DETECTING HEPATITIS B VIRUS ASSOCIATED CELLULAR CHANGES IN NEEDLE ASPIRATE SAMPLES OF HBV LINKED HEPATOCELLULAR CARCINOMA PATIENTS OF DIFFERENT STAGES

Ratna Kumari[1#]*, Anindita Tripathy[1] , Manoj Kumar Sahu[2], Kanishk uthanasingh[2], Manas Behera[2]

[#]DBT BIOCARe Scientist,

[1]Disease Biology Lab, KIIT School of Biotechnology, KIIT University, Bhubaneswar,751024, Odisha, India

[2]Department of Gastroenterology and Hepatobiliary Sciences, IMS & SUM Hospital, Bhubaneswar,751024, Odisha, India

Subtitle: *Hepatitis B virus induced cytopathology in needle aspirate samples of hepatocellular carcinoma*

Abstract

Hepatocellular Carcinoma (HCC) is the major cause of cancer-related mortality worldwide and in most cases it is either presented with or followed after viral-hepatitis. Hepatitis B Virus (HBV) infection in hepatocytes is the major culprit for most HCC cases among Asians. HBV has high oncogenic potential and is regarded as group-I human carcinogen by World Health Organisation. The mechanism of HBV linked HCC is still under active investigation; moreover, the cytopathic changes in hepatocytes after HBV infection is vaguely studied. The
progression of HBV cytopathology in infected hepatocytes is apparently associated with pathogenesis of HCC. The study of cytopathic changes in hepatocytes after HBV infection is
one of the important aspect for understanding the mechanism of HBV linked HCC. In the present study we endeavoured to explore the HBV induced cytopathology of infected
hepatocytes through FOLDSCOPE.

IndexTerms – Hepatitis B Virus (HBV), Hepatocellular Carcinoma (HCC), Cytopathology, Fine Needle Aspiration Cytology (FNAC), FOLDSCOPE.

Introduction

Hepatocellular Carcinoma (HCC) is the major cause of cancer-related mortality worldwide and viral-hepatitis is one of the main risk factor for HCC. Most cases of HCC in Asian countries is due HBV infection (60%) while in western countries it constitutes only 20%, where HCV infection is the major causal factor [1]. HBV has high oncogenic potential and is
regarded as group-I human carcinogen by World Health Organisation [2]. The mechanism of
HBV linked HCC is still under active investigation, but the virus is known to cause indirect and direct effect. Indirectly the virus can cause inflammation, regeneration, fibrosis, and cirrhosis after infecting hepatocytes. It has been also demonstrated that the virus DNA can be
directly integrated into the chromosome of infected host's hepatocyte [3]. Sometime this integration can occur in the critical location of cellular genome causing changes in cellular growth/proliferation pattern. The viral gene product called Hepatitis B x-gene (HBx) product is a transcriptional activator of various cellular genes involved in growth/proliferation like p53 and Ras-Raf-MapK pathway genes [4]. To study the cytopathic changes in hepatocytes after HBV infection is one of the important aspects for understanding the mechanism of HBV
linked HCC. The progression of HBV cytopathology in infected hepatocytes is apparently associated with pathogenesis of HCC. As very little is known about the cytopathic effect of HBV in hepatocytes so in the present study we investigated the cellular changes associated with HBV linked HCC. One of the distinctive features of HBV infection in hepatocytes is the wide unpredictability of the disease course which varies from duration to the severity of the liver disease. The HBV infection can range from acute cytocidal to persistent chronic infection. In both cases the cellular effect of viral infection is largely unknown, which is rather known for various other infectious viruses (e.g. Rotavirus, Flavivirus, Herpesvirus). In
fact, the cellular effects caused by these viruses are reason behind the gastro-intestinal symptoms, necrosis, inflammation, or phagocytosis in the affected individuals. In the present
study we endeavoured to identify the cytopathic effect of HBV on infected hepatocytes through FOLDSCOPE and its utility as a onsite disease diagnostic tool.

