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Histopathological Changes Due to Interaction of Visceral Larva Migrans and Diabetes Mellitus

Sahil Kumar[1*], Rajesh Katoch[1], Nawab Nashiruddullah[2], Shagufta Azmi[2] and Raghubir Singh[3]

[1]Division of Veterinary Parasitology, F.V.Sc. & A.H., SKUAST-J, Jammu, J&K, INDIA
[2]Division of Veterinary Pathology, F.V.Sc. & A.H., SKUAST-J, Jammu, J&K, INDIA
[3]Division of Veterinary Public Health, F.V.Sc. & A.H., SKUAST-J, Jammu, J&K, INDIA

[*]Correspondence author: S Kumar; Email: jimmysns@gmail.com.

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ABSTRACT

The study was aimed to investigate the histopathological changes due to interaction of Visceral Larva Migrans (VLM) and diabetes mellitus in Wistar rats (*Rattus norvegicus*) and its potential zoonotic risk after being consumed accidently. A total of seventy two adult Wistar rats were taken (N=72) and divided into four groups of 18 rats each viz; group I (healthy control), group II (diabetic control), group III (VLM infected healthy rats) and group IV (VLM infected diabetic rats). Experimental rats exhibited haemorrhages in the liver, lungs and brain on 10, 20 and 30 days post infection (dpi). The accumulation of mononuclear cells in the hepatic parenchyma was observed on 10 dpi. Thrombosis was seen in some blood vessels at 20 dpi. Fibrous connective tissue proliferation in triad areas around the biliary tubules were seen at 30 dpi as compared to control group. Massive hyperplasia of the bronchiolar lymphoid tissue, bronchiolar epithelial and sub-mucosal smooth muscle hyperplasia were seen on 20 and 30 dpi. The brain of rat with diabetes and without diabetes showed the degenerative changes on 10, 20 and 30 dpi.

Keywords: VLM, diabetes mellitus, Wistar rats, degenerative changes

Visceral Larva Migrans is a condition in humans caused by migratory infective larvae (L_2) of nematode *Toxocara canis*, though the larvae of *T. cati*, *T. leonina*, *Capillaria hepatica* and *Lagochilascaris minor* etc. have also been incriminated (Soulsby, 1982; Agnihotri *et al.*, 1987). Human infection can occur by accidental ingestion of embryonated eggs of *Toxocara* species. Children are most likely to be infected, probably because of their undeveloped immune system, the amount of eggs ingested (Habluetze *et al.*, 2003) and frequent reinfections (Chorazy and Richardson, 2005). Embryonated eggs of *Toxocara canis* are mostly present in the environment and on accidental ingestion; the infective larval stage migrates through the soft tissues of the body of many paratenic hosts like mice, rabbits, pigs, monkeys and humans (Tomimura *et al.*, 1976). Migration of *T. canis* through tissues produces pathological changes and the larvae become widely disseminated throughout the body, where they can live either encapsulated and/or free in the liver, lung, brain etc. (Bisseru, 1969; Glickman and Summers, 1983).

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fats and proteins. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin (Dixit and Joshi, 1985). It is a common metabolic disorder associated with various disease such as arteriosclerosis, nephritis and hypertension (Edem, 2009). Diabetes mellitus is one of the most common endocrine disorders and is characterized by hyperglycemia resulting from defects either in insulin secretion or insulin action or both (David, 1996). Various complications have been noted with DM such as cardiovascular disease, peripheral vascular disease, stroke, diabetes neuropathy, amputation, renal failure and blindness (Mayer, 1993).

In fact the diabetes shows multifaceted effect on blood glucose level, renal function, tissue degeneration and lipid profile while VLM also causes various pathological changes in various vital organ. Keeping this in view the study was done to find the histopathological changes due to interaction of VLM and Diabetes mellitus.

