plasma albumin levels, the use of magnesiuric diuretics, impaired Mg2+ absorption in the distal jejunum, and the indirect impact of alcohol on renal tubules.[11]

Several mechanisms linked to alcoholism can induce Mg2+ deficiency, including increased urinary Mg2+ excretion, malnutrition, gastrointestinal losses, and deficiencies in phosphate and vitamin D.[12] Acute alcohol consumption disrupts G protein signalling pathways within hepatocytes, resulting in heightened cyclic adenosine monophosphate (cAMP) formation. This, in turn, activates the protein kinase C (PKC) pathway, impeding PKC translocation to the membrane and subsequently augmenting renal Mg2+ excretion through the sodium/Mg2+ exchanger.[13,14]

In cirrhosis, diminished intracellular Mg2+ levels adversely affect mitochondrial bioenergetics, which heavily rely on appropriate mitochondrial Mg2+ concentrations. Impaired mitochondrial function compromises oxidative phosphorylation in hepatocytes, leading to 17% reduction in adenosine triphosphate (ATP) production and subsequent hepatocyte damage. Subsequent liver regeneration processes contribute to additional fibrosis and excessive extracellular matrix (collagen) deposition in the liver, exacerbating the cirrhotic condition.[15,16]

Several studies have explored the mechanisms by which Mg2+ deficiency can trigger inflammation and exacerbate the condition of cirrhotic patients. Firstly, Mg2+ being a natural calcium antagonist, modulates the signal transduction cascade.[17] Consequently, diminished extracellular Mg2+ concentrations may culminate in heightened intracellular calcium levels, thereby instigating an inflammatory cascade. Hence, oral Mg2+ supplementation and elevated intracellular Mg2+ levels may confer beneficial anti-inflammatory effects.[18,19] Secondly, Mg2+ deficiency triggers a systemic stress response by activating neuroendocrinological pathways, resulting in heightened production of neuro-mediators like substance P, which can provoke an inflammatory reaction. Thirdly, the activation of nuclear factor-κB (NF-κB) may be one of the major mechanism through which Mg2+ deficiency precipitates an inflammatory response.[20]

Research conducted by Altura et al,[21] revealed that reduced extracellular Mg2+ concentrations in cultured canine cerebral vascular smooth muscle cells can trigger lipid peroxidation and activate NF-κB. In instances of diminished Mg2+ content within liver cells, local leukocytes and macrophages exhibit heightened activity, releasing numerous inflammatory cytokines and recruiting additional inflammatory cells to the liver.[22] This inflammatory cascade inflicts damage upon liver cells, initiating a reparative process characterized by fibrosis, which exacerbates liver cirrhosis. Prior investigations have underscored the pivotal role of reactive oxygen species (ROS) in hepatic fibrogenesis.[13,23,24] ROS exhibit direct fibrogenic effects by promoting the proliferation and activation of hepatic stellate cells (HSCs). Upon stimulation, quiescent HSCs transition into activated myofibroblasts, becoming the principal pro-fibrotic cell type and overproducing type I collagen, thereby perpetuating additional inflammatory and fibrotic alterations within the liver.

Furthermore, upon scrutinizing Mg2+ levels across various classes of cirrhosis, a significant decline in Mg2+ levels was noted with increasing severity of cirrhosis. This observation aligns with findings from studies conducted by Saxena et al,[2] and Ali et al.[25] This phenomenon may be attributed to the progressive intestinal lining damage accompanying cirrhosis advancement, which restricts Mg2+ absorption from dietary sources. Additionally, as liver damage escalates, damaged cells may uptake more Mg2+ in a compensatory manner, further depleting serum Mg2+ levels.

CONCLUSION

In conclusion, the intricate interplay between Mg2+ levels and liver function underscores their mutual influence. Liver pathologies contribute to Mg2+ depletion, while low Mg2+ levels exacerbate the progression of liver diseases. Therefore, integrating Mg2+ supplementation into the comprehensive management of all cirrhosis patients is essential. By doing so, not only can liver function be preserved, but the trajectory of liver disease progression may also be attenuated, offering the prospect of improved clinical outcomes and overall patient well-being.

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