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# Assessment of the Histochemical Occurrence of Ferric Iron and Calcium Ion in Liver Biopsies and their Correlation with Liver Disease: A Retrospective Study

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**Abstract:- Liver disease is a major global health concern, with iron overload and calcium dysregulation implicated in the pathogenesis of several liver diseases. This retrospective case-control study aimed to determine the presence or absence of ferric iron and calcium ion histologically in formalin-fixed paraffin-embedded liver biopsies diagnosed with various liver diseases. The study found that the staining techniques employed in this study were reliable and effective for detecting ferric iron and calcium ion in FFPE liver biopsies, but no significant presence of these ions was detected in any of the samples. Therefore, the study did not find evidence to support the hypothesis that patients with liver disease should take iron and/or calcium supplements. These findings highlight the need for further research to fully understand the role of iron and calcium in liver disease.**

***Keywords:-*** liver disease, histological examination, liver biopsies, Prussian blue stain, retrospective case-control study.

***Running title:*** *Occurrence of Ferric Iron and Calcium Ion in Liver Biopsies and Correlation with Liver Disease*.

## I. INTRODUCTION

Liver disease is a growing global health concern, with an estimated 844 million people affected worldwide [1]. While there are various causes of liver disease, iron overload and calcium dysregulation have been implicated in the pathogenesis of several liver diseases, including non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), and hepatitis C virus (HCV) infection [2]; [3]. However, the role of iron and calcium in liver disease is still not fully understood.

Histological examination of liver biopsies is a commonly used diagnostic tool for liver disease. Formalin-fixed paraffin-embedded (FFPE) liver biopsies have the advantage of being readily available in clinical settings and can provide valuable information on the presence or absence of iron and calcium in liver tissue. However, the reliability of histological staining methods for detecting these ions in formalin-fixed paraffin-embedded (FFPE) liver biopsies diagnosed with various diseases has been debated [4].

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Many studies have shown relationship between various liver diseases and deposition of calcium and Ferric iron where in some studies, hypothesis are raised that patients with liver disease should take iron and/or calcium supplements, the study aimed to determine the presence or absence of ferric iron and calcium ion histologically in FFPE liver biopsies diagnosed with various liver diseases. Additionally, we sought to correlate the presence of these ions with different liver diseases, correlate the hypothesis of calcium and iron intake in liver disease, and assess the reliability of histological staining methods for detecting ferric iron and calcium ion in FFPE liver biopsies.

## II. MATERIALS AND METHOD

### *A. Study Area*

### *B. Study Design*

The study design is a retrospective case-control study. The cases are liver biopsies diagnosed with various diseases obtained from the histopathology archive of University of Maiduguri Teaching Hospital during the period of January to December 2019. The negative control is normal liver tissue obtained from postmortem. The study will collect data from the histopathology archive of the hospital, which means that the data collection is not prospective.

### *C. Sampling Technique*

Selection and preparation of liver biopsy samples: Formalin-fixed paraffin-embedded liver biopsy samples were obtained from the pathology department of a hospital. The samples were selected based on their diagnosis of various diseases, including but not limited to liver cirrhosis, hepatitis, and liver cancer.

### *D. Laboratory Analysis*

The samples were cut into thin sections (3 microns) using a microtome and mounted on glass slides.

Staining for ferric iron: The presence or absence of ferric iron in the liver biopsy samples was determined using the Prussian blue stain. The sections were deparaffinized, rehydrated, and then incubated with a mixture of 2% potassium ferrocyanide and 2% hydrochloric acid for 30 minutes. The sections were then washed and counterstained with eosin stain for 1 minute.

Staining for calcium ion: The presence or absence of calcium ion in the liver biopsy samples was determined using a calcium stain. The sections were deparaffinized, rehydrated, and then placed in 1.5% silver nitrate solution, in a dark cupboard for 20 minutes. They were washed in deveral changes of distilled water then subsequently reduced in freshly prepared 0.5% hydroquinone for 5 minutes, rinsed in water and treated with 2.5% Sodium thiosulphate (hypo) for 5 minutes and washed again. The sections were counterstained with neutral red.

All slides were finally dehydrated, cleared and mounted before histological examination.

Histological examination: The stained sections were examined under a microscope by a trained pathologist to determine the presence or absence of ferric iron and calcium ion in the liver biopsy samples. The pathologist was blinded to the diagnosis of the biopsy samples.

Results: The results of the study were presented photomicrographically as plates.

### *E. Inclusion and Exclusion Criteria*

The inclusion criteria for this study are liver biopsies that were diagnosed with different diseases and obtained between January and December 2019, and that were preserved using formalin and paraffin embedding techniques. Biopsies from both male and female patients of all age groups are eligible for inclusion.