MATERIALS AND METHODS

A total number seventy two (N=72) adult male Wistar rats, weighing 200-250 gm procured from Indian Institute of Integrative Medicine (CSIR), Jammu, J&K, India were used in the study at Division of Veterinary Parasitology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R.S. Pura, J&K, India. The animals were maintained under standard managemental conditions. All the experimental animals were kept under observation during entire period of study. The protocol for conducting the experiments was duly approved by IAEC (Institutional Animal Ethics Committee).

The egg containing second stage larvae of *Toxocara canis* (N=500) were fed to adult Wistar rats by gastric intubation. Streptozotocin (STZ) solution was prepared freshly in 0.1 M sodium citrate (pH 4.5). For induction of diabetes experimental animals were fasted over night with free access to water and STZ was injected intraperitoneally @ 60mg/kg bodyweight (Chang, 2000; Ramesh and Pugalendi 2006). After 72 hours diabetes was confirmed by hyperglycemia in all rats by testing their blood glucose level using glucometer. A total of seventy two number adult Wistar rats were taken (N=72) and divided into four groups viz; I, II, III and IV of 18 rats each. The rats of group I served as healthy control, group II served as diabetic control, group III served as VLM infected healthy rats and group IV served as VLM infected diabetic rats. The design is summarized as follow in Table 1. The rats were sacrified (N=6) from each group on 10, 20 and 30 dpi to study various histopathological changes in liver, lung, brain etc.

After thorough gross examination, small representative pieces of less than 5 mm thickness from respective visceral organ viz., liver, lungs and brain were collected in 10% neutral buffered formalin solution. After 3-4 days of fixation, the tissues were washed in running water for 7-8 hours, dehydrated in ascending grades of ethyl alcohol, cleared in benzene and embedded with melted paraffin wax (melting point 58°C). The paraffin blocks were prepared and the sections were cut at 4-5 µ thickness with a hand operated microtome and stained by routine haematoxylin and eosin stain (Luna, 1968).

RESULTS AND DISCUSSION

Histopathological changes in different organs due to visceral larva migrans in Wistar rats on 10, 20 and 30 dpi.

Liver: Histopathologically, control group did not show any microscopic lesion of pathological significance in liver and revealed normal hepatic architecture with regularly arranged portal triads (PT) and central veins (CV). No fatty change was seen in any zone of the hepatic lobule. Portal triads were free of inflammation (Fig. 1a).

There was accumulation of mononuclear cells in the hepatic parenchyma on 10 dpi (Fig. 1b). Liver showing reactive peri-vasculitis and biliary hyperplasia with accumulation of mononuclear cells. Thrombosis was also seen in some blood vessels at 20 dpi (Fig. 1c). Fibrous connective tissue proliferation in triad areas around the biliary tubules were seen on 30 dpi (Fig. 1d).

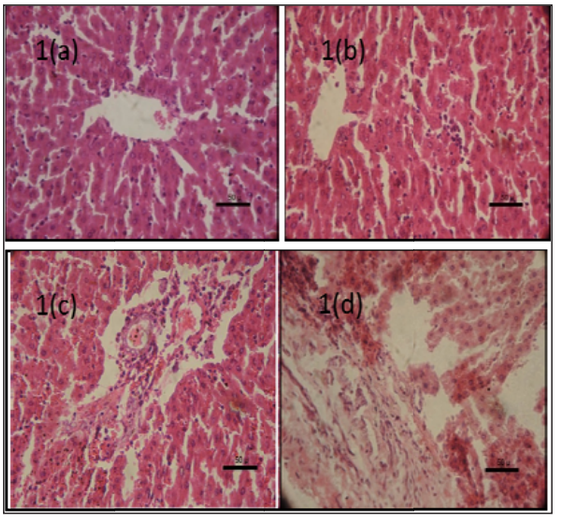

Fig. 1: Photomicrograph of rat Liver (H&EX400). **1(a)** Prominent central vein with normal hepatic lobules and hepatocytes. **1(b)** Accumulation of mononuclear cells in the hepatic parenchyma (10 dpi). **1(c)** Reactive peri-vasculitis and biliary hyperplasia, accumulation of mononuclear cells with thrombosis in some blood vessels (20 dpi). **1(d)** Fibrous tissue proliferation in tried areas around the biliary tubules (30 dpi)

