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Altered trace elements, antioxidant vitamin C, and malondialdehyde levels are associated with the pathophysiology and development of pre-hepatic jaundice: A case-control study

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Abstract

Objectives: Pre-hepatic jaundice results from an imbalance between bilirubin production and clearance, often linked to hemoglobinopathies. Antioxidant vitamin C, malondialdehyde, and trace elements play roles in jaundice, yet their specific associations remain unclear. The objective is to assess and compare these biomarkers in pre-hepatic jaundice patients and healthy controls, aiming to identify potential diagnostic markers and understand distinctive characteristics related to the disease's pathogenesis.

Methods: This case–control study enrolled 50 pre-hepatic jaundice patients and 50 healthy controls, utilizing advanced techniques for biomarker quantification. We completed blood sample collection from study participants between 1 September 2023 and 31 December 2023. This study investigates the correlation between various biomarkers and pre-hepatic jaundice using serum samples with a focus on antioxidant vitamin C, malondialdehyde, and trace elements.

Results: This study demonstrates elevated concentrations of malondialdehyde in patients with pre-hepatic jaundice, suggesting alterations in bilirubin metabolism and increased oxidative stress. We found that the serum levels of malondialdehyde were significantly higher in pre-hepatic jaundice patients compared to healthy controls. Our observations revealed a notable decrease in the average serum vitamin C levels in patients with pre-hepatic jaundice compared to healthy controls. The patients had lower serum Zn levels and higher serum Cu and Mn levels compared to the healthy controls. The correlation study demonstrates robust positive correlations among these biomarkers in pre-hepatic jaundice. As the levels of vitamin C rise, the levels of the other criteria often fall, and vice versa. There is an inverse relationship between higher levels of vitamin C and lower levels of malondialdehyde. The current investigation identifies possible changes in antioxidant vitamins, malondialdehyde levels, and trace elements, which provide significant insights for targeted interventions.

Conclusions: The present research highlights the integrated significance of vitamin C, malondialdehyde, and trace elements in the progression of the disease.

Keywords

Pre-hepatic jaundice, antioxidant vitamins, malondialdehyde, trace elements, urobilinogen, unconjugated bilirubin, pathogenesis

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Introduction

The hepatic bile's one of two major functions is the elimination of bilirubin along with excess cholesterol, xenobiotics, etc., that are not sufficiently water soluble to be removed from the body by urinary excretion. Hence, any process that hinders the regularity of biliary excretion results in higher bilirubin levels, a condition known as jaundice.[1] Jaundice can be classified into three different types, such as—pre-hepatic jaundice (PHJ), hepatic jaundice, and obstructive jaundice.[2] PHJ, among the three forms, is distinguished by an imbalance between bilirubin production and clearance, allowing serum unconjugated bilirubin (SUB) to overflow into body tissue prior to reaching the liver. This phenomenon occurs majorly due to excessive breakdown of erythrocytes, which are a result of various haemoglobinopathies.[3] As a result, jaundice is considered to be a symptom of an underlying disease rather than a disease itself. Having said that, one of the major causes of jaundice is haemoglobinopathy, followed by pathological conditions such as hepatitis, obstruction of bile flow, and so forth.[3] Globally, half of full-term babies and 80% of preterm babies suffer from jaundice. According to reports, the frequency of newborn jaundice is highest in the African area (667.8 per 10,000 live births), followed by Southeast Asia (251.3 per 10,000) which affects populations worldwide, especially in sub-Saharan and South Asian countries.[4] Besides, according to a report published by WHO in 2006, 5% of the population is at risk of developing any semblance of hemoglobinopathies, which also puts the population at the risk of developing PHJ. Moreover, PHJ is also prevalent throughout the world, particularly in the sub-Saharan and south Asian countries of the world.[5] In Bangladesh, pregnant women with jaundice caused 19%–25% of maternal deaths and 7%–13% of neonatal mortality.[6] To learn more about PHJ, it is important to grasp specific criteria and their relationship to PHJ. The symptoms of PHJ are: In addition to the typical jaundice, which is characterized by yellowing of the skin and eyes, other symptoms may include abdominal pain fever (with chills or cold sweats), abnormal weight loss, itchiness, dark urine, or pale stool.[7]

To begin with, bilirubin, a yellow pigment, forms as the end product of heme breakdown, mainly from old red blood cells reaching the end of their lifespan. This breakdown often occurs in the spleen and macrophages.[3] There are two types of bilirubin—conjugated and unconjugated. In diseased states, specially hemoglobinopathies, where red blood cell breakdown occurs frequently, only a limited amount of SUB is processed in the liver to be made conjugated, as the enzymatic reaction that causes the bilirubin conversion is saturable. As a result, diseased states where there is excess bilirubin production (hemoglobinopathies) occurs, or where the liver fails to convert the bilirubin into its water-soluble form cause bilirubin levels to rise.[8] The elevated bilirubin levels manifest as jaundice, either pre-hepatic or hepatic.