However, biopsies that are inadequate for histological analysis, biopsies from patients who have received treatment for liver disease prior to biopsy, and biopsies that show signs of significant necrosis, inflammation, or fibrosis that may make it difficult to observe ferric iron and calcium ions in the liver tissues, were excluded from the study.

## III. RESULTS

Based on the histological analysis of formalin-fixed paraffin-embedded liver biopsies diagnosed with various liver diseases, the staining techniques employed in this study namely Pearl's prussian Blue and Modified Von Kossa stain were found to be very reliable and effective for the histochemical demonstration of ferric iron and calcium ion respectively in FFPE liver biopsies. However, no significant presence of ferric iron or calcium ion was detected in any of the samples therefore the study did not find evidence to support the hypothesis that patients with liver disease should take iron and/or calcium supplements.

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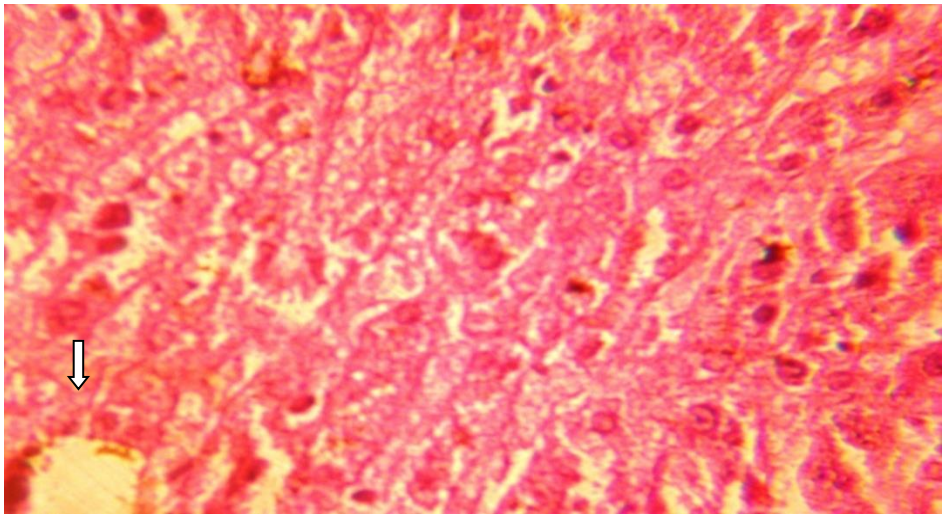

Plate 1: Showing normal liver; Open arrow shows the hepatic central vein. Haematoxylin and Eosin (x100)

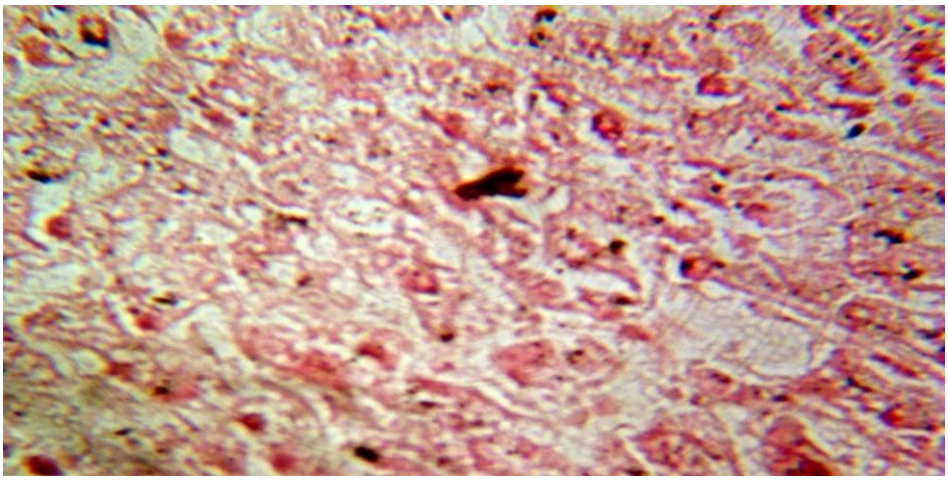

Plate 2: Normal liver; modified Von Kossa (x100)