Lungs: Histopathology of control group rats showed the normal histological appearance of alveoli and bronchi (Fig. 2a). On 10 dpi reactive fibrous parasitic tracts in the lung parenchyma communicating from the airways (bronchioles) to the sub-pleural surface. Surrounding alveoli showed marked congestion together with varying degrees of atelectasis and emphysema. There was also marked hyperplasia of the bronchiolar associated lymphoid tissue (BALT) extending to the surrounding alveolar parenchyma (Fig. 2b). Massive hyperplasia of the bronchiolar lymphoid tissue (BALT), bronchiolar epithelial and sub-mucosal smooth muscle hyperplasia were seen on day 20 and 30 dpi (Fig. 2c).

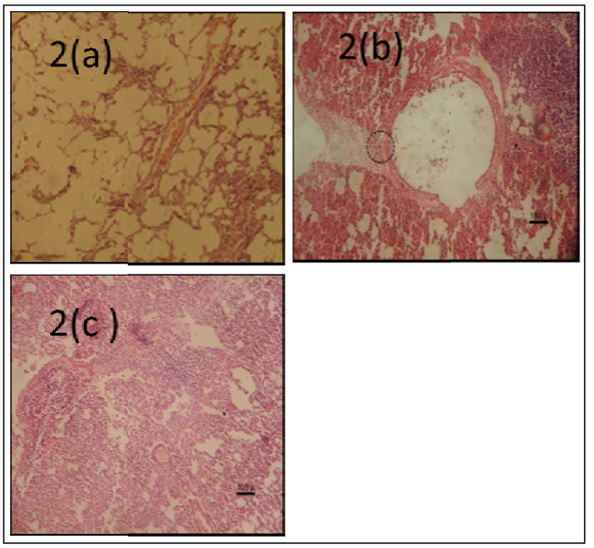

Fig. 2: Photomicrograph of rat Lung (H&E X400). **2(a)** Normal lung. **2(b)** Reactive fibrous parasitic tracts in the lung parenchyma communicating from the airways (bronchioles) with congestion, atelectasis and emphysema. **2(c)** Massive infiltration of mononuclear cells in the surrounding alveolar parenchyma

Brain: Histopathological examination of brain in control group did not show any microscopic lesion of pathological significance and showed normal meninges of brain (Fig. 3a). At 10 dpi brain of rat showed haemorrhages in the cerebral parenchyma (Fig. 3b). Brain showed haemorrhage and glial accumulation in cerebral parenchyma at 20 and 30 dpi (Fig. 3c).

Histopathological changes in different organs due to visceral larva migrans infection in diabetic Wistar rat on 10, 20 and 30 dpi.