Urine urobilinogen (UU) is a colorless by-product of bilirubin breakdown which is done by bacteria in the intestines. Majority of the UU is reabsorbed in the blood to be sent to the liver, and the remaining is excreted through biliary excretion.[9] UU levels serve as a crucial biomarker for bile flow and liver function. There is considerable evidence to conclude that autooxidation is an important factor in hemolysis, which is the most prominent cause of PHJ. Hence, there is a possibility that antioxidant levels may decrease in PHJ.[10] While some studies have shown that in the state of hyperbilirubinemia, the anti-oxidant vitamin levels (vitamins C and E) do decrease, there is not enough research to find a concrete association between antioxidant vitamin levels and PHJ.[11] In other studies, it was concluded that malondialdehyde (MDA) levels, which is a by-product of lipid peroxidation, increased in patients with hyperbilirubinemia, although a strong positive correlation was not found.[12] Based on previous research, trace element level is also an important parameter that should be considered in the assessment of the functionality of the liver. Increased liver dysfunction has been shown to change trace element metabolism, favoring excess zinc (Zn), copper (Cu), manganese (Mn) insufficiency.[13] In this context, according to literature, trace elements in excess is considered to be one of the causative factors of jaundice.[14] Studies have examined neonatal vitamin D levels in connection with hyperbilirubinemia. Meta-analyses have revealed that neonates suffering from hyperbilirubinemia have decreased levels of vitamin D in comparison to healthy babies. These findings indicate that reduced levels of vitamin D may elevate the likelihood of developing hyperbilirubinemia. Importance: monitoring neonatal vitamin D levels is crucial for identifying individuals with an elevated risk of hyperbilirubinemia.[15]

This study aims to assess and compare the levels of antioxidant vitamins, trace elements Zn, Cu, Mn, and MDA in patients with PHJ and healthy controls (HCs). The purpose is to investigate the potential relationship between these parameters and PHJ in adults, with the goal of identifying specific biomarkers associated with the disease. The findings of this research have the potential to accelerate the process of diagnosing PHJ and reveal distinctive attributes associated with the development of PHJ. Moreover, this article is the first assessment of these parameters in the Bangladeshi population. We conducted this research with a specific focus on the characteristics that are crucial for maintaining good health, including antioxidants. These parameters could potentially be employed as a dietary supplement if any abnormalities are detected in their values.

Methods and materials

Study design and participants

We assumed population proportion, margin of error, and confidence level as 3%, 5%, and 95%, respectively. Based

on this calculation, the minimum number of cases should be 45. Therefore, we recruited 50 PHJ patients and 50 matched HCs were enrolled in this 1:1 case–control study. We completed participants enrollment for this study between 1 September 2023 and 31 December 2023. We involved a qualified hepatologist to evaluate study participants.

The patients were chosen from several medical facilities in the Noakhali district, including Janani General Hospital Ltd., Good Heal Hospital Private Ltd., and Motherland Hospital and Heart Care Center Ltd. in Chattogram, Bangladesh. Comprehensive physical examinations were performed to detect the existence of any additional complications. The study participants excluded any preexisting medical conditions such as hepatic disorders, organ failure, medication use, smoking, or substance addictions that could have influenced the concentrations of trace elements, antioxidant vitamins, and MDA. The controls were matched to the age, sex, and body mass index (BMI) of patients from various Noakhali city regions. Preexisting questionnaires were utilized in the process of gathering sociodemographic information. We have performed a pilot test among 10% of participants for clarity of questions by the respondents. Additionally, other biographical characteristics were assessed for both the cases and the controls.

Sample collection

Following an overnight fast, 5 mL of blood were drawn from each participant's cephalic vein. The samples were left to clot at room temperature for an hour. Serum samples were taken from collected blood samples and kept in microtubes at −80°C until analysis, following a 15 min centrifuge at $1000 \times g$.

