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Prenatal Hepatic changes induced by Lamivudine in Swiss Albino Mice

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Abstract:

Aims and Objectives: Lamivudine is a reverse transcriptase inhibitor used in pregnant females infected with hepatitis B or HIV virus to prevent maternal to child transmission. The present study is aimed to observe the toxic effects of Lamivudine on liver in growing embryo.

Material and Methods: Lamivudine was given to pregnant Swiss Albino mice by oral gavage in doses of 18mg / kg b.w and 30mg / kg b.w from 6-15th day of gestation. The control mice were fed distilled water by the same route on the same gestational days. The pregnant mice were sacrificed on 18th day of gestation by cervical dislocation and livers were dissected out from the foetuses, observed for any gross malformation and then processed for histological study.

Result: In the gross examination of liver, there was a reduction in size and presence of haemmorhagic patches on the liver. On histological examination, the treated liver was found to show degeneration of hepatoblasts, resulting in large vacuolar spaces, and damaged sinusoids.

Conclusion: Lamivudine causes toxic changes in foetal liver and should thus be used judiciously in pregnant females.

Keywords: anti-retroviral drug, haemoprogenitor cells, lamivudine, teratogenic drug

Introduction:

With an increasing number of pregnant HIV-infected women receiving Anti-Retroviral Therapy worldwide, concerns have been raised over the possible side effects linked to exposure in early pregnancy, mainly in the first trimester. This trimester is the period of greatest susceptibility to teratogens, since it is when germ-layer formation and organogenesis occur.[1,2]

Lamivudine (2',3'-dideoxy-3'-thiacytidine) commonly called 3TC is a potent nucleoside analog reverse transcriptase inhibitor (nRTI). Like all nucleoside analogues, 3TC must be metabolized to its triphosphorylated form, 3TC-triphosphate, to be an active compound. Its incorporation in elongating DNA molecule results in irreversible chain termination.[3] Lamivudine crosses the human placenta, and at birth the plasma levels of lamivudine found in the maternal, the umbilical cord and the fetal blood are similar. 3TC clearance is prolonged in neonates compared to that in infants and older children.[4,5]

Guan-Guan Su *et al.*, 2004 studied the efficacy and safety of lamivudine treatment for chronic hepatitis B in pregnancy.[6] The data in their study indicated Lamivudine was able to neutralize hepatitis activity and reduction in viral load might result in fewer

pregnancy complications and prevent maternal-infant HBV transmission. This benefit may well outweigh the risk of Lamivudine's early toxic effects on the infant during the first trimester. However, they have not recommended its use during pregnancy especially during the first trimester because animal studies have demonstrated lethal effects on the rabbit fetus.

Thus due to inconclusive reports about the potential hepatic toxicity in embryo, we have taken this study.

Material and Methods:

The present study was conducted in the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi. 30 adult female Swiss albino mice weighing 25 to 30 grams and average age of 80 to 100 days were used after approval of institutional ethical committee. All the institutional and national guidelines for the care and use of laboratory animals were followed.

The female adult mice in their pre-oestrous phase were transferred in the evening to the cages containing adult mice in the ratio of 2:1. The presence of vaginal plug on the following morning indicated mating has occurred and was designated as day zero (GD0) of gestation. In case of doubt the plug was examined microscopically for the presence of sperms.

The mice were divided into three groups; lamivudine was obtained from GlaxoSmithCline Company (Epivir 100 mg) and was given to pregnant Swiss Albino mice by oral gavage; in doses of 18mg/kg b.w (Group II) and 30mg/kg b.w (Group III) from 6-15th day of gestation. The control (Group I) mice were fed distilled water by the same route on the same gestational days. The pregnant mice were sacrificed on 18th day of gestation by cervical dislocation and livers were dissected out from the foetuses, observed for any gross malformation and then processed for histological study. The sections were stained with haematoxylin & eosin stain and mallory trichrome stains.

Observations and Results:

On gross examination of the fetal livers, there was reduction in size in a dose dependent manner and few haemmorhagic patches were observed on the external surface of the liver (Fig. 1). In H&E staining, the microscopic picture of developing fetal liver of control shows the following characteristic features: (Fig. 2. A, B)

1. Developing sinusoids lined by haemoprogenitor cells.
2. The reticulum shows hepatoblast, hemoprogenitor cells and megakaryocytes suspended in it.
3. The central vein is well developed but the radiating hepatocyte pattern is absent.

In the low magnification of low dose lamivudine treated group there is a destruction of cells in the liver parenchyma resulting in vacuolar spaces (Fig. 2. D). In the high power view there is degeneration of hepatoblast and loss of reticulum giving rise to large vacuolar spaces (Fig. 2. E).

