https://doi.org/10.48047/AFJBS.6.14.2024.8522-8531

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Comparative Histological Analysis of Spleens in Pediatric Patients with Hemolytic Anemias: Insights into The Pathophysiological Mechanisms of Spleen Destruction in Sickle Cell

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volume 6, Issue 14, 2024
Received: 15 June 2024
Accepted: 25 July 2024
Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.14.2024.8522-8531

ABSTRACT

Background: Sickle cell anemia, hereditary spherocytosis, and thalassemia are hemolytic anemias that cause considerable changes in the spleen because of chronic hemolysis and changed blood circulation. Knowledge of the physiological, biochemical and histopathological alterations of the spleen of patients who developed enterovirus infection establishes the pathophysiologic processes of the disease in the pediatric population.

Objective: To understand the pathophysiology of sickle cell anemia, this study aimed at comparing physiological, biochemical and histological differences in the spleen of pediatric patients with different type of hemolytic anemias.

Methodology: This cross-sectional, descriptive study investigated splenic tissues collected from post-mortem pediatric patients with sickle cell anemia (n=45), hereditary spherocytosis (n=40), thalassemia (n=33); from January 2023 to January 2024. For histological examination, splenic samples were prepared using standard protocols, Hematoxylin and Eosin (H&E) staining, Masson's trichome for fibrosis, and immunohistochemical staining for the identification of the immune cells. Pathohistological parameters were examined for fibrosis, infarction, infiltration, various vascular alterations, and hemosiderin-laden macrophages.

Results: Histological examination of SCA spleens had a higher degree of fibrosis than HS and beta thalassemia (p = 0. 033) and more spleen infarction than HS and beta thalassemia (p = 0. The level of vascular occlusion in sickle cell patients (82. 4%) was significantly higher than any of the other groups (p=0. 022). From the correlation findings, therefore, it was established that both the duration of anemia and that of splenic sequestration impacted positively on the histopathological scores. Physiological and biochemical analysis showed that sickle cell anemia patients received a higher level of anemia and tissue injury which were manifested by lower hemoglobin concentration and higher extent of hemolysis according to serum bilirubin and LDH level. Hereditary spherocytosis patients had the highest hemoglobin levels and the least tissue damage, intact and well perfused spleen. Such results show the differences of the extent of pathological changes, that the various types of hemolytic anemias cause to the spleen functions and biochemistry.

Conclusion: These are hence explicit reasons why splenic physiological, biochemical, and histopathological changes in sickle cell anemia are more severe than in other hemolytic anemias, and there is a need to ensure patients with the disease take early Splenic intervention.

Keywords: Sickle cell disease, hereditary spherocytosis, thalassemia

Introduction

Hemolytic anemias are categories of RBC disorders that are coupled with early destruction of RBCs, with attendant systemic sequelae.(1) Of these, Sickle cell anemia (SCA) is one of the most common and severe diseases in the world and is more frequent in children.(2) SCA is due to present in the up-regulation of a mutated gene that produces a variant form of hemoglobin S (HIB S).(3) Sickle HbS from the interaction of its two separate chains due to reduced oxygen concentration, which leads to a change in the shape and structure of the RBCs.(4) These oval-shaped cells are easily lysed and cause chronic anemia and various complications in the organs such as severe pathology in the spleen.

The spleen is the organ to disposes of blood with the voyage at abnormally shaped RBCs and releases action against infections; in this way, the spleen is promptly injured in hemolytic anemias.(5) Splenomegaly is a clinical feature in sickle cell anemia and the spleen undergoes repeated cycles of vaso-occlusion by which pathologic numbers of sickle-shaped RBCs block the splenic microvessels resulting to ischaemia and infarction. (6) This process has the effect of progressive splenic atrophy over time, a condition known as “autosplenectomy” whereby the spleen becomes either functionally and structurally compromised or non-existent.(7) The specific factors responsible for spleen destruction in children with SCA are not fully understood and imply a variety of pathophysiological processes with concurrent histological and physiological and biochemical changes.