Quantification of serum trace elements

In continuation of our previous work, we evaluated blood levels of trace elements using both flame atomic absorption spectrometry (FAAS) and graphite furnace atomic absorption spectrometry (GFAAS) following our earlier methods.[16] Serum samples were diluted 1:10 with deionized water. Calibration curves were constructed for Cu, Mn, and Zn using five mineral concentrations (0.5, 1.0, 2.0, 5.0, and 10.0 mg/L). Elemental concentrations were determined based on absorbance measurements at specific wavelengths: 327.4 nm for Cu, 279.8 nm for Mn, and 213.9 nm for Zn. Standard solutions were analyzed alongside each of the 10 test samples to ensure analytical accuracy and precision. Limits of detection (LoDs) were established by analyzing five blank solutions. LoDs were found as follows (μg/L): ^{66}Zn-0.05, ^{63}Cu-0.03, and ^{55}Mn-0.07.

Quantification of serum MDA and antioxidant vitamin

The quantification of MDA, which is generated through lipid peroxidation, was performed on plasma samples using the thiobarbituric acid reactive substances assay, with a slight modification to the method described by Islam et al.[17] Briefly, 900 μL of 0.9% saline was combined with 100 μL of serum in each sample. Subsequently, 30 μL of 50 mM butylated hydroxytoluene and 2 mL of newly developed thiobarbituric acid reagent were added. After 15 min of incubation at 60°C, the mixture was cooled on ice for 5 min, and then it was centrifuged for 10 min at 5000 rpm. Spectrophotometric measurements of the supernatant absorbance were made at 535 nm, using 1,1,3,3-tetraethoxy-propane as the reference standard. This technique made it easier to quantify MDA, which in turn made it possible to assess the degree of lipid peroxidation in the plasma samples under analysis. Serum vitamin C level was determined following the method described by Sarwar et al.[18] Quantification of ascorbic acid was performed using a UV-VIS spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan) with phenyl hydrazine as an indicator.

Statistical analysis

The statistical software utilized for the statistical analysis was SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). We performed normality tests for our data and observed that all data were normally distributed without any significant skewness; therefore, the parametric method of statistical tests. We applied independent sample *t*-test for group differences in serum parameters. We have each item of data that were shown as mean ± SEM, or mean ± standard error mean. We have drawn error bar graphs for visual presentation of serum parameters in patients and in controls. Using Pearson's correlation analysis, the relationship between the different research factors was ascertained. We considered all statistical analyses significant at *p*-value less than 0.05.

Ethical consideration

The Research Ethics Committee of the University of Asia Pacific has approved the study protocol (UAP/REC/2023/219). Informed written consent was taken from all the attendees prior to data collection. The investigations were carried out in conformity with the principles outlined in the Helsinki Declaration.

Results

Characteristics of the study participants

We presented the sociodemographic characteristics of all the study subjects in Table 1. Female PHJ patients constituted 40%, with 60% being male; in HCs, females were 44%, and males were 56%. In the PHJ patients' group, 40% of participants were between the ages of 25–34, and 60% participants were above or equal to the age of 35. In case of BMI, all participants of both groups had a BMI in the normal range. Regarding educational background, 72% of the PHJ patients

Table 1. Sociodemographic characteristics of the study population.

Characteristics	PHJ patients ($n = 50$), mean ± SEM	Healthy controls ($n = 50$), mean ± SEM	p-Value
Age (years)	35.60 ± 0.77	34.52 ± 0.38	0.211
25–34	20 (40%)	26 (52%)	
35 and above	30 (60%)	24 (48%)	
Sex			
Male	30 (60%)	28 (56%)	0.685
Female	20 (40%)	22 (44%)	
BMI (kg/m^2)	22.72 ± 0.22	22.58 ± 0.21	0.656
18.5–25 (normal)	50 (100%)	50 (100%)	
Above 25 (obese)	0 (0%)	0 (0%)	
Education level			
Illiterate	36 (72%)	32 (64%)	0.700
Secondary level	7 (14%)	9 (18%)	
Higher secondary level	4 (8%)	5 (10%)	
Graduate and above	3 (6%)	4 (8%)	
Occupation			
Service	1 (2%)	1 (2%)	0.999
Business	18 (36%)	15 (30%)	
Student	1 (2%)	1 (2%)	
Jobless	19 (38%)	22 (44%)	
Others	10 (20%)	11 (22%)	

BMI: body mass index; SEM: standard error mean.

were illiterate, 14% of patients completed the secondary level, 8% of patients completed the higher secondary level, and only 6% of the patients graduated. The ratios for the HCs educational qualification were statistically similar. In this study, 38% of patients and 44% of HCs were jobless. Compared to HCs (30%), participants with business as occupation were in a higher proportion of PHJ patients (36%), and for both the groups, only 2% of the participants were service holders.