In the low magnification of high dose lamivudine treated group there is extensive damage of liver parenchyma in the perivenular area (Fig. 2. G). and in the high power of high dose lamivudine treated group the parenchyma is extensively degenerated with loss of portal triad. Also there is damage of progenitor cells, hepatoblasts and megakaryocytes resulting in empty spaces and damaged sinusoids. (Fig. 2. H)

In the slides, in Mallory trichrome staining, the normal foetal liver shows orange stained haemoprogenitor cells, the red stained hepatoblasts, and the purple stained megakaryocyte; the underlying reticulum is stained blue. (Fig. 2. C)

In low dose, the density of haemoprogenitor cells is reduced, and the megakaryotes are few and lying interspersed with the progenitor cells. The density of hepatoblast is also reduced. (Fig. 2. F) In high

dose lamivudine-treated group, there is an underlying damage to the reticulum which results in large vacuolar spaces which also hampers the development of central vein. The density of progenitor cells is vastly decreased due to apoptosis; the megakaryocytes are few in number. (Fig 2. I)

Fig. 1: Group I (control) showing normal feature of liver. Group II, III (LD, HD) showing reduction in size and (red arrow) showing haemorrhagic patches.

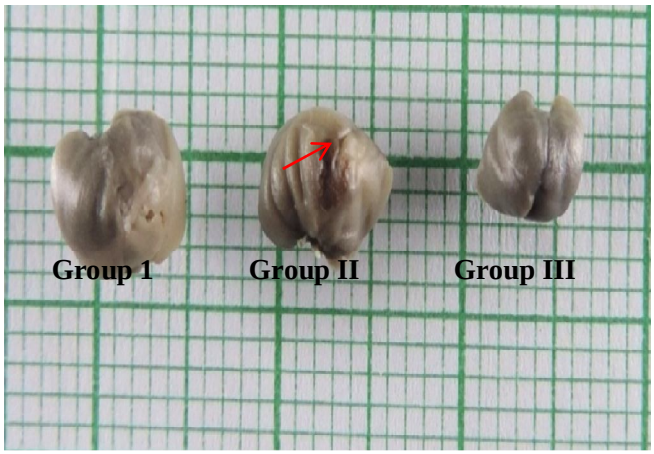


Fig 2: Photomicrograph of liver of mice fetus of Group-I (control) showing

(A) central vein (CV), developing sinusoids (SIN), hepatoblast and haemoprogenitor cells.(H&E100X)

(B) haemoprogenitor cells (red arrow), hepatoblast(yellow arrow) and developing sinusoids. (green arrow) (H&E400X)

(C) haemoprogenitor cells (blue arrow), hepatoblasts (red arrow) and megakaryocytes(black arrow) MT stain (400X).

Group-II Low dose (LD) showing:

(D) destruction of cells in the liver parenchyma resulting in vacuolar spaces (red arrow) (H&E100X)

(E) degeneration of heapatoblast (red arrow) and loss of reticulum giving rise to large vacuolar spaces.(green arrow) (H&E400X)

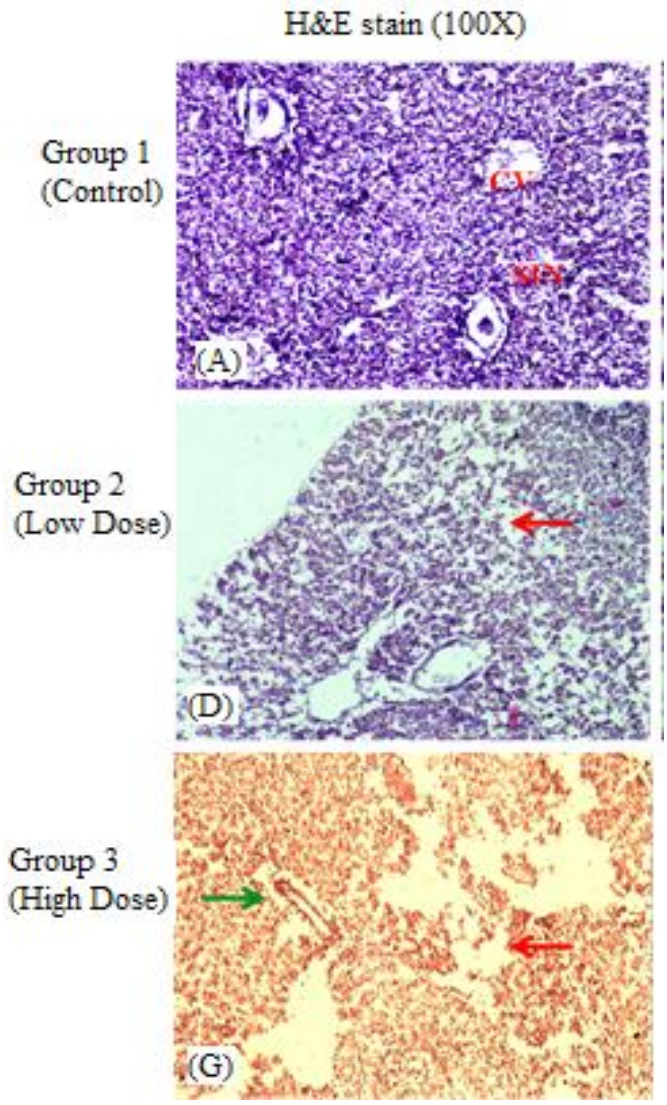
(F) reduced density of haemoprogenitor cells (blue arrow), hepatoblasts (red arrow) and megakaryocytes(black arrow) MT stain (400X).