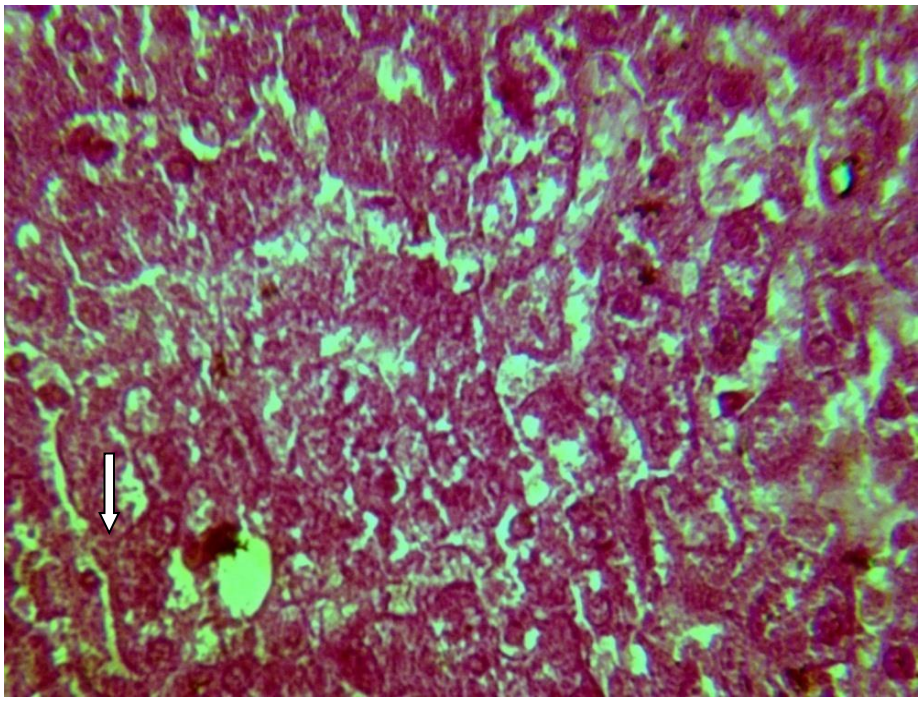

Plate3: Normal liver. Arrow is showing the hepatic central vein. Pearls prussian blue (x100)

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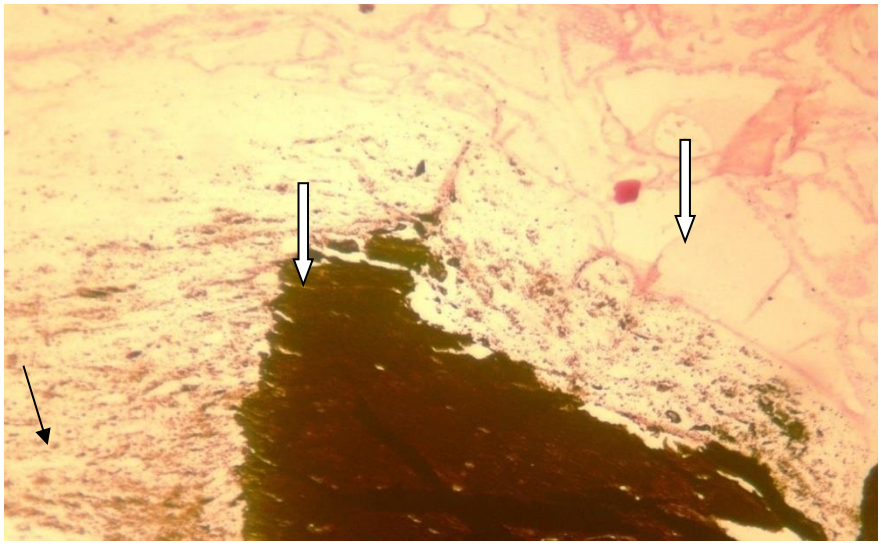

Plate 4: Calcified thyroid tissue used as positive control for calcium ion demonstration. Arrows showing calcification represented by thick black area. Modified Von Kossa (x100)