Clinical profiles and laboratory findings

The clinical outcome and laboratory findings of the study subjects are presented in Table 2. We found higher serum levels of MDA in PHJ patients (5.73 ± 0.03 µg/mL) than in HCs (2.87 ± 0.06 µg/mL) ($p < 0.001$). We also observed significantly lower mean serum Vit-C concentrations in PHJ patients (9.21 ± 0.17 µg/mL) compared to HCs (17.13 ± 0.09 µg/mL). The serum concentrations of Zn were lower in the patients (0.46 ± 0.01 mg/L) compared to HCs (0.77 ± 0.00 mg/L). The serum concentrations of Cu were higher in the patients (0.08 ± 0.00 mg/L) compared to HCs (0.05 ± 0.00 mg/L). The serum concentrations of Mn were higher in the patients (0.91 ± 0.01 mg/L) compared to HCs (0.64 ± 0.00 mg/L). Error bar graphs are showing concentrations differences of serum parameters between the groups for better comparison (Supplemental Figure 1).

Table 2. Clinical information and laboratory findings of the study population.

Parameters	PHJ patients ($n = 50$), mean ± SEM	Healthy controls ($n = 50$), mean ± SEM	p-Value
UU (mg/dL)	5.28 ± 0.18	0.52 ± 0.03	<0.001
SUB (mg/dL)	18.6 ± 0.19	0.58 ± 0.03	<0.001
Vit C (ug/ml)	9.21 ± 0.17	17.13 ± 0.09	<0.001
MDA (ug/ml)	5.73 ± 0.03	2.87 ± 0.06	<0.001
Zn (mg/L)	0.46 ± 0.01	0.77 ± 0.00	<0.001
Cu (mg/L)	0.08 ± 0.00	0.05 ± 0.00	<0.001
Mn (mg/L)	0.91 ± 0.01	0.64 ± 0.00	<0.001

PHJ: pre-hepatic jaundice; Vit-C: vitamin C; MDA: malondialdehyde; Zn: zinc; Cu: copper; Mn: manganese; UU: urine urobilinogen; SUB: serum unconjugated bilirubin; SEM: standard error mean.

Table 3. Correlation analysis of various research parameters among study population.

Correlation parameters	PHJ patients		Controls	
	r	p	r	p
UU and SUB	0.886	<0.001	−0.119	0.410
MDA and SUB	0.892	<0.001	0.984	<0.001
Zn and SUB	−0.985	<0.001	−0.984	<0.001
Cu and SUB	0.991	<0.001	0.985	<0.001
Mn and SUB	0.974	<0.001	0.984	<0.001
SUB and Vit C	−0.948	<0.001	−0.985	<0.001
UU and Vit C	−0.914	<0.001	0.129	0.370
MDA and Vit C	−0.956	<0.001	−0.998	<0.001
Zn and Vit C	0.908	<0.001	0.998	<0.001
Cu and Vit C	−0.934	<0.001	−0.999	<0.001
Mn and Vit C	−0.915	<0.001	−0.999	<0.001
MDA and UU	0.873	<0.001	−0.140	0.330
Zn and UU	−0.840	<0.001	0.140	0.330
Cu and UU	0.869	<0.001	−0.124	0.390
Mn and UU	0.844	<0.001	−0.129	0.370
Zn and MDA	−0.843	<0.001	−0.999	<0.001
Cu and MDA	0.898	<0.001	0.998	<0.001
Mn and MDA	0.820	<0.001	0.999	<0.001
Cu and Zn	−0.975	<0.001	−0.999	<0.001
Mn and Zn	−0.966	<0.001	−0.998	<0.001
Mn and Cu	0.959	<0.001	0.999	<0.001

PHJ: pre-hepatic jaundice; Vit C: vitamin C; MDA: malondialdehyde; Zn: zinc; Cu: copper; Mn: manganese; UU: urine urobilinogen; SUB: serum unconjugated bilirubin.

