Giardiasis in Streptozotocin – Induced Diabetic C57BL/6 mice

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ABSTRACT

Giardia lamblia an intestinal flagellated protozoan parasite that may give rise to diarrhea with or without malabsorption. Streptozotocin (STZ) is a broad – spectrum antibiotic that is toxic to the insulin β cells of pancreatic islets and has been used investigationally in a wide variety of large and small animal species. Parasitological and histopathological studies were done on the course of G. lamblia infection through the streptozotocine - induced diabetic C57BL/6 mice this was done through intragastric infection by ± 200.000 G. lamblia cyst, the results revealed in non-diabetic vs diabetic group; the infection rate was 86.6% vs 96.2%, the prepate nt period (4.04± vs 2.8±0.84), death rate (4.28% vs 50%), with a significant increase in the mean excreted cysts count /2hours till the end of the experiment respectively. Aggrevated histopathological changes of the liver were observed in diabetic compared to non-diabetic mice as hydropic and fatty degeneration inflammatory cells infiltration and in the invading number of the parasites. It could be concluded that experimentally there is an element of immunesuppresion evokes by diabetes and further studies will be in need.

KEYWORDS: Giardia lamblia, Streptozotocin diabetes.

INTRODUCTION

The enteric protozoan Giardia lamblia is one of the most common non viral causes of diarrhea in human, it infects a wide range of vertebrates including human.[36]

In the last two decades, major immunodeficiency syndromes have strongly influenced medical parasitology. The immuno-compromised host is generally defined as a person who has one or more defects in the normal defense mechanisms predisposing him or her to an increased risk of severe or even life threatening infections. Patients with immuno-compromised status have an increased probability of acquiring a primary infection with more aggressive manifestations, or reactivation of a latent infection. Protozoa in such patients were common as cryptosporidiosis, isos – psoriasis, cyclosporiasis, giardiasis and toxoplosmosis.

Diabetes mellitus (DM) is becoming a major chronic disease burden all-over the world, including Egypt (looker et al., 2010) the diabetic patients are considered as the immune-compromised group of patients. Several aspects of immunity are altered in patients with diabetes [18] According to Foster (1989) the affection of the immune mechanisms in diabetes mellitus is mainly physical, microangiopathy that reduces tissue perfusion and defective polymorph nuclear leukocytes functions including mobilization, adhesion chemotaxis, phagocytes and bactercidial activity in addition, only 25% of the diabetic patients have complement deficiency, though there is no reported increased susceptibility to infections with this complement deficiency. Masur [25] reported minor cell - mediated immune response in diabetic patients including defect in interleukin -2 (IL-2) that
stimulates the proliferate function of b-cells, he added that protozoa and helminthic infections were associated with major immunological function defects (humoral and cellular) this statement was confirmed by Deresinski [12] who reviewed and assigned that all the infections associated with diabetes mellitus are due to fungal, bacterial and viral organisms, but neither protozoal nor helminthes.

Streptozotocin (STZ) was initially isolated from Streptomyces achromogenes in 1960, after which it was shown to be a broad-spectrum antibiotic possessing antitumor, oncogenic, and diabetogenic properties [24]. Its diabetogenic property is characterized by selective destruction of pancreatic islet $\beta$-cells, causing insulin deficiency, hyperglycemia, and polyuria, all of which mimic human type 1 diabetes mellitus [21].

Several species, including the mouse, rat, rabbit and monkey, are sensitive to the pancreatic $\beta$-cells, cytotoxic effect of STZ. Currently, STZ is often used to induce diabetes in all of those animal and is routinely used for that purpose in the mouse.

Many studies recorded the prevalence of giardiasis among diabetic patients, in Egypt, Abaza et al. [1], Antonios et al., Elnady et al. and Sabah and Temsah [33] recorded 6%, 0.0%, 22% and 3% respectively. In Iran Alkhalgi et al [4] reported 9%. Magnitude of diabetes and the wide range of giardiasis prevalence urged the authors to evaluate this discrepancy experimentally through diabetic murine giardiasis.

**MATERIAL AND METHODS**

A - Animal:

C57BL/6 male mice ~ 20g, 8-12 week old were purchased from Theodor Bilhaz Research Institute (TBRI), caged in groups in plastic cages and kept under standard housing condition.