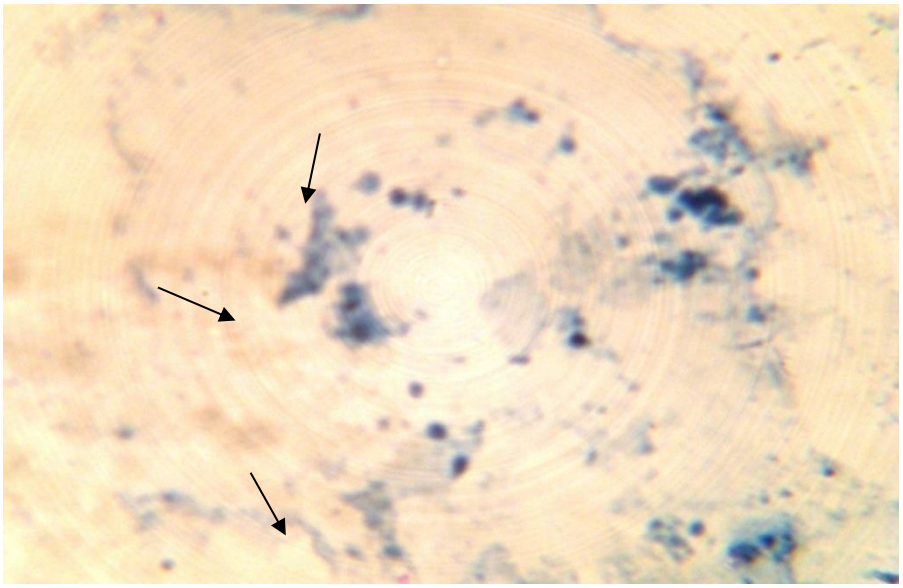

Plate 5: Formalin fixed paraffin embedded Ferric ammonium sulfate used as positive control for ferric iron demonstration. Arrow is showing areas with presence of ferric iron. Pearl's Prussian Blue (x100)

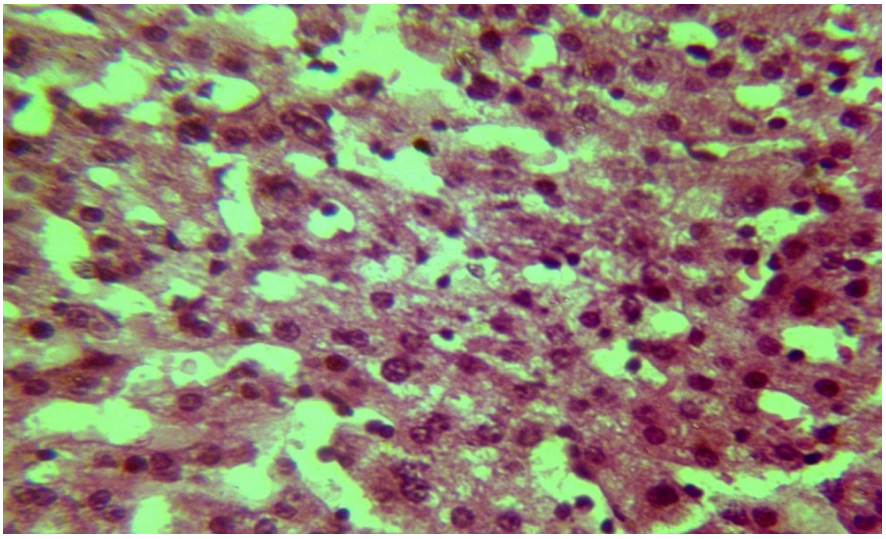

Plate 6: Chronic active hepatitis: Haematoxylin and Eosin (x100)

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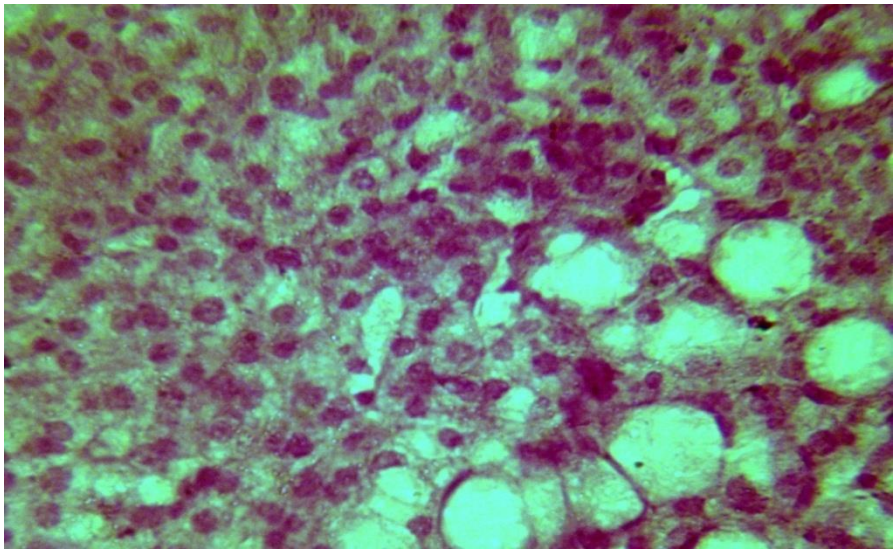

Plate 7: Chronic active hepatitis; Modified Von Kossa method (x100)