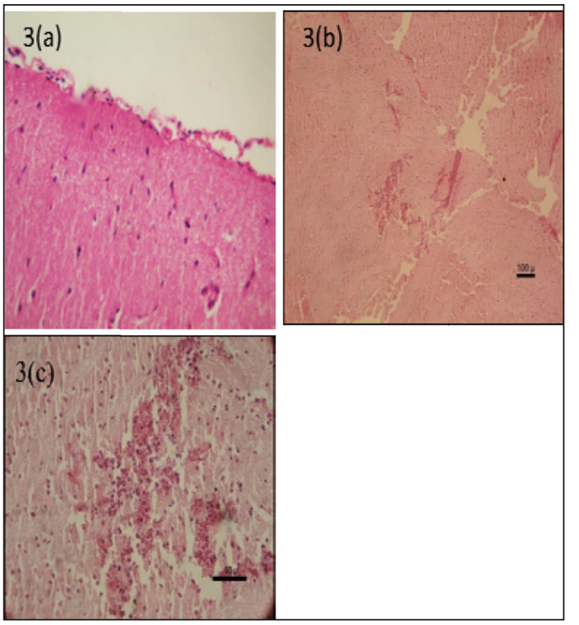

Fig. 3: Photomicrograph of rat brain (H&EX400). **3(a)** Normal brain. **3(b)** Brain showing haemorrhage in the cerebral parenchyma (10 dpi). **3(c)** Brain showing haemorrhage and glial accumulation in cerebral parenchyma (20, 30 dpi)

Liver: Histopathologically there is accumulation of mononuclear cells in the hepatic parenchyma at 10 dpi (Fig. 4a). On 20 dpi liver showed biliary hyperplasia (plate 4b). Sinusoids were dilated with disruption of hepatic cords. Hepatocytes appear degenerated and atrophied; the central veins were congested at 30 dpi (Fig. 4c).

Lungs: Histopathologically hyperplastic bronchiolar epithelium with complete obliteration of the lumen with surrounding bronchiolar associated lymphoid tissue (BALT) hyperplasia in the sub-mucosa were seen at 10 dpi (Fig. 5a). On 20 dpi reactive fibrous parasitic tracts in the lung parenchyma communicating from the airways (bronchioles) and extending to the lung parenchyma was seen. Massive infiltration of mononuclear cells in the surrounding alveolar parenchyma was also noticed (Fig. 5b). The cross section of a dead parasitic larva in the alveolar lumen surrounded by reactive mononuclear inflammatory cells was observed with bronchiolar sub-mucosal lymphoid proliferation, smooth muscle hyperplasia and infiltration of macrophages and some multinucleated giant cells was also observed on 30 dpi (Fig. 5c, 5d).

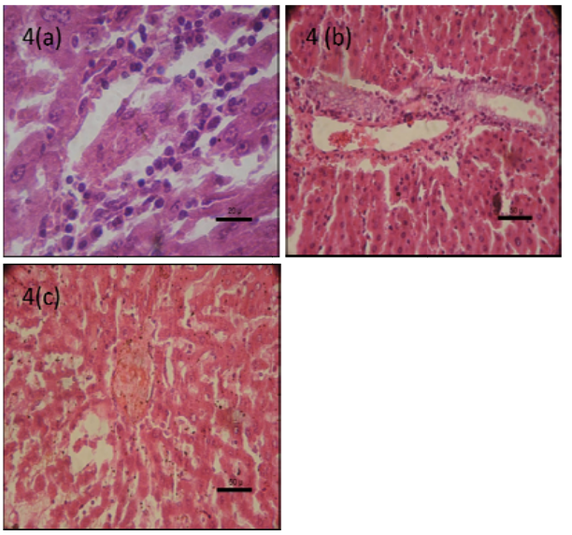

Fig. 4: Photomicrograph of rat liver. **4(a)** Mononuclear cells in the hepatic parenchyma (10 dpi). **4(b)** Liver showing biliary hyperplasia (20 dpi). **4(c)** Liver showing sinusoids dilated with disruption of hepatic cords. Hepatocytes appear degenerated and atrophied. The central venis are congested. (30 dpi)