All procedures met the International Guiding Principles for Biomedical Involving Animals, as issued by the International Organization of Medical Sciences (www.cioms3ch/)

A – Animals:

- Animals were divided to:
  1. Normoglycemic (non – diabetic) and II Hyperglycemic (diabetic) ones.
  1. Normoglycemic group subdivided to:
    1a : normal control group : 20 mice neither infected non treated with STZ
    1b : infected control group : 60 mice infected orally with $\pm$ 200,000 G. lamblia, cysts
  2. Hyperglycemic (diabetic) groups (Wu and Huan, 2007)
    2a- drug control group : 20 mice this group received STZ as 40 mg / kg / mouse through ip administration for 5 consecutive days.
    2b - Infected diabetic group : 80 mice infected orally with $\pm$ 200,000 G. lamblia cysts and on 2nd day of infection received STZ as 40 mg / k g / mouse through ip administration on 5 consecutive days to induce diabetes

B. The parasite:

Cysts of G. lamblia ($\pm$ 200,000 / mouse) were obtained from freshly voided stool on 2 successive days of patient heavily infected with G. lamblia only according to Romia et al (1990). Cysts purification and preparation for mice infection was done (Cheesbrough, 2004).

Examination of infected groups for the presence of G. lamblia cysts or trophozoites was done from the 2nd day post-infection till day 21.

C. The drug:

Streptozotocin (STZ) used for induction of DM purchased from Sigma chemical (St Louis, MO, USA). It was dissolved in cold citrate buffer (10 Mm, pH 4.5), it should be prepared fresh for each use and injected intraperitoneally (ip) within 10 munities 40mg/kg/mouse [41]

Determination of blood glucose: test the blood glucose level from the tail vein using glucocheck blood glucose meter, sc, on days 3, 7, 10, 14, 17, 21 to check for STZ injection induced hyperglycemia [41]

Study the course of G. lamblia in both normoglycemic (non – diabetic) and hyperglycemic (diabetic) in C57BL/6 male mice was done through the following:

1. Parasitolgical study include determination of:
   - Infection rate - prepatent period
   - Patent period - mean number of exerted parasite/2 hours

   These were done according to [31]
II. Histopathological study:
Mice from enrolled groups were killed by ether inhalation on the 7th and 14th days post-infection: from each mouse, the 1st part of the duodena and liver specimens were cut, fixed in 10% neutral buffered formalin and processed for paraffin embedded sections, 5μm - thick were stained with haematoxylin and eosin (H x & E) according to Drury and Wallington.

Results:
Blood glucose level in C57BL/6 mice treated with ip injection of STZ in both non-infected (Drug control group (Ia) became gradually hyperglycemic reaching highest level on days 17th post STZ with mean blood glucose level of 328.9 ± 16.9 vs 115.9±9.8 mg/dl in normal control group (Ia).
Parasitological and histopathological results are given in tables (1-4) and figures (1-14).

I- Parasitological results:

Table 2: Infection rate in experimental giardiasis of non-diabetic and diabetic mice groups:

<table>
<thead>
<tr>
<th>Group of infected mice</th>
<th>No. of + ve infected mice</th>
<th>Rate of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic mice</td>
<td>60</td>
<td>86.6%</td>
</tr>
<tr>
<td>Diabetic mice</td>
<td>80</td>
<td>96.2%</td>
</tr>
</tbody>
</table>

II-Histopathological results:

Table 3: Death rate in different studied mice groups:

<table>
<thead>
<tr>
<th>Studied mice groups</th>
<th>Total no examined</th>
<th>% of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Drug control drug</td>
<td>20</td>
<td>20%</td>
</tr>
<tr>
<td>non-diabetic infected</td>
<td>60</td>
<td>4.28%</td>
</tr>
<tr>
<td>Diabetic infected</td>
<td>80</td>
<td>50%</td>
</tr>
</tbody>
</table>

Table 4: Mean ± SD of excreted cysts count (1 x 10³) / 2 hours in normal infected control group (non-diabetic) and diabetic mice groups

<table>
<thead>
<tr>
<th>Post – infection days (P. I)</th>
<th>Groups Non-count</th>
<th>Groups diabetic mean ± SD cyst count</th>
<th>Groups diabetic mean ± SD cyst count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>19.6 ± 6.8</td>
<td>21.1 ± 6.9*</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>4.2 ± 1.7</td>
<td>40.3 ± 7.2*</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>12.1 ± 1.4</td>
<td>70.1 ± 6.3*</td>
</tr>
<tr>
<td>4</td>
<td>4.2 ± 1.7</td>
<td>28.9 ± 3.61</td>
<td>90.2 ± 1.9*</td>
</tr>
<tr>
<td>5</td>
<td>12.1 ± 1.4</td>
<td>71.8 ± 3.91</td>
<td>9.8 ± 1.9*</td>
</tr>
<tr>
<td>7</td>
<td>28.9 ± 3.61</td>
<td>31.1 ± 5.31</td>
<td>59.7 ± 7.6*</td>
</tr>
<tr>
<td>9</td>
<td>41.5 ± 7.65</td>
<td>16.2 ± 1.5</td>
<td>41.4 ± 0.3*</td>
</tr>
<tr>
<td>11</td>
<td>71.8 ± 3.91</td>
<td>3.8 ± 0.42</td>
<td>29.2 ± 2.3*</td>
</tr>
<tr>
<td>14</td>
<td>31.1 ± 5.31</td>
<td>0</td>
<td>16.8 ± 1.9*</td>
</tr>
</tbody>
</table>