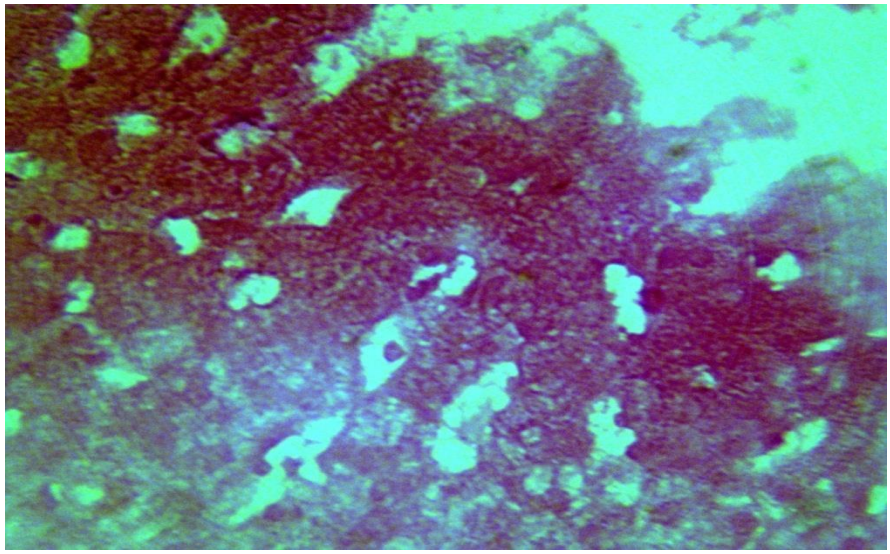

Plate 8: Chronic active hepatitis; Pearl's prussian blue (x100)

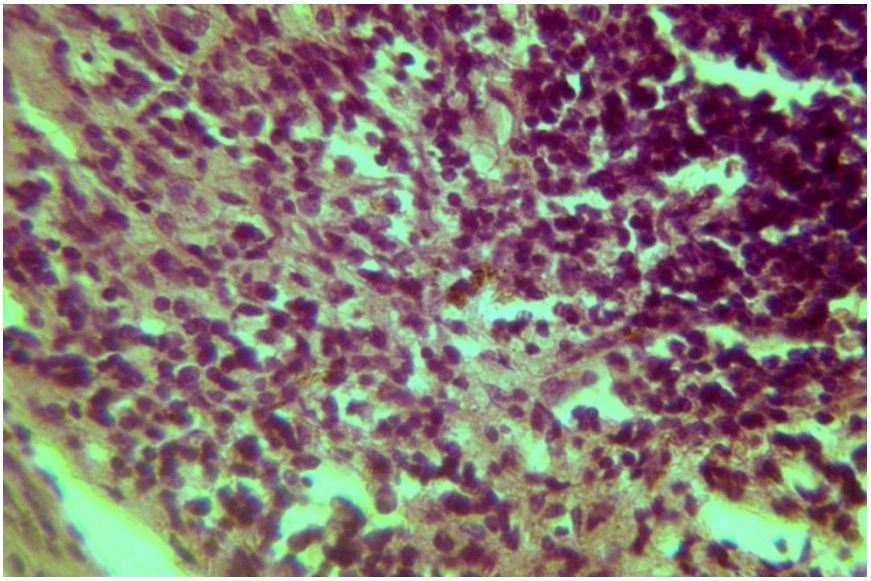

Plate 9: Liver cirrhosis; Haematoxylin and Eosin (x100)

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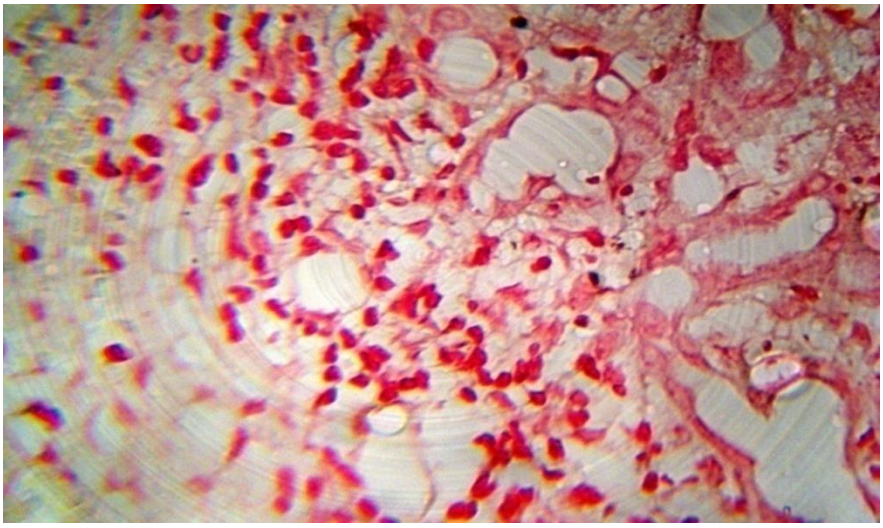

Plate 10: Liver cirrhosis; Modified Von Kossa Method (x100)