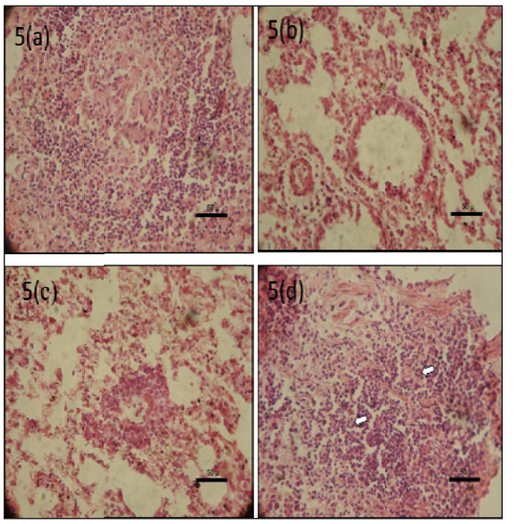

Fig. 5: Photomicrograph of rat Lung (H&EX400). **5(a)** Hyperplasic bronchiolar epithelium with complete obliteration of the lumen in lung (10 dpi). **5(b)** Reactive fibrousis seen, parasitic tracts in the lung parenchyma, accumulation of mononuclear cells and eosinophils (20 dpi). **5(c)** Cross section of a dead parasitic larva in the alveolar lumen surrounded by reactive mononuclear inflammatory cells (30 dpi). **5(d)** Bronchiolar sub mucosal lymphoid proliferation, smooth muscle hyperplasia, infiltration of macrophages and some multinucleated giant cells (arrow, 30 dpi)

Brain: On 10 and 20 dpi brain of rat showed degenerative changes in the myelin tracts with proliferation and gliosis (Fig. 6a) while on day 30 dpi no significant changes were seen.

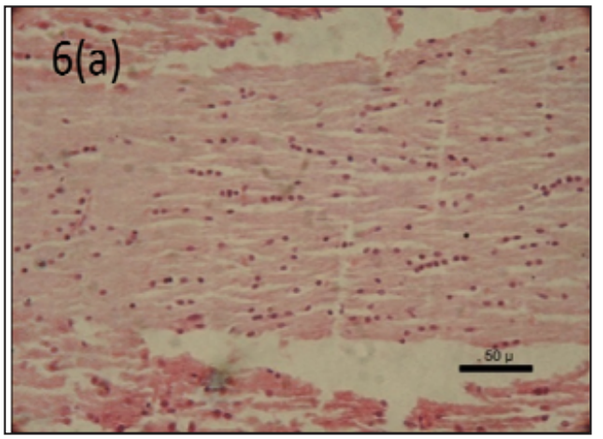

Fig. 6(a): Photomicrograph of rat brain showing degenerative changes in the myelin, proliferation and gliosis (H&EX400)

In the present study, no larva was detected in kidneys and muscles in both larval recovery and histopathological methods. It is stated that because of their mobility, larvae are not always found in histopathologic lesions (Fenoy *et al*., 2001). The accumulation of mononuclear cells in the hepatic parenchyma was observed on 10 dpi. Thrombosis was seen in some blood vessels at 20 dpi. Fibrous connective tissue proliferation in triad areas around the biliary tubules at 30 dpi and the changes were incompatible with the liver of rat showing reactive peri-vasculitis and biliary hyperplasia (Cardillo *et al.,* 2009). Lescano *et al.*, 2004; Hrckova *et al.,* 2001; Roberts, 2009 also reported the same histopathological changes in liver which appears hemorrhagic and necrofied with central infiltration in liver cords and hemorrhages between kupffer cells (Roberts, 2009). The polymorphocyte infiltration, fat cells in the tissue, destruction and necrosis of nucleus were reported in a study that may be due to immune response and the presence of fat cells in the tissue, destruction and necrosis of nucleus could explain the hyperplasia of the tissue (Lescano *et al.,* 2004; Hrckova *et al.,* 2001). Wasim and Mahmood (2011) reported congestion of sinusoids, granuloma and fatty degeneration of hepatocytes. The granuloma contained a centre of closely eosinophils and macrophages surrounded by larger macrophages with pale

Table 1: Experimental design to study histopathological changes due to interaction of VLM and diabetes mellitus in Wistar rats.