Correlation analysis among the parameters

The results of a correlation analysis between various research parameters measured in two groups: PHJ patients and control subjects are presented in Table 3. Here the correlations among the different serum parameters among PHJ patients are briefly presented. Serum vitamin C levels were negatively correlated with serum SUB, UU, MDA, Cu, and Mn levels and positively correlated with serum Zn levels

($p < 0.001$). Serum SUB levels were positively correlated with serum UU, MDA, Cu, and Mn levels and negatively correlated with serum Zn levels ($p < 0.001$). However, serum UU levels were positively correlated with serum MDA, Cu, and Mn levels and negatively correlated with serum Zn levels ($p < 0.001$). Moreover, serum MDA levels were positively correlated with serum Cu and Mn levels and negatively correlated with serum Zn levels ($p < 0.001$). Also, serum Zn levels were negatively correlated with serum Cu and Mn levels and serum Mn and Cu levels were positively correlated with each other ($p < 0.001$).

Discussion

Jaundice is a hallmark of underlying disorders, especially hemoglobinopathies and indicates an imbalance in the removal of bilirubin and other chemicals by the liver's bile.[19] This study focused on investigating the relationship between numerous parameters, including antioxidant vitamin, MDA, and trace elements in PHJ. The research study uncovered significant findings in people with PHJ in comparison to individuals without the condition. PHJ patients had heightened levels of MDA, suggesting disturbances in bilirubin metabolism and exacerbated oxidative stress. Furthermore, alterations in the levels of antioxidant vitamin (vitamin C), Zn, Cu, and Mn demonstrate a possible disruption in the body's antioxidant defense mechanism and metabolism of trace elements. These findings support the fundamental premise that jaundice, specifically PHJ, occurs as a result of elevated bilirubin synthesis, generally associated with the excessive destruction of erythrocytes.[20] Patients with PHJ display abnormal levels of trace elements (Zn, Cu, and Mn), suggesting a disturbance in their antioxidant defense mechanisms. Increased concentrations of MDA indicate the presence of oxidative stress caused by disruptions in bilirubin metabolism.[21] To summarize, comprehending these associations can direct therapeutic administration and emphasize the significance of maintaining equilibrium in antioxidant levels.

The measurement of factors related to oxidative stress is a widely utilized method for assessing the level of cellular damage.[22] Prior research has shown that levels of MDA rise and antioxidant capabilities diminish in cases of both acute and chronic hepatitis.[23] The findings of our study demonstrate a substantial and statistically significant elevation in serum MDA levels among PHJ patients (5.73 ± 0.03 µg/mL) compared to HCs (2.87 ± 0.06 µg/mL) ($p < 0.001$), as presented in Table 2. These findings were consistent with the findings revealed by several other scientific publications.[24–29] Researchers have documented elevated levels or enhanced production of reactive oxygen species (ROS) in the plasma of individuals with liver damage and in animal models of liver disease. Inflammation has been identified as a key cause of liver injury following pathophysiological remarks, and it may be associated with the formation of ROS in the liver. Kupffer cells and neutrophils, once activated, ROS in the liver as a reaction to inflammatory cytokines. ROS excessively remove hydrogen atoms from lipoproteins, leading to lipid peroxidation. The major cause of this process is MDA.

Trace elements are vital micronutrients that are necessary for the growth, development, and maintenance of healthy tissues. The significance of trace elements in body metabolism is paramount. Deficiencies of these substances can lead to diseases, while an excessive amount of them can be hazardous to human health.[30] Zn is a vital constituent of numerous metalloenzymes that play a crucial role in almost all metabolic processes. A previous study demonstrated a substantial ($p < 0.001$) decrease in serum zinc levels in the case group (0.50 ± 0.03 mg/L) compared to the control group (0.68 ± 0.10 mg/L). Additionally, there was a significant link between hyperbilirubinemia and zinc levels, with a p-value of <0.001.[31] Another study revealed that the levels of zinc in jaundice patients (0.0012 ± 0.002) were considerably lower compared to healthy newborns (0.0021 ± 0.0036).[32] In our current research (Table 2), we have found that the levels of Zn in the serum were significantly lower in the PHJ patients (0.46 ± 0.01 mg/L) compared to the HCs (0.77 ± 0.00 mg/L; $p < 0.001$). Zn inhibits the disruption of the cell membranes' lipid composition, and a deficiency in Zn may affect the structure of red blood cell membranes. The inadequate production of certain enzymes involved in bilirubin metabolism may occur as a consequence. Additionally, hypozincemia can lead to abnormalities in the structure of red blood cell membranes, leading to hemolysis.[33]