II. OBJECTIVE OF THE STUDY

(i) To perform cytomorphic analysis of needle aspirates from HCC patients of different stages using different stains to visualize cellular features with FOLDSCOPE.

(ii) To corroborate the cytology data of HCC patient with well established diethylenitrosomine (DEN) induced rodent hepatocellular carcinoma model.

III. RESEARCH METHODOLOGY

In order to achieve these objectives, we procured needle aspirates of HBV linked HCC patients of different stages from IMS & SUM Hospital (Bhubaneswar, India). We also performed DEN induced hepatocellular carcinoma study in male wistar rats as mentioned below.

IV. HCC PATIENTS SAMPLE

Fine Needle Aspiration Cytology (FNAC) samples from HCC patients of different stages were obtained from department of Gastroentrology & Hepatobiliary Sciences, IMS & SUM Hospital, Bhubaneswar, India. The FNAC procedure was performed by experienced surgeon/pathologist and the aspirates was immediately spread on glass slides. It was further stained with H and E stain and subsequently subjected for cytomorphic

analysis with FOLDSCOPE. The protocol was approved by Institutional Ethical Committee (IEC) and written consent was taken by the patients.

V. IN VIVO EXPERIMENT AND HEPATOCARCINOGENESIS MODEL:

In vivo studies were performed with male Albino Wistar rats of 120-150g body weight, which were procured from the laboratory animal facility of KIIT School of Biotechnology. They were housed in standard temperature/ humidity conditions and environment (12hr light/dark cycle). All animals were provided standard pellet diet and water ad libitum. The experiments were performed as mentioned in Tripathy et al., 2018 [5]. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC, KIIT School of Biotechnology, Bhubaneswar, India).

VI. RESULTS

1. Hematoxylin and Eosin stained sections from Fine Needle Aspirates (FNAC) samples of HCC patients:

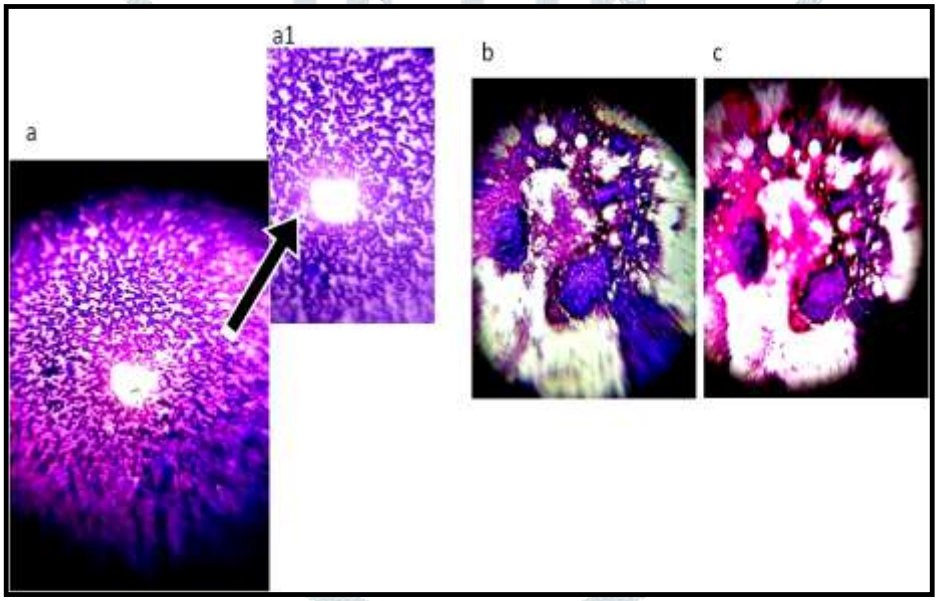

Fig1: HCC patients belonging to different stages (lower stage (a) and higher stage (b-c). The photomicrgraphs taken with FOLDSCOPE demonstrates cellular clusters of small cells with enlarged central vein and disorganized architecture with widened trabecular/acinar structures (Fig a and a1). Fig b and c represents cancer cells in FNAC samples of HCC patients with widened trabecular and multilayered cells. Images were taken with FOLDSCOPE attached with Lenovo K6 Note android cell phone camera.