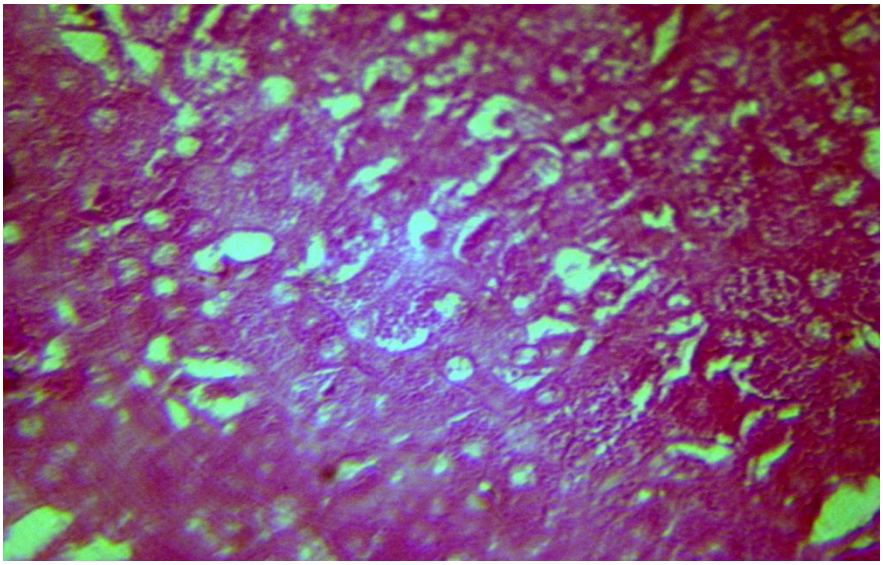

Plate 11: Liver cirrhosis; Pearl's prussian blue Reaction (x100)

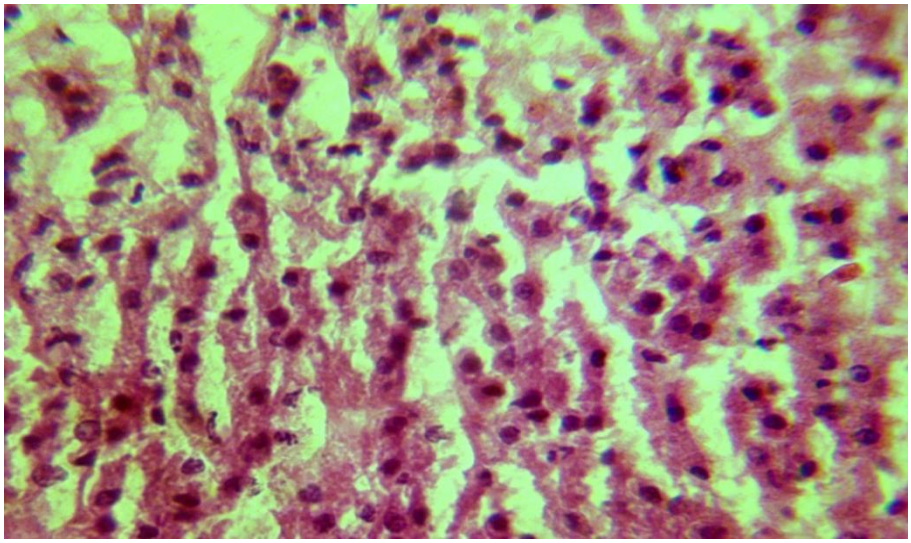

Plate 12: Liver tissue showing fatty changes (arrow). Haematoxylin and Eosin (x100)

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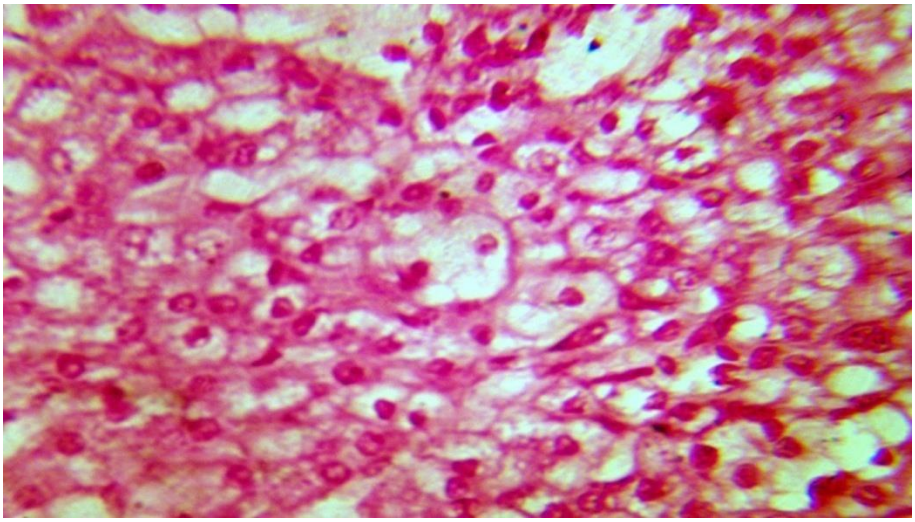