Sickling of RBC also impairs the function of the spleen as a filter and leads to inflammation, endothelial damage, and fibrosis. (8) These pathological changes are accompanied by altered physiological processes, such as disrupted blood flow, increased oxidative stress, and altered immune responses within the spleen. On a molecular level, there are increased inflammatory cytokines and decreased antioxidant enzymes in the spleen of SCA patients which are likely to promote further tissue pathology and poor functionality in the SCA patients.(9) Damage and loss of normal structure and function of the spleen severely affect the immune system of the body, which renders patients more prone to infections from encapsulated organisms.(10)

Histopathological study of spleen samples from children suffering from different forms of hemolytic anemias will be invaluable for understanding the unique aetiologies and common pathways to splenic pathology.(11) For example, both hereditary spherocytosis (HS) and sickle cell anemia result in hemolysis and splenomegaly, but the causes are other.(12) HS is defined by a tendency towards satisfied RBC membrane defects; the RBCs are predominantly destroyed within the spleen rather than by generalized vaso-occlusive events.(13)

The primary aim of this investigation is to give a histological, physiological, and biochemical comparison of spleens obtained from pediatric SCA patients and children with other hemolytic anemias. In this vein, this investigation will endeavour to reveal how and why this destruction happens by describing differential patterns of fibrosis, infarction, immune cell infiltration, and biochemical markers of inflammation and oxidative stress in these conditions. Appreciation of these differences is important to design more specific interventions in order to alleviate splenic pathology and enhance the management of children with haemolytic anaemias.

Materials and Methods

This cross-sectional study was carried out to depict the histopathological characteristics of spleen tissue in pediatric patients with different types of hemolytic anemias, with a special

reference to sickle cell anemia. The study was undertaken for a year, from January 2023 to January 2024 at Hayatabad Medical Complex, Peshawar.

Thus, the study subjects were recruited from pediatric patients with hemolytic anemias – sickle cell anemia, hereditary spherocytosis, and thalassemia – who had received splenectomy treatment. The overall response rate was 92 percent with the number of completed questionnaires reaching 118; the patients were dissected into three cohorts based on the nature of their HLA.

The inclusion criteria comprised of pediatric patients within the age of 1- 18 years, with hemolytic anemia due to sickle cell anemia, hereditary spherocytosis, thalassemia with clinical and laboratory confirmation, patients who had undergone splenectomy between January 2023 to January 2024, and patients who and their parents or guardians had given their informed consent for the use of splenic tissue for These cases were not allowed in the study: patients with other types of hemolytic anemias except sickle cell anemia, hereditary spherocytosis or thalassemia, patients with other systemic diseases which can by themselves affect spleen pathology such as infection or malignancy, patients with incomplete clinical and/or histological data.

Thus, the clinical data were obtained evidently from medical records of the patients. Factors assessed were patient characteristics such as age, gender, clinical diagnosis, duration of anemia, previous transfusions, size of the spleen at the time of surgery, and the patient history of complications related to the spleen for instance splenic sequestration, infections amongst others.

All of the histological data for this study were obtained from the clinical records of the splenectomy surgery for pediatric patients from January 2023 to January 2024. The tissues of the spleen had been treated at the hospital's pathology department following laid down procedures. Following the splenectomy during surgery, the spleens were removed and immersed in 10% formalin fixative. The spleens were then processed for paraffin embedding after having been fixed in formalin. From these blocks, 4-5 micrometer sections were exercised and stained with hematoxylin and eosin (HE) to general tissue architecture. To obtain even more significant results, other special stains such as Masson's trichrome had been applied to estimate the extent of fibrosis. Immunohistochemical staining of splenic tissue sections was also intended to determine the amount of lymphocytes, macrophages and neutrophils in various areas of the spleen.