2. Detecting Hepatitis B virus associated cellular changes in needle aspirate samples of HBV linked Hepatocellular carcinoma patients of different stages:

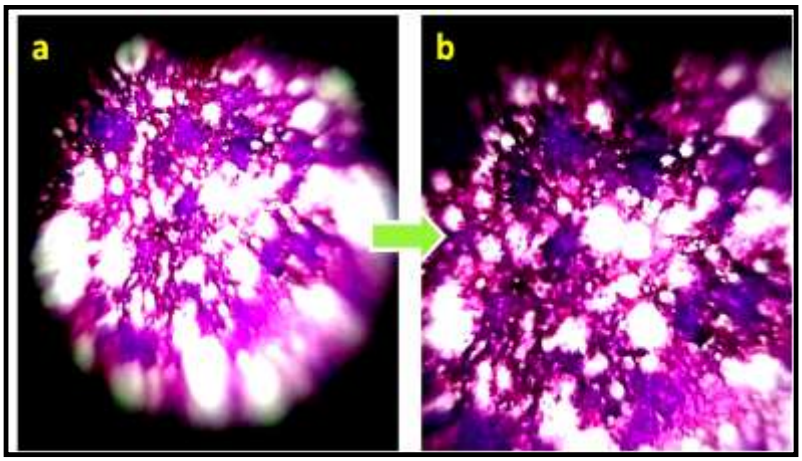

Fig2: HCC patients belonging to higher stage (a-b). The photomicrographs demonstrates cancer cells in FNAC samples of HCC patients with widened trabecular and multilayered cells. Figure b is the zoom out (3X) image of figure a taken with foldscope attaching OPPOF7 android cell phone camera.