Plate 13: Liver tissue showing fatty change; Modified Von Kossa(x100)

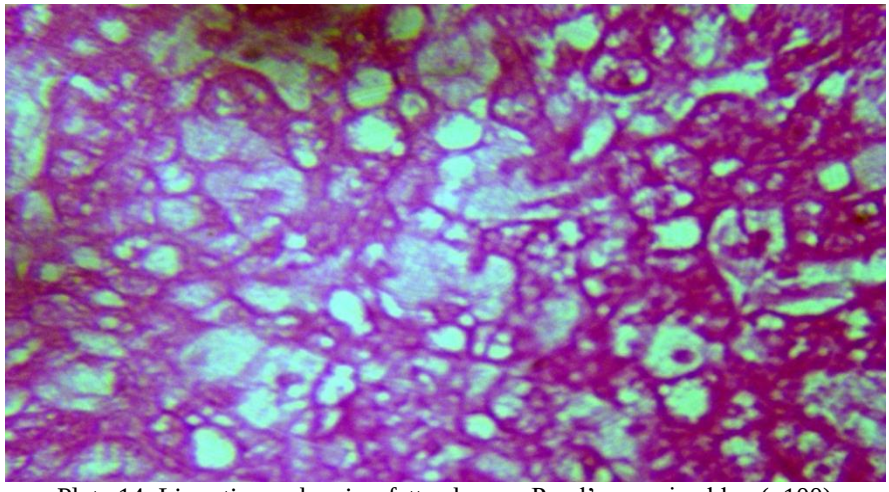

Plate 14: Liver tissue showing fatty change, Pearl's prussian blue (x100)

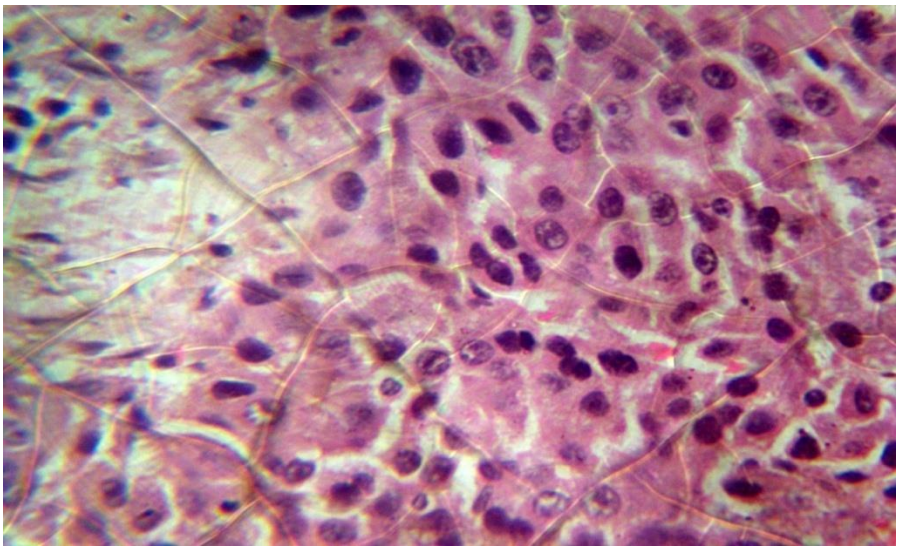

Plate 15: Primary liver cell carcinoma, Haematoxylin and Eosin (x100)

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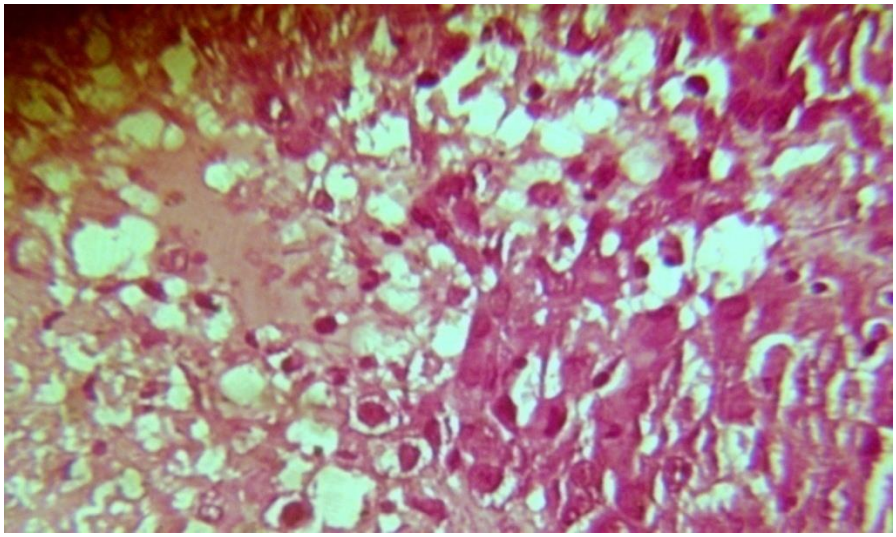