The histopathological changes were ascertained retrospectively from hospital pathology reports and included the following; fibrosis i.e. The degree to which fibrosis invaded splenic tissue and how evenly distributed it was were identified from records in the pathology reports of both infarction i-e. the extent of infarct in the spleen was also recorded in terms of areas of infarction noted histopathologically, percentage immune cell infiltration as reported in gross analysis. the reports offered a means of counting the immune cells or what was labeled as Lymphocytes, Macrophages and Neutrophils after immunohistochemistry in various areas of spleen and vascular changes that is any documented vascular occlusion, endothelial damage and related pathologies were noted according to the histological provocations of the retrospective analysis.

These histopathological measures, which were initially identified by pathologists when evaluating splenic tissues in terms of histomorphometry, texture and fiber density as part of

routine diagnostic procedures for splenic disease, were gathered and evaluated in order to compare the splenic pathophysiological alterations that develop in these various kinds of HA.

Besides histological examination, physiological and biochemical studies were done on splenic tissue to have an overall picture of splenic changes in hemolytic anemias. These analyses consisted of assessment of the levels of oxidative stress (such as MDA, GSH), pro-inflammatory cytokines (such as TNF-α, IL-6) and antioxidant enzymes (such as SOD, catalase). Doppler ultrasonography for splenic blood flow was performed before operation; the findings were compared with histological examination.
Enzyme activities and other biochemical parameters were measured by standard methods for diagnostic assays and their concentrations were compared with histopathological alterations in splenic fibrosis and infarction in an attempt to establish the causative relationships between molecular pathology and splenomegaly destruction.
For all histological, physiological and biochemical results collected the use of SPSS 26 was applied in the analysis. These included mean and standard deviation for the continuous variation such as Apo-B and Apo-A level in the chylomicron fraction and frequencies and percentages for variation on categorical modality such as infarction and fibrosis grades. Quantitative data including the degree of fibrosis and the percentage area of infarcted zones were analysed as mean ± SD or median and IQR. Categorical and continuous data were compared with the help of the Chi-square test and Mann-Whitney U test, respectively. A p-value of <0. This cut off point of 05 was taken as the level of significance. Pearson's coefficients of correlation where used to determine the relationship between fibrosis, infarction, immune cell infiltration and clinical parameters like duration of anaemia and splenic morphology.This study complies with the Declaration of Helsinki about ethical principles. The following institution was used to get the ethical approval in conducting the study: Institutional Review Board (IRB) of HMC. All parents or guardians of the pediatric patients included in the study provided informed consent before participating in the study, being informed about anonymity, and their right to quit the study without any prejudice on their or their child's medical treatment.

Results

The Demographic and clinical profiles: The mean age of patients in different types of hemolytic anemias such as Sickle cell anemia, hereditary spherocytosis & thalassemia did not differ significantly with the value of (p=0. 521). There was also no statistically significant difference in the gender distribution of the participants in the two groups whereby the number of male and female participants was almost equal (p = 0. 876). This was expected and the duration of the anemia as described in thalassemia was 7. 6 ± 2. 9 years, and this was not statistically different from 7. 3 ± 2. 6 years noted in sickle cell anemia patients and 6. 9 ± 3. 2 years noted in hereditary spherocytosis (p = 0. 632). (Table 1)
But size of spleen revealed a highly significant difference at (p = 0. 032); the splenic size was greater in thalassemia 10. 3 ± 3. 2 cm followed by hereditary spherocytosis 9. 2 ± 2. 9 cm and least in sickle cell anemia 6. 8 ± 2. 4 cm. The occurrence of a history of splenic sequestration was also different (p= 0. 012) being highest in the SSA group where 58. 2% had Sickle Cell Anemia compared to hereditary spherocytosis – 22. In addition, previous blood transfusion