Cu is a crucial constituent of numerous metalloenzymes, such as ceruloplasmin, cytochrome c oxidase, superoxide dismutase, dopamine β-hydroxylase, ascorbate oxidase, lysyl oxidase, and tyrosinase.[34] Cu stimulates the process of angiogenesis, which involves the formation of blood vessels, particularly those that provide nourishment to tumors. Exhausting copper levels can impede the growth and reappearance of tumors. Chelating copper has the potential to prolong the lifespan of individuals with advanced and challenging-to-treat malignancies, such as triple-negative breast cancer.[35] Liver diseases such as cirrhosis, obstructive jaundice, and cholestasis are associated with increased levels of Cu in the bloodstream. In newborns with jaundice, the elevated levels of Cu in the blood may originate from within the cells (specifically, red blood cells).[36] A prior investigation demonstrated a statistically significant elevation in the average serum Cu concentration among infants with hyperbilirubinemia.[37] In our investigation (Table 2), we found that the levels of Cu in the serum were greater in the patients (0.08 ± 0.00 mg/L) compared to the HCs (0.05 ± 0.00 mg/L; $p < 0.001$). Similarly, to this study, Schulpis et al.[34] found that the levels of copper in the blood serum doubled in infants with moderate hemolytic jaundice and nearly tripled in neonates with severe hemolytic jaundice. Also, another research found that the levels of copper were substantially higher (0.198 ± 0.341) in patients with jaundice compared to the control group (0.238 ± 0.411).[32]

Mn obtained from food is efficiently absorbed by the liver and then transported to various tissues, where it contributes to

the transfer of albumin.[30] Elevated serum levels of Mn are observed in newborns with mild hemolysis. The high levels of Mn are primarily found in erythrocytes, particularly reticulocytes, rather than in serum concentrations. It is therefore possible that Mn enters the serum as a result of hemolysis.[38] Previous studies identified a direct association between the levels of manganese in the blood and the levels of bilirubin in children with jaundice who were receiving prolonged total parenteral nutrition. Interestingly, rats who are poisoned with manganese experience the development of cholestasis. Increased concentrations of manganese have also been documented in cases of hepatitis and postnecrotic cirrhosis.[39] Another study found that levels of manganese were substantially higher (0.0277 ± 0.0391) in individuals with jaundice compared to the control group (0.015 ± 0.031).[32] In our study (Table 2), we also found that the levels of Mn in the blood serum were greater in patients with PHJ (0.91 ± 0.01 mg/L) compared to healthy individuals (0.64 ± 0.00 mg/L). Nevertheless, it is advisable to refrain from consuming excessive quantities of manganese, as it has the potential to be toxic. It is necessary to customize medical management to meet the unique needs of each patient during administration.[40]

Oxidants are substances that contain molecules with unpaired electrons, known as free radicals. These free radicals, such as reactive oxygen, nitric, carbonic, or sulfuric species, are produced as a result of metabolic redox reactions.[41] These reactions occur in response to conditions of low oxygen (hypoxia) or high oxygen (hyperoxia), ischemia-reperfusion, inflammation, activation of the immune response, mitochondrial dysfunction, and the Fenton reaction.[42–44] Antioxidants, including superoxide dismutase, catalase, glutathione peroxidase, vitamins C and E, minerals, and glutathione (GSH), have the ability to counteract or eliminate free radicals, thereby providing protection to tissues from oxidative stress-induced damage.[45]

Vitamin C is a powerful antioxidant that plays a crucial function in safeguarding cells from oxidative stress and hemolysis.[11] Insufficient data exist to establish a definitive correlation between levels of antioxidant vitamins and PHJ. Prior research[11] has demonstrated a correlation between a decreased level of antioxidants and an increased risk of hyperbilirubinemia in newborns. Hemolysis of red blood cells is a significant factor in the production of high levels of bilirubin in newborns. Neonatal erythrocytes are vulnerable to peroxide-induced hemolysis because they are constantly exposed to high levels of oxygen and have plasma membranes that are rich in polyunsaturated fats. Elevated amounts of lipid peroxidation markers were seen in both plasma and erythrocytes upon birth, particularly in preterm newborns. This observation was a result of deficient levels of antioxidant vitamins and/or insufficient activity of antioxidant enzymes.[11] They revealed clear evidence of the correlation between deficient levels of vitamin E and C and neonatal hyperbilirubinemia. On the other hand, Elfarargy et al.[46] discovered a substantial decrease in the levels of Vitamin C, D, and E in the serum of both jaundiced neonates and healthy neonates without jaundice on the third day of life ($p < 0.001$).[46] Our analysis (Table 2) found that patients with PHJ had considerably decreased average serum vitamin C levels compared to HCs ($p < 0.001$). Thus, a deficiency in vitamin C may be responsible for the occurrence of hyperbilirubinemia in individuals. Additionally, the optimization of vitamin C consumption may be beneficial in the treatment of neonatal jaundice. It is crucial to underscore that these antioxidant vitamins are essential for overall health, and it is crucial to maintain the appropriate dosages.[47]