Plate 16: Primary liver cell carcinoma; Von Kossa Method (x100)

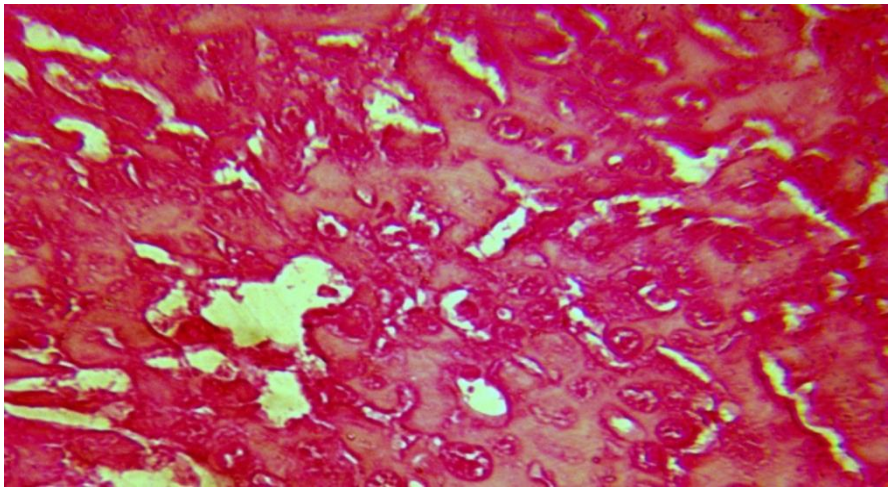

Plate 17: Showing primary liver cell carcinoma; Pearl's prussian blue(x100)

## IV. DISCUSSION

The present study aimed to determine the presence or absence of ferric iron and calcium ion histologically in formalin-fixed paraffin-embedded liver biopsies diagnosed with various diseases namely: chronic active hepatitis, fatty change, liver cirrhosis, metastatic adenocarcinoma, metastatic squamous cell carcinoma, multiple non neoplastic liver cyst and primary liver cell carcinoma as diagnosed. However, the results of the study did not reveal any positive correlation between the presence of ferric iron and calcium ion and various liver diseases. These findings are in contrast to the results of previous studies that have reported a positive correlation between the presence of these ions and liver diseases.

One such study conducted by [5] reported that the accumulation of iron in the liver is associated with the development of liver fibrosis. Similarly, another study by [6] reported that the deposition of calcium in the liver is associated with the development of hepatocellular carcinoma. However, in our study, we did not observe any such correlations, which may be attributed to the small sample size or the retrospective nature of the study.

However this study have shown that the staining techniques employed in this study namely Pearl's prussian Blue and Modified Von Kossa stain were found to be very reliable and effective for the histochemical demonstration of ferric iron and calcium ion respectively in FFPE liver biopsies because all the control slides tested positive for the elements of interest, and this is in concordance with [7] and [8] who said it is important to note that Pearl's Prussian Blue and Von Kossa staining methods were previously validated and have been used in various studies to detect the presence of these ions in liver biopsies, moreover, the controls for both Calcium ion and Ferric iron were positive. Therefore, the lack of positive results in this study may not be attributed to the reliability of the staining methods.

In conclusion, the results of this study do not support the previously reported correlations between the presence of ferric iron and calcium ion and liver diseases, it did not also found evidence to support the use of iron and/or calcium supplements in patients with liver disease. However, these findings highlight the need for further studies to determine the role of these elements in the pathogenesis of liver diseases as well as investigate other potential benefits or risks associated with these supplements in the patient population.

## V. RECOMMENDATION

Although the findings of this study do not support the previously reported correlations between the presence of ferric iron and calcium ion and liver diseases, it is important to note that these ions may still play a role in the pathogenesis of liver diseases. Therefore, further studies with larger sample sizes and more comprehensive analyses using more specific techniques to determine which element can be controlled in the liver to improve the diagnosis and prognosis of liver disease in addition to determining the role of these ions in the development of liver diseases.

It is important to note that the study had some limitations, such as the small sample size and retrospective design. Further studies with larger sample sizes and prospective designs are needed to confirm these findings.

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