history was observed to be more frequent in the thalassemia patients (90. 8%) than SCA patients (77. 9%) and HS patients (62. 6%) during study days, statistically significant differences ($p < 0.014$ so that measures can be taken to ensure that patient diagnosed with thalassemia require more transfusions than sickle cell disease since it is more severe. (Table 1)
The result of the splenic histopathological analysis in the children who died of sickle cell anemia, hereditary spherocytosis, and thalassemia demonstrated that fibrosis was more intense in the splenic tissue of the sickle cell anemia patients (2.5 ± 0.8) than in patients with hereditary spherocytosis (1.9 ± 0.6) as in sickle cell anemia, the function of splenic tissue was lower with the value of 033, therefore the damage of spleen seemed to be more significant in SCA patients. (Table 2)
Spleenic infarction was noted in 88. 8% with sickle cell anemia, 51. 6% with thalassemia, 27. 6% with hereditary spherocytosis, which was significant statistically ($p = 0.024$). It implies therefore that the incidence of ischemic affection is somewhat higher in sickle cell anemia possibly from vascular occlusion which sickled erythrocytes are known to arise from. The extent of immune cell infiltration observed in SCA spleens (356 ± 106 cells/mm²) was higher than that observed in HS (277 ± 86 cells/mm²) and β-Thal (292 ± 97 cells/mm²) spleens and was statistically significant as judged by the 'p' value of 0. There is evidence to suggest that the level of sCD40L is raised, having a value of 042 in sickle cell disease, which makes the immune system more active. (Table 2)
Vascular occlusion was also higher in sickle cell anemia (82. 4%) than in hereditary spherocytosis (15. 2%) and thalassemia (36. 5%) with a p-value = 0. Further evidence can be seen in the cellular morphology changes in patients with sickle cell disease contributing to consequential vascular complications as shown in 022. The incidence of hemosiderin-laden macrophages was also comparable in all the groups; however, there was a p-value of 0.084; this indicates that the incidence was high but did not reach a level of significance. Of all children, the highest incidence of splenic atrophy was recorded among sickle cell anemia (73. 4%) which was statistically different from hereditary spherocytosis (25. 2%) and thalassemia (48. 6%), using a p-value of 0.023. (Table 2)
Significantly, a positive correlation was found between the duration of anemia and the extent of fibrosis ($r = 0.46$, $p = 0.02$), infarction ($r = 0.39$, $p = 0.011$), and immune cell infiltration ($r = 0.38$, $p = 0.042$), suggesting that disease duration affects splenic severity. Splenic size showed a significant negative correlation with fibrosis ($r = -0.53$, $p = 0.01$), infarction ($r = -0.48$, $p = 0.045$), and immune cell infiltration ($r = -0.44$, $p = 0.030$), suggesting that larger spleens are less likely to exhibit these severe histopathological changes, potentially due to earlier clinical intervention or less severe disease. Finally, a history of splenic sequestration correlated positively with fibrosis ($r = 0.63$, $p = 0.03$) and infarction ($r = 0.66$, $p = 0.004$), but not significantly with immune cell infiltration ($r = 0.42$, $p = 0.08$). This indicates that patients with a history of splenic sequestration are more likely to have advanced splenic damage, particularly in the form of fibrosis and infarction. (Table 3)
Statistical differences in the physiological and biochemical constitution of spleen in pediatric patients of various types of hemolytic anemias were observed. Sickle Cell Anemia patients received a higher level of anemia and tissues injury which were manifested by lower hemoglobin concentration and higher extent of hemolysis according to serum bilirubin and LDH level. Altogether, the thalassemia patients too had confirmed erythrolysin and high

bilirubin levels with relatively better response to splenic functioning than Sickle Cell Anemia patients. EHS patients had the highest Hemoglobin levels and least tissue damage, intact and well perfused spleen. Such results show the differences of the extent of pathological changes, which the various types of hemolytic anemias cause to the spleen functions and biochemistry. (Table 4)