Group	No of animals per group	Day '0' streptozotocin	5 day post streptozotocin, adminstration *T. canis* infective eggs given orally
Group I (healthy control)	18	Nil	Nil
Group II (diabetic control)	18	60 mg/kg b.wt.	Nil
Group III (VLM infected healthy rats)	18	Nil	500
Group IV (VLM infected diabetic rats)	18	60 mg/kg b.wt.	500

vesicular nuclei. Multinuclear giant cells were also been reported.

Histopathologically, the liver of diabetic and *Toxocara canis* infected Wistar rats were accumulated with the mononuclear cells in the parenchyma at 10th dpi. On 20 dpi biliary hyperplasia with dilatation of sinusoids and disruption of hepatic cords and 30 dpi hepatocytes appeared degenerated and atrophied with congestion in the central veins. The study by Hanna and Seham, 2012 on streptozotocin induced diabetes rat illustrated the liver sections with mononuclear cell infiltration extending through hepatic tissue, kupffer cell engulfing debris and hyperplasia of bile duct and fatty changes. In many previous morphological studies there were many pathological changes, degenerated hepatocytes with polymorphic nuclei, dilated sinusoids and mononuclear cell infiltrate extending through hepatic tissue in diabetic animals (Cameron *et al.,* 2005).

The rats in the control group showed the normal histological appearance of alveoli and bronchi and on the 10 dpi, reactive fibrous parasitic tracts in the lung parenchyma communicating from the airways (bronchioles) to the sub-pleural surface were visible. Surrounding alveoli showed the marked congestion together with varying degrees of atelectasis and emphysema. There were marked hyperplasia of the bronchiolar associated lymphoid tissue (BALT) extending to the surrounding alveolar parenchyma, massive hyperplasia of the bronchiolar lymphoid tissue (BALT), bronchiolar epithelial and sub-mucosal smooth muscle hyperplasia on day 20 and 30 day of the post infection. Kuziemski *et al.*, 1999 study too reported lung manifestations indicated by disseminated pulmonary lesions of VLM syndrome due to *T. canis* infection. Behrman *et al.*, 2004 found the granulomas in lungs and fibrous parasitic tracts in the lung parenchyma and bronchioles. Wasim and Mahmood, 2011 in his study on histopathology of lungs found the cellular infiltration with localized foci of eosinophils and neutrophils.

Histopathologically, the lung of diabetic and *Toxocara canis* infected Wistar rats in cross section showed the dead parasitic larva in the alveolar lumen surrounded by reactive mononuclear inflammatory cells with bronchiolar sub-mucosal lymphoid proliferation, smooth muscle hyperplasia and infiltration of macrophages, some multinucleated giant cells on 30 dpi. Noor *et al.*, 2008 in their study showed the finding in lungs same as our study which includes marked infiltration and hyperplasia.

The brain of rat with diabetes and without diabetes showed the degenerative changes on 10 and 20 dpi which might be the period of migration of larva in this site. The myelin tracts were found with proliferation and gliosis while on day 30 dpi significant changes in the brain were negligible as the larval may migrate from this site to other body organs and the immune response of the body of the host might clear the earlier changes. Othman *et al.,* 2010; Nathalia *et al.*, 2015 study also reported the larval tract in the cerebrum with vesicular congestions and hemorrhages. Jannecek *et al.*, 2014 found the cerebrum as a preferential location for the larva in the brain and degenerative changes with signs of migration visible. Ozdemir *et al.,* 2009 observed mild neurological changes viz., congestion, and gliosis in diabetic rats.

CONCLUSION

It can be concluded that VLM in healthy rats had moderately effect on histopathological alterations

as compared to VLM in diabetic rats. Further, the aggrevation of parasitemia on the recovery of larva were found in abundant on 20 dpi might be due to the immune-supression and inherent cellular changes in the liver and lungs. The results from the study may help in providing the answers to the questions on the relationship of diabetes which bring with itself the immune supression in hosts and aggrevation of parasitic and other infectious diseases such as *Toxocara* infections and may contribute to the development of means to combat or prevent such diseases in India and worldwide.

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