Probability * P < 0.05 Significant

II-Histopathological results:
Fig. 1: Transverse section in the duodenum of a normal control mouse showing the normal villus pattern (V), the simple columnaz epithelium lining (EC), goblet cell (g) and musculosa (M) (H & E. X 125)

Fig. 2: Transverse section in the duodenum of drug control mouse showing: broadened shorted and oedematous villi with cellular infiltration (1) and goblet cells hyperplasia. (H & E. X 125)

Fig. 3: Section of normal liver (H & E. X 100)
Fig. 4: Section in the liver of drug control mouse showing: an area of focal necrosis (1→) with many lymphocytes infiltration. The portal tract exhibits heavy lymphocytic infiltration (2→) and portal vein (P.V.) congestion (3→). Liver tissue is more or less normal. (H & E, X 100)

Fig. 5: Transverse section in the duodenum of a normal control mouse 7 days P.I showing: dedached, broaded and matted villi with heavy cellular infiltration. (H & E, X 100)

Fig. 6: A transverse section in the duodenum of normoglycemic infected mouse 14 days P.I showing: showing markedatrophic changes in the mucosa in the form of thickening and shortening of the villi with flattened and degenerated tips and even atrophy of some villi (1→). Inflammatory cellular infiltration and oedema in the C.T. core and lamina propria are also seen (2→). (H & E, X 100)
Fig. 7: Transverse section in the duodenum of normoglycemic infected mouse 14 days P.I showing: *G. lambia* trophozoites (1→) in the lamina propria. (H & E X 1000)

Fig. 8: Section in the liver of normoglycemic infected mouse 7 days (P.I) showing: mild hydropic degeneration and mild parenchymal infiltration. (H & E X 300)

Fig. 9: Section in the liver of normoglycemic infected mouse 14 days P.I showing: *G. lambia* trophozoites inbetween the liver cords (arrows). Liver parenchyma shows hydropic degeneration (1→) (H & E X 400)
Fig. 10: Transverse section in the duodenum of a diabetic infected mouse 7 days P.I showing: marked atrophic changes, some of the villi appeared short with blunt, degenerated tips (1→). In some areas, the villi ruptured or fused together (2→), with marked cellular infiltration of the C.T. core and lamina propria (3→). (H. & E. X 100)

Fig. 11: Transverse section in the duodenum of a diabetic infected mouse 14 days P.I showing: structurless necrosed and detached villi. *G. lamblia* trophozoites in the crypts (1→). In the musculosa (2, 3→) and empty vesicle (4→). (H. & E. X 100)

Fig. 12: Section in liver of diabetic infected mouse 7 days P.I showing. *G. lamblia* trophozoites inbetween the the epithelium lining of a bile duct (B.D.) and in the C.T. of porta hepatis (2→) and (2→) and (2→). (H. & E. X 1000)
Fig. 13: A section in liver of diabetic infected mouse 14 days P.I showing: sever hydropic (HD), and fatty degeneration (FD). severely parenchymal infiltration and isolated liver cell necrosis (LN). (H & E X 300)

Fig. 14: Section in liver of diabetic infected mouse 14 days P.I showing: numerous G. lamblia trophozoites infiltrating the liver parenchyma (arrows), hydropic degeneration (1→), focal necrosis (2→). (H & E X 400)

Discussion:

Giardia Lamblia is an intestinal flagellated protozoan parasite has been identified as the etiological agent in numerous water borne outbreaks of diarrheal disease. [2]

This parasite is endemic throughout developing countries [22]. An increased incidence of giardiasis in immunosuppressed patients recorded throughout different studies [26,35].

Diabetic patients considered as being of immune compromised group of patients. In the present study, the effect of experimentally induced diabetes in C57BL/6 mice, on the course of giardiasis comparing with non-diabetic ones was studied. Parasitological and histopathological studies were done to explore the diabetic effect.

The parasitological parameters revealed: in normal infected group the infection rate (86.6%), Buert [7] and Irikov and Kovalenko [19] reported 100%.

These variations in the infection rate may be due to the difference in the infecting dose, the virulence of G. Lamblia strains and the variability in host immune response.

In diabetic mice group the infection rate was higher than among the non-diabetic one (96.2% vs 86.6%), considering that diabetes is one of the immune suppressor agents so the present results run with those recorded by Romia et al. [32].

While the prepatent period was in non-diabetic mice was 2.8± 0.84 days it was 4.04±1.06 in diabetic mice, Nitipan et al [27] recorded an average of 4 days.