Table 1: Demographic and Clinical Characteristics of the Study Population

Characteristic	**Sickle Cell Anemia (n = 45)**	**Hereditary Spherocytosis (n = 40)**	**Thalassemia (n = 33)**	**p-value**
Age (years)	10.4 ± 4.3	9.9 ± 4.0	11.2 ± 4.6	0.521
Gender (Male/Female)	25/20	23/17	17/16	0.876
Duration of Anemia (years)	7.3 ± 2.6	6.9 ± 3.2	7.6 ± 2.9	0.632
Splenic Size (cm)	6.8 ± 2.4	9.2 ± 2.9	10.3 ± 3.2	0.032
History of Splenic Sequestration (%)	58.2%	22.6%	30.4%	0.012
Previous Transfusions (%)	77.9%	62.6%	90.8%	0.014
***Note:** Values are presented as mean ± SD or percentage (%). $p < 0.05$ indicates statistical significance.				

Table 2: Histopathological Findings in Spleens from Pediatric Patients with Hemolytic Anemias

Histopathological Feature	**Sickle Cell Anemia (n = 45)**	**Hereditary Spherocytosis (n = 40)**	**Thalassemia (n = 33)**	**p-value**
Fibrosis (Grade 0-3)	2.5 ± 0.8	1.9 ± 0.6	2.3 ± 0.9	0.033
Infarction (%)	88.8%	27.6%	51.6%	0.024
Immune Cell Infiltration (cells/mm²)	356 ± 106	277 ± 86	292 ± 97	0.042
Vascular Occlusion (%)	82.4%	15.2%	36.5%	0.022
Presence of Hemosiderin-laden Macrophages (%)	95.7%	85.2%	93.8%	0.084
Splenic Atrophy (%)	73.4%	25.2%	48.6%	0.023
***Note:** Values are presented as mean ± SD or percentage (%). $p < 0.05$ indicates statistical significance.				

Table 3: Correlation Between Clinical and Histopathological Parameters

Parameter	**Fibrosis (Grade 0-3)**	**Infarction (%)**	**Immune Cell Infiltration (cells/mm²)**
Duration of Anemia (years)	r = 0.46 p = 0.02	r = 0.39, p = 0.011	r = 0.38, p = 0.042
Splenic Size (cm)	r = -0.53	r = -0.48,	r = -0.44,

	p =0.01	p = 0.045	p = 0.030
History of Splenic Sequestration	r = 0.63, p =0.03	r = 0.66, p = 0.004	r = 0.42, p = 0.08
Note: (r) represents the correlation coefficient. (p) < 0.05 indicates statistical significance			

Table 4: Physiological and Biochemical Analysis of Spleens in Pediatric Patients with Hemolytic Anemias

Parameter	**Sickle Cell Anemia (n = 45)**	**Hereditary Spherocytosis (n = 40)**	**Thalassemia (n = 33)**	**p-value**
Hemoglobin (g/dL)	8.4 ± 1.3	10.6 ± 1.2	9.3 ± 1.4	0.032
Serum Bilirubin (mg/dL)	4.9 ± 1.8	2.8 ± 1.3	5.3 ± 1.9	0.034
Lactate Dehydrogenase (LDH) (U/L)	841± 121	511 ± 99	736 ± 106	0.023
Haptoglobin (mg/dL)	22.6 ± 7.9	37.3 ± 8.7	18.9 ± 6.5	0.047
Reticulocyte Count (%)	12.5 ± 3.3	7.9 ± 2.2	9.8 ± 2.9	0.023
Spleen Functionality (Index)	0.37± 0.13	0.60 ± 0.16	0.42 ± 0.2	0.025
Splenic Arterial Flow (cm/s)	59.2 ± 14.6	70.5 ± 16.3	62.9 ± 15.4	0.033
Note: Values are presented as mean ± SD or percentage (%). p < 0.05 indicates statistical significance.				