3. Hematoxylin and Eosin stained sections from Fine Needle Aspirates (FNAC) samples of HCC patients:

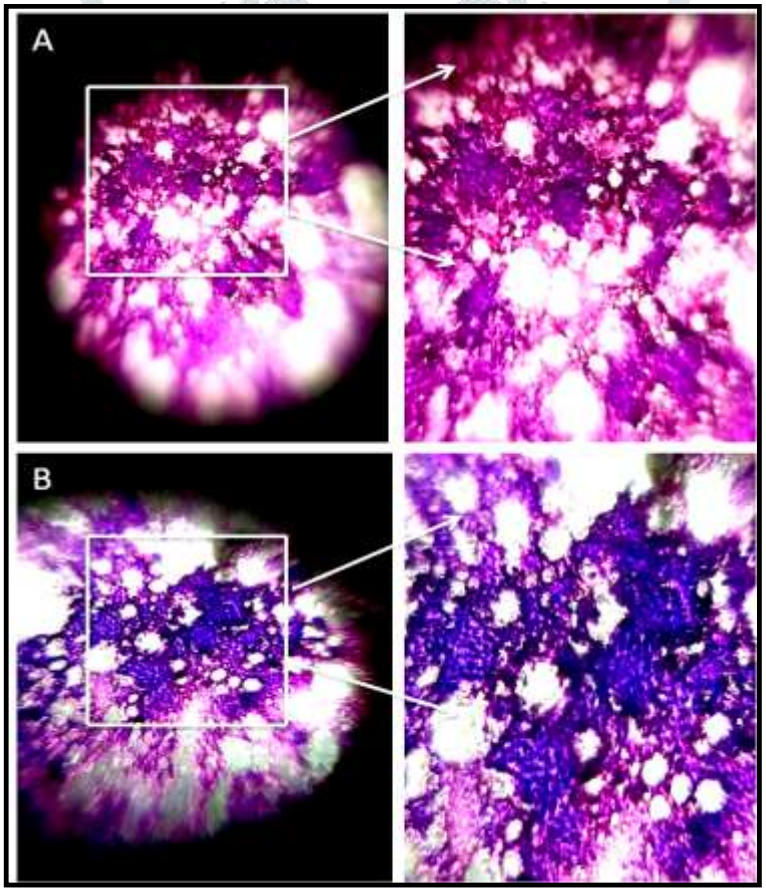

Fig3: Figure A & B represents cancer cells in FNAC patient's samples of different HCC stages patients (of different ages). The photomicrgraphs taken with foldscope demonstrates higher stages of different HCC patients which show more positive thick staining with enlarged vacuoles between clusters of multilayered cells along with widened trabecular and disorganized architecture. The white arrow shows zoom out (3X)

images of Fig A & B respectively which were taken with foldscope attaching Lenovo K6 Note android cell phone camera.

4. Photomicrographs of Hematoxylin and Eosin stained sections from Fine Needle Aspirates (FNAC) samples of HCC patients of different stages:

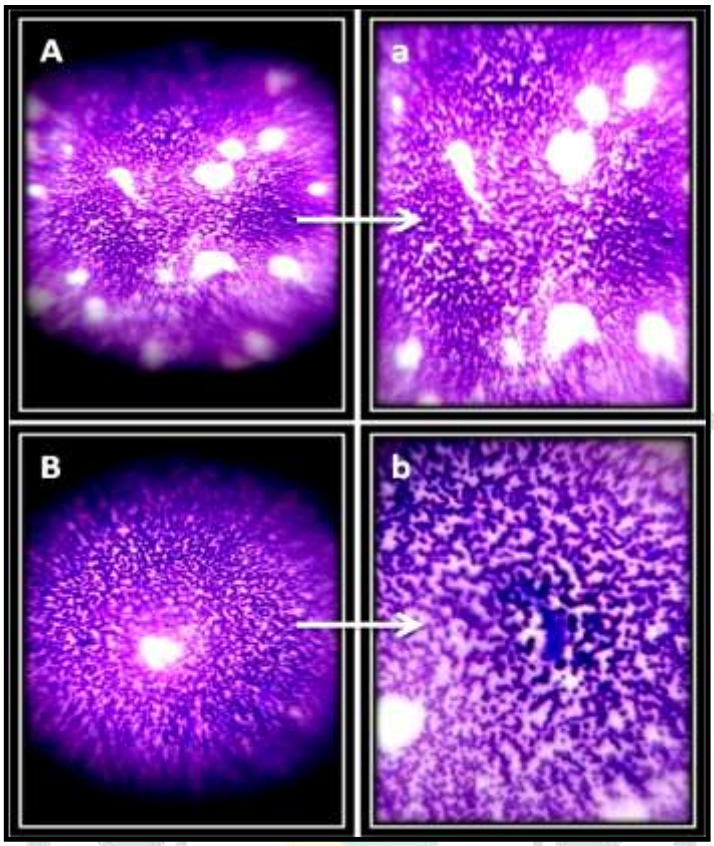

Fig4: HCC patients belonging to different stages (of different ages) mainly lower stages. The photomicrographs taken with foldscope demonstrates cellular clusters of small cells with enlarged central vein and the thick purple staining shows the cluster of small cells with disorganized architecture. Figure a and b shows zoom out (4X) images of Fig A & B respectively taken with foldscope attaching OPPO F7 android cell phone camera.

5. Photomicrographs of H & E stained Rat liver section –DEN induced hepatocarcinogenesis after Lithium Chloride treatment:

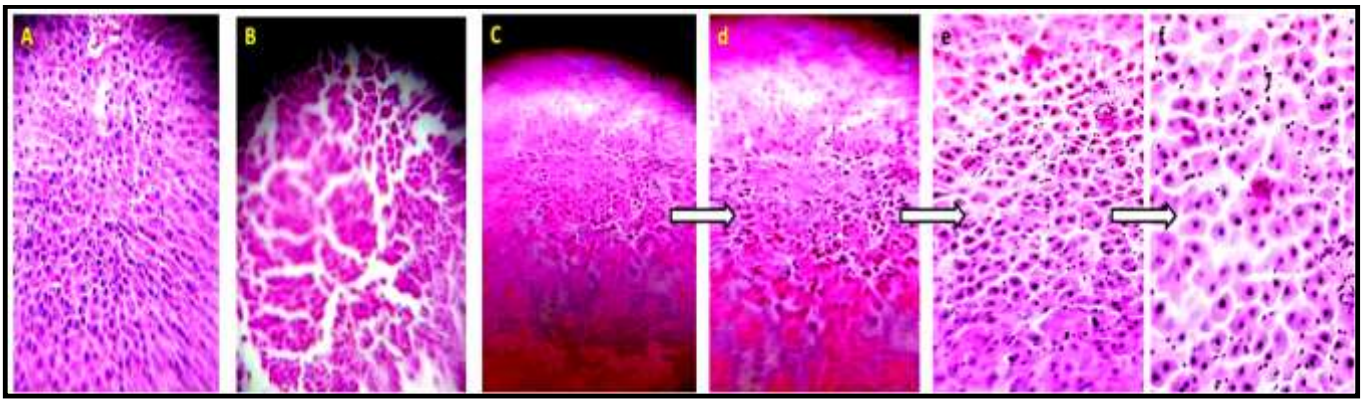

Fig5: Panel A shows untreated rat liver sections, with normal liver architecture, cuboidal hepatocytes, and radiating hepatic cords surrounding the central vein. Panel B shows DEN treated rat liver sections, with disorganized liver architecture, dilated sinusoids, and expanded portal vein. Panel c,d,e, and f shows post Lithium Chloride treated rat liver sections with cuboidal hepatocytes like untreated rat liver sections. d, e, f are the zoom out images of image c, which were taken on OPPO-F7 android cell phone camera attaching with foldscope. d is 1.2X , e is 2.5X and f is 4X zoom out.

6. Photomicrographs of Oil red O stained in DEN treated Rat liver section – The DEN Induced hepatocarcinogenesis:

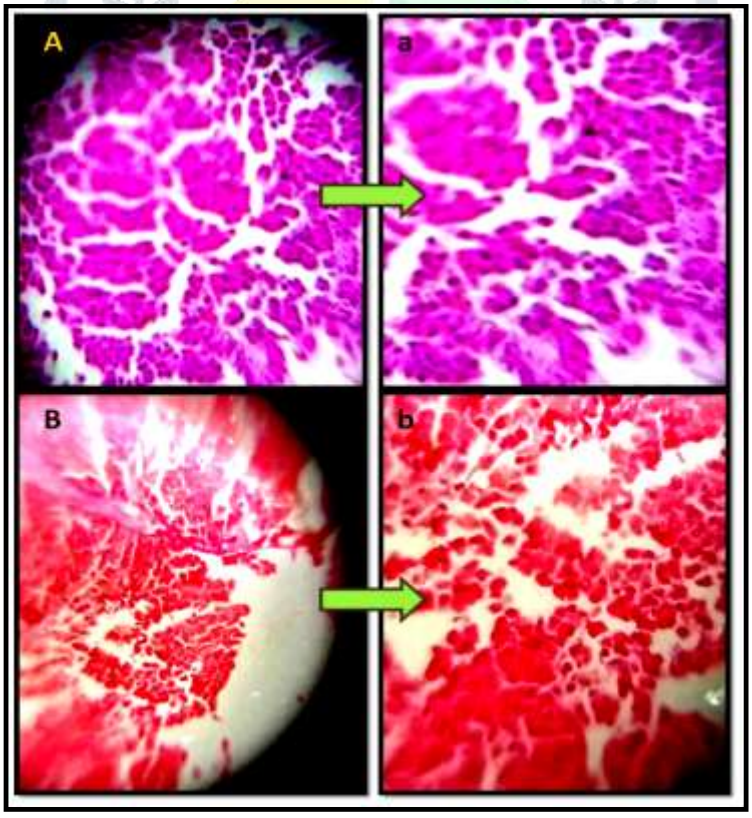

Fig6: Panel A shows H&E stained DEN treated rat liver section, with disorganized liver architecture and dilated sinusoids. Panel B shows the same tissue section, with Oil red-O staining where the positive red

staining demonstrates more lipidogenesis in this sample. a & b images are zoom out (3X) images of panel A & B respectively.