In diabetic group the mean of prepatent period was shorter (2 day) indicating the earlier attainment of infection in this group which in accordance with Rashid et al [29]. The maximum cyst excretion rate was in non-diabetic group was (71.8 ± 3.91)/2 hours at day (11) then decline to disappeared by day (21), this run with those recorded by Rashid et al [29] and Williamson et al [40]. While in diabetic group the maximum cyst
excretion was (90.2 ± 15.5) / 2 hours on the day (9) which in parallel was those recorded by Degerly et al [11]. The higher cyst excretion rate and its earlier attainment in the diabetic mice group in the present study may indicate the role of diabetes in aggravating the intensity of infection. As regard the duration of infection, excretion of Giardia cysts in non-diabetic group started from the 4th post infection and maintained up to day (20) (P.I) this was achieved by daily stool examination of both mice groups using formal ether sedimentation methods.

The duration of infection in diabetic mice group could not be recorded because the experiment was terminated by day 21 post – infection however it was expected that clearance of the parasite from such mice might need a long time to occur because cyst excretion was noticed till the last day of the experiment (21 days post – infection).

The death rate was found to be zero % among normal control (non – infected non received STZ) and infected control (non – diabetic) group was 4.28%. On the other hand, the death rate was found to be 20% and 50% among drug control mice group and diabetic mice group . this may be attributed to drug toxicity in addition to the possibility of immunosuppression or the side effects of the drug that induced diabetes, Liu et al [23] recorded more or less the same results on using dexamethasone.

The histopathological changes occurring in the first part of the duodenum of non-diabetic and diabetic mice after G. lamblia infection on day 7th and 14th as recorded in the present study revealed a wide range of changes ranging from mild to complete villus atrophy . One week post – infection apical necrosis with detached parts of the villi in the lumen , decrease in the villus to crypt rates with heavy cellular infiltration of the lamina propria occasionally G. lamblia trophozoites were seen in between the epithelial cells of the mucosa . these changes reached its maximum peak by 14 days post- infection in addition to increase in the number of goblet cells and the invading trophozoites

These mucosal changes are in agreement with those recorded by Williamson et al [40], El – Sayed et al., aloisio et al. Alissos et al. and William and Ramzy [39] in animals and Buret [7] and Erne et al. on human cases the author added by the day 21 post – infection , the abnormal pattern of the mucosal changes could be observed in a milder degree with a reduction in the inflammation cellular reaction . This could be explained by starting resolution of the infection.

The authors attributed these mucosal changes to the direct in jury by grasping action of the adhesive discs of Giardia trophozoites , toxic byproducts elaborated by the parasites [9]. Also these injures could be attributed to the secretion of the surface mannose – binding lection of G.lamblia or to cystein proteases [8,20]

Histopathological changes in diabetic infected mice were detected earlier and more evident than in the non – diabetic mice along all the period of the experiment this may be related to STZ administration toxicity which in turn led to aggravation of the intensity and invade action of Giardia trophozoites with its subsequent damage effects , also it could be attributed to the effect of diabetes on the host immunity. Vinayak et al. [38] and Shukla and Sardha [34] reported similar results on using immune suppressor agents through the course of experimental giardiasis while Farthing recorded less effects.

Concerning the effect of STZ drug on the liver in drug control group , numerous inflammations cellular infiltration with mild hydropic degeneration were recorded.

In non-diabetic infected mice group, liver histopathological changes resembles those of the normal control group with increased cellular inflammatory cell, mild hydropic, cloudy swelling and fatty degeneration by day 7th post – infection , these aggravated by day 14th with multiple invasion of many Giardia trophozoites this may indicate that the trophozoites invade the liver from duodenum or via the bile duct. by the day 21 Rashid et al. [29] recorded that a gradual improvement in liver tissues were observed . Awadalla et al. [6] added also granulomatous hepatitis and cholangitis . These could be attributed to the same factors affecting the intestinal host pathological.

In diabetic mice group , severe hepatitis with hydropic and fatty degeneration obtained by 7 days post – infection with increased number of invading which indicate earthier and more severe than in non – diabetic mice proceeded by 14 day post – infection to focal necrosis with the crease in the host pathological changes are parallel with the increase in the density of the parasite in this mice group.From the previous data it can be concluded that giardiasis led to host pathological changes in the small intestine and the liver as extra intestinal sites causing different degrees of enteritis and hepatitis , these change are more pronounced in diabetic mice group induced by streptozotocin (STZ) infection , so further experimental studies on STZ induce diabetes for exploration and giardiasis must be put into consideration as differential diagnosis in diabetic patients presenting with gastrointestinal trouble and should be treated promptly.

REFERENCES