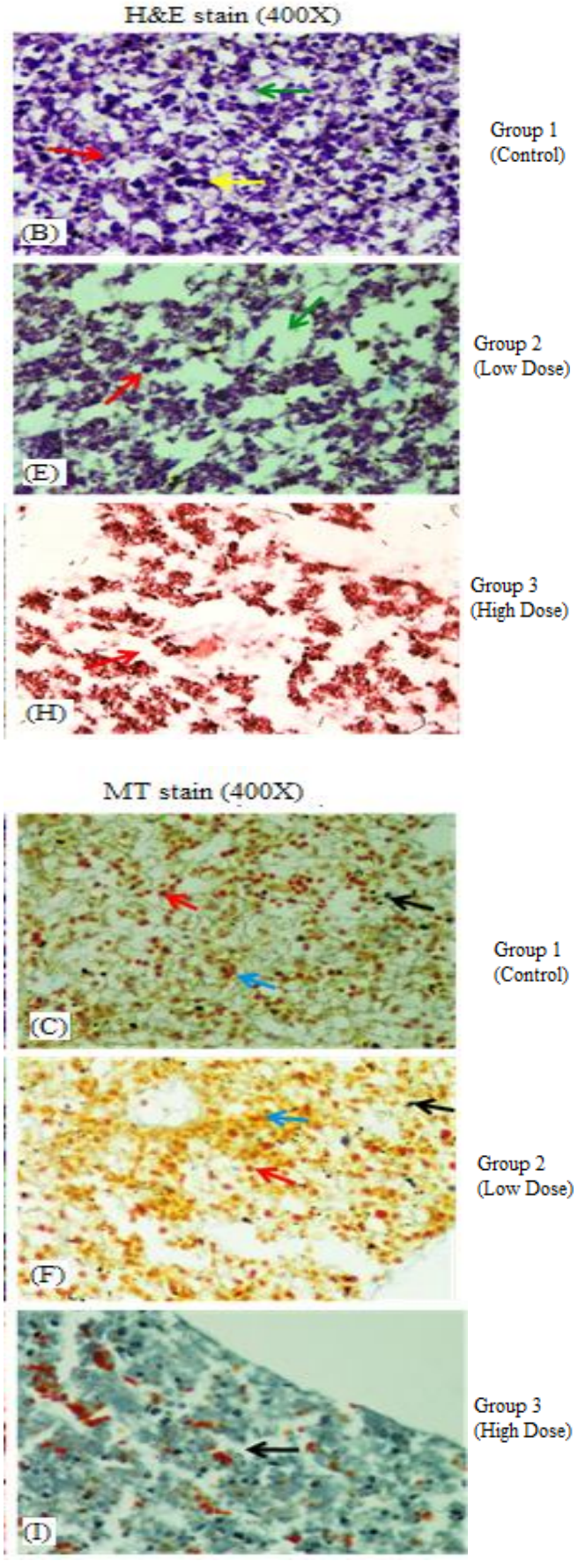
Group-III High dose (HD) showing:

(G) extensive damage of liver parenchyma (red arrow) in the perivenular area (green arrow) (H&E100X)

(H) extensively degenerated parenchyma with complete loss of cytoarchitecture. (red arrow) (H&E400X)

(I) damaged reticulum resulting in large vacular spaces(black arrow) MT stain (400X).





Discussion:

Pregnant women having an HIV infection or hepatitis B have more complications and high mortality.[4,7] The complications of such pregnancy can be abortion, preterm birth, birth defect or death of foetus.[8] Lamivudine is not recommended to be used during embryonic period.[10] The occurrence of anaemia and mild elevation of liver enzyme levels have been seen in children taking lamivudine treatment. Hepatotoxicity is one of the adverse effects reported about lamivudine along with pancreatitis, hyperglycaemia etc.[11]

On histological examination of liver in the lamivudine treated group there is damage of the liver parenchyma and degeneration of hepatoblast and progenitor cells resulting in empty vacuolar spaces. Also the sinusoids of liver appeared to be destroyed resulting in loss of cytoarchitecture. This might be explained with increased production of reactive oxygen species (ROS) resulting from mitochondrial dysfunction due to lamivudine that damages intracellular proteins, lipids and mitochondrial DNA resulting in lactic acidosis and cell death. These findings are in unison to the studies which reported hepatic steatosis, elevated hepatic transaminases and lactic acidosis in pregnant women receiving lamivudine.[9]

Thus we can say that lamivudine is toxic in fetal mice when given prenatally specially in large doses and when given during first trimester. Thus it should be used cautiously and judiciously in pregnant mothers, especially during first trimester.

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The author(s) declare that they have no conflict of interests.