7. Photomicrographs of Oil red O stained Rat liver section – The different stages of DEN induced hepatocarcinogenesis:

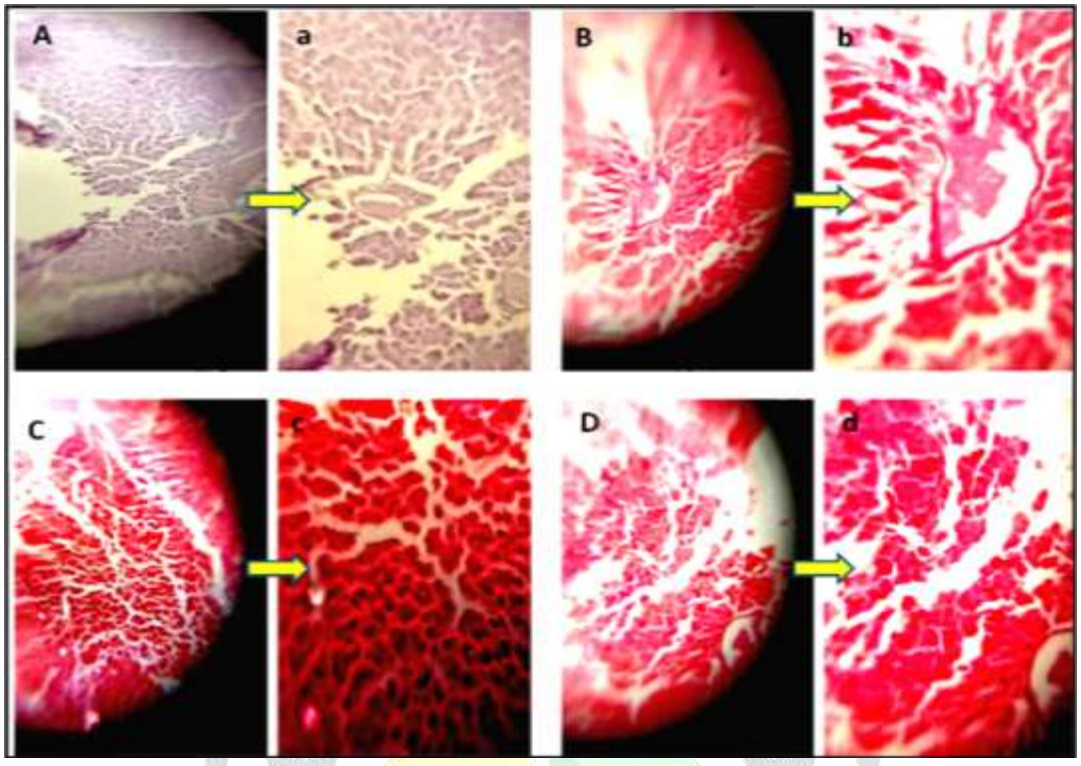

Fig7: Panel A shows untreated rat liver sections, with normal liver architecture where Oil red-O staining is negative. Panel B, C, D shows DEN treated rat liver sections, with positive Oil red-O staining. B, C, D are the Initiation, Promotion and Progression stages of hepatocarcinogenesis. It is more in Promotion stage (Panel C) as there are more lipidogenesis at this stage. a, b, c & d images are zoom out (4X) images of panel A, B, C & D respectively taken with foldscope attaching OPPO-F7 cell phone camera.