This study expands upon the traditional scope by conducting a systematic investigation of various parameters, including antioxidant vitamins, MDA, and trace elements, simultaneously. This approach allows for a comprehensive knowledge of their respective roles in the setting of PHJ. This research goes beyond the previous literature by examining the connections between hemoglobinopathies, jaundice, antioxidant vitamins, MDA, and trace elements. The study's findings support the idea that PHJ is not an independent disease but rather a combination of many factors that impact bilirubin metabolism, oxidative stress, and antioxidant defense mechanisms. Adding trace elements to the analysis introduces a new aspect, as these components have been found to affect liver function and could enhance our comprehension of PHJ.

This study has particular significance because it investigates the correlations among antioxidant vitamins, MDA, and trace elements, specifically in cases with adult PHJ. This study offers a more comprehensive understanding of the biochemical changes related to PHJ by simultaneously examining numerous biological markers. By identifying strong positive and negative connections, this research gains a more detailed insight and sets itself apart from earlier studies that just examined individual indicators in isolation.

Clinical implications

Understanding the correlations identified in this study has significant clinical implications. Elevated levels of MDA, along with altered antioxidant vitamin and trace element levels, could serve as risk assessment markers and helps to initiate quick interventions for PHJ. These findings offer clinicians valuable insights for early risk assessment and targeted interventions. This suggests that the levels of antioxidant vitamins, MDA, and trace elements may fluctuate together in PHJ. Conversely, a strong negative correlation was observed between antioxidant vitamin and the aforementioned parameters, suggesting an inverse relationship. As vitamin C levels increased, the levels of MDA and trace elements tended to decrease.

Strengths and limitations

The strength of this study lies firstly in the fact that this study took into account various parameters that were previously unaccounted for to be associated with adult PHJ patients.

There was also the utilization of advanced techniques to reduce the error margin, and given the global prevalence of jaundice, especially in regions like sub-Saharan Africa and South Asia,[48] the study's findings may have broad relevance. This study, however, could have multiple limitations. First off, the results can be impacted by the patient demographics' lack of variety. Larger cohorts might increase the study's validity, but the study's small sample size may have an influence on how broadly the results can be applied. It was not taken into account how the patients' prescriptions were affecting them.

Conclusion

Using cutting-edge approaches and evaluating features that were previously unknown constitute the importance of this investigation. The evaluation of these unknown biomarkers in individuals with prehepatic jaundice from Bangladesh is noteworthy since it is the first of its kind. This research reveals significant correlations between several biomarkers and PHJ, enabling medical professionals with possible relevant risk assessment and information for focused therapies. Expanding the study to a larger, more diverse population and studying the impact of drugs on biomarkers are potential avenues for future research that can deepen our understanding and lay the groundwork for more targeted diagnostic methods.

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Author contributions

SMRD, AD, and ZLA: conceptualization, methodology, data curation, investigation, formal analysis, and writing – original draft. SAK, RN, and MSS: data curation, and project administration. MSI and MRI: conceptualization, methodology, supervision, and writing – review and editing.

Availability of data and materials

The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics approval

The Research Ethics Committee of the University of Asia Pacific has approved the study protocol (UAP/REC/2023/219). Informed written consent was taken from all the attendees prior to data collection. The investigations were carried out in conformity with the principles outlined in the Helsinki Declaration.

Informed consent

We obtained written consent from each participant after a well brief to them about the purpose of the study. The primary caregiver gave the sign on behalf of patients if independent thinking capacity was suspected or from those with no formal education.

Trial registration

Not applicable.

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Supplemental material

Supplemental material for this article is available online.

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