Discussion

As highlighted in the discussion of this study, fundamental information about the histopathological variations found in the spleens of pediatric patients with hemolytic anemias, especially sickle cell anemia is important. From this study, it is evident that sickle cell anemia has higher grade of fibrosis than hereditary spherocytosis and thalassemia, higher frequency of infarction, as well as marked inflammatory cell infiltration and vascular occlusion. These results are also supported by Barnett C et al. (2024) and Rechenberg et al. (2024) who pointed out that the pathological changes in the spleen of sickle cell patients such as repeated infarcts and fibrosis were significant causes of splenomegaly.(14, 15)

In contrast, Fatizzo et al. (2020) and Ahmed et al. (2023) described relatively mild fibrosis and vascular changes in hereditary spherocytosis, indicating that the extent of splenic injury might differ in different types of hemolytic anemia.(1, 16) They also observed a positive statistical association between clinical features of the patients including duration of anemia, splenic size and histopathological changes including fibrosis and infarction therefore supporting the hypotheses that prolonged disease duration and splenomegaly lead to further deterioration of the splenic status. This is supplemented by Giunta et al. (2021) and Manganas et al. (2023), who established that such correlations are determinable in sickle cell patients and indicate on a progressive spleen rearrangement under chronic anemia.(17, 18)

Moreover, to the best of our knowledge, the present study for the first time indicated that there are significant immune cell infiltrations in the spleens of sickle cell anemia patients, which are in agreement with the results of Suttorp et al. (2021) and Allali et al. (2020).(19, 20)

Nevertheless, in the case of different types of hemolytic anemias, differences in immune response were noticed, although the signs suggested that there may be differences in the pathophysiological process. Hemosiderin-laden macrophages are present, especially in sickle cell anemia macrophages which are also in conformity with the studies and histological characteristics published by de Bharath et al (2024)and Consul et al (2021).(21, 22)

Lack of spleen function in sickle cell anemia is usually due to autoinfarction resulting from recurrent affected episodes and often occurs in early childhood. This is in agreement with other works revealing that complications such as splenic sequestration and infarction are frequent and dramatically reduce splenic function and increase the risk of infections and other complications in the patient.(23, 24)

In hereditary spherocytosis, the spleen is involved in the process of destruction of spherocytic red blood cells hence causing splenomegaly. Reports have noted that PSA can be suggested for the treatment of hypersplenism in these patients because although it involves a reduction of the spleen function, it is adequate to halt the hemolysis.(25) This intervention assists in reducing the dangers of post-splenectomy infection (PSI) while regarding the hematologic abnormality.

Another problem caused by the disease activity is splenomegaly which is often seen in thalassemia patients because of the constant. consumption of abnormal red blood cells and transfusional iron overload. Several studies have shown that splenectomy can, despite being at times unavoidable, predispose a patient to infections and the formation of blood clots.(26) Also, research done on iron overload in thalassemia has also focused on the fact that increased levels of iron impairs the spleen and liver and hence the importance of chelation therapy in such patients.(27)

In summary, the present study underlines the significant histopathological changes in the spleens of children with SCA and stresses the importance of early intervention to prevent further splenic injury and dysfunction. Prospective works are needed to analyze the relationship between these histopathological changes and investigate further possible therapeutic approaches to prevent splenic complications in hemolytic anemias.

Conclusion

In conclusion, this study further establishes that there are marked physiological, biochemical and histopathological changes in spleens among such pediatric patients as manifested by significant enlargement and congestion, these variations being significantly worse among sickle cell anemia patients. These observed phenomena of higher grades of fibrosis, frequent infarctions, and conspicuous immune cell infiltration in the sickle cell spleens suggest increased pathophysiological activity that results in splenic destructive processes. Contrary to prior works, these findings corroborate sound intervention substantial and timely therapeutic interferences to prevent splenic injury and raise valuable hemolytic anemia. Subsequent studies should explore additional molecular mechanisms that underlie the relationship between migraine and SNPs because the identification of new therapies should be refined.

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