8. Photomicrographs of Oil red O stained DEN treated Rat liver section in promotion stage – higher magnification pictures of DEN induced hepatocarcinogenesis:

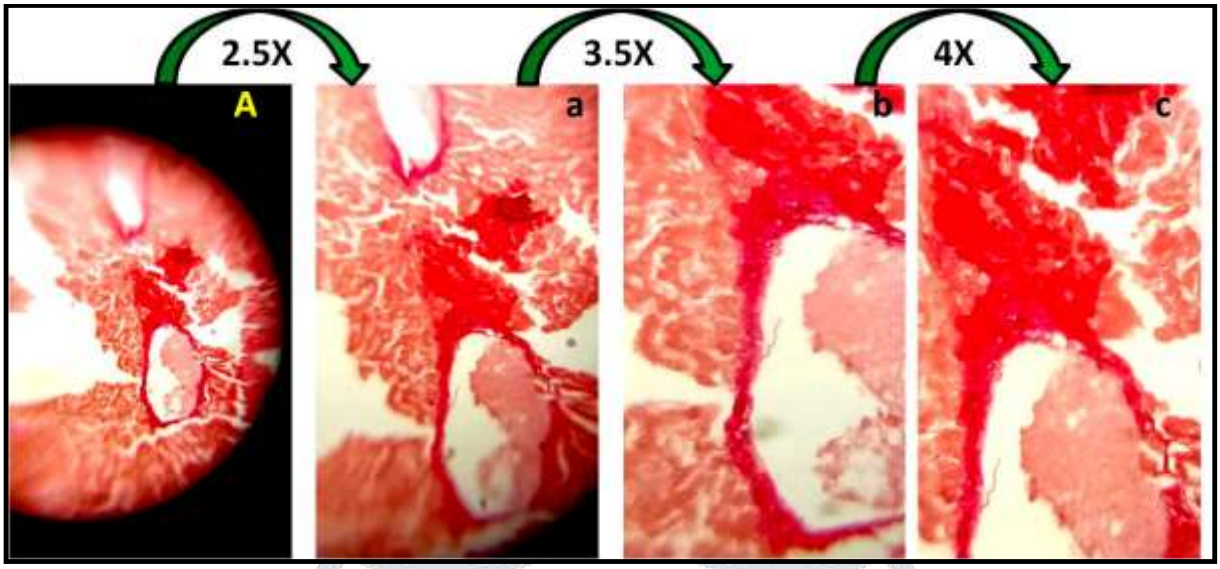

Fig8: The photomicrographs shows higher magnification of DEN treated rat liver sections in Promotion stage with Oil red-O staining. Figure A shows a more red staining demonstrating a higher accumulation of lipids at this stage of carcinogenesis. a, b and c are the 2.5X, 3.5X and 4X zoom out images of figure A respectively taken with foldscope attaching Lenovo K6 Note android cell phone camera.

CONCLUSION

The use of FOLDSCOPE provides an opportunity to work on the samples quickly and easily leading to an immediate interpretation. Our results demonstrated that with FOLDSCOPE we can clearly distinguish the changes in hepatic-tissue architecture of HCC as compared to control-tissues in DEN induced hepatocarcinogenesis model. Although medical devices continue to develop into sophisticated instruments, many significant medical decisions are still based on conventional microscopes. Use of cell-phone camera with Foldscope surely makes visual pathologic inspection quick and easy. The cytopathic effect of HBV on infected
hepatocytes has important impact on the study of HCC pathogenesis and further adds to its current understanding. On-site cytopathology is not widely available and the use of FOLDSCOPE certainly increases the possibility of on-site cytopathology. Our results clearly
demonstrated the potential utility of this paper origami microscope- The FOLDSCOPE as an
On-Site Disease Diagnostic Tool particularly for the cytopathological analysis.

Competing interest

The authors declare that they have no competing interest.

Authors Contributions

RK conceived the study, participated in its design and coordination, supervised the project. RK wrote the first draft of the manuscript. AT performed all the experiments and helped in drafting the manuscript. All the authors were involved in the critical revision of the manuscript.

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