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Comparative Analysis Of Amyloid A In Brain And Pancreas Of Male Rats During Diabetes And Leishmania Infection

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ABSTRACT

BACKGROUND:*Serum amyloid A (SAA) is a key acute-phase protein associated with inflammation, metabolic dysfunction, and infection. While SAA levels have been extensively studied in serum, little is known about its tissuespecific expression in metabolic and parasitic disease contexts. The purpose of this research was to examine the levels of SAA in brain and pancreatic tissues of diabetic and Leishmania donovani infected rats using an ELISA-based approach.*

METHODS:*Adult male rats were divided into four groups: control, diabetic (induced via alloxan, 130 mg/kg), Leishmania donovani infected (15 × 10^6 promastigotes intraperitoneally), and co-infected diabetic rats. After four weeks, brain and pancreas tissues were harvested, homogenized, and analyzed for SAA concentrations using a commercial ELISA kit. Data were statistically compared between groups using one-way ANOVA.*

RESULTS:*Amyloid A levels varied across tissues and experimental conditions. In the brain, the highest accumulation was observed in co-infected rats, followed by diabetic and Leishmania-infected groups, relative to controls. Pancreatic amyloid levels increased in diabetic and Leishmania-infected rats but were lower in the co-infected group. Serum levels were slightly reduced in diseased groups compared to controls, with co-infected rats showing values close to baseline.*

CONCLUSION:*Diabetes and Leishmania infection, individually and in combination, influenced tissue-specific amyloid A accumulation, with the most notable elevation occurring in the brain during co-infection. These findings suggest differential inflammatory responses in peripheral and central tissues under metabolic and parasitic stress.*

KEYWORDS: Serum amyloid A, ELISA, Diabetes mellitus, Leishmania donovani, Brain, Pancreas, Inflammation

INTRODUCTION

Diabetes mellitus (DM) is a long-term metabolic disease marked by persistently high blood sugar levels brought on by deficiencies in either the action or production of insulin, or both. Because of its rising incidence and link to major side effects including cardiovascular disease and neuropathy, it is a major worldwide health problem, nephropathy, and retinopathy. The long-term effects of diabetes are largely attributed to chronic hyperglycemia, which promotes oxidative stress, inflammation, and progressive damage to various organs, including the pancreas and nervous system . About 537 million persons worldwide had diabetes in 2021, and by 2030, that figure is expected to increase to 643 million, according to the International Diabetes Federation (1). Leishmania donovani is an obligate intracellular protozoan parasite and the primary causative agent of visceral leishmaniasis (VL), also known as kala-azar. It is transmitted by the bite of infected female phlebotomine sandflies and primarily infects bone marrow, spleen, and liver macrophages. The most serious kind of leishmaniasis is visceral leishmaniasis, which may be lethal if treatment is not received. In the Indian subcontinent, the illness is endemic, East Africa, and parts of Latin America, where the majority of global VL cases occur (2,3). Serum Amyloid A (SAA) is a highly conserved acute-phase protein predominantly produced by the liver in response to inflammation. It plays a crucial role in modulating immune responses, including the recruitment of immune cells and the regulation of cytokine production. Elevated SAA levels have been linked to a number of pathological conditions, including chronic inflammatory diseases, metabolic disorders such as diabetes, and cardiovascular complications. Beyond its role as a biomarker of inflammation, emerging evidence suggests

that SAA actively contributes to disease progression by promoting tissue remodeling, lipid metabolism alterations, and insulin resistance. This makes it not only a marker but also a potential mediator in chronic disease states (4).

Diabetes causes chronic inflammation and elevates Serum Amyloid A (SAA), which promotes immune dysregulation. Similarly, Leishmania donovani infection triggers a strong inflammatory response, including increased SAA. The overlap in inflammatory pathways, especially via SAA and cytokine signaling, suggests that diabetes may worsen the immune response to

L. donovani, increasing the risk of severe infection, tissue damage, and brain involvement (5,6)

MATERIALS AND METHODS

Animals and Experimental Groups

We used Twenty adult male albino rats then were divided into four groups (n = 5 per group): control group healthy rats, diabetic group received a single intraperitoneal injection of alloxan monohydrate (130 mg/kg), L. donovani-infected group: injected intraperitoneal with 15×10^6 promastigotes, co-infected group: diabetic rats infected with L. donovani

Induction of Diabetes

Diabetes was produced using a single intraperitoneal injection of alloxan monohydrate at a dosage of 130 mg/kg body weight, dissolved in sterile normal saline. Rats had a 12-hour fasting period before injection. Seventy-two hours after injection, fasting blood glucose levels were assessed using a glucometer. Rats exhibiting glucose levels over 200 mg/dL (11.1 mmol/L) were

Leishmania donovani Infection

Leishmania donovani promastigotes were grown in NNN medium. Promastigotes (15×10^6 cells/rat) were delivered intraperitoneally to rats in both the Leishmania-infected and diabetic + Leishmaniainfected groups. Infections were let to develop for 4 weeks prior to euthanasia. **Tissue and Serum Collection**

After four weeks, rats were anesthetized and anatomized. Blood samples were collected, centrifuged, and sera stored at −20°C for ELISA. The brain and pancreas were harvested and placed in plane tube and kept at a deep freeze

Tissue Homogenization

Each tissue sample was weighed and placed in sterile microcentrifuge tubes containing 1 mL of ice-cold PBS supplemented with protease inhibitor cocktail . Homogenization was performed using a mechanical homogenizer until a uniform lysate was obtained. Homogenized samples were centrifuged and carefully transferred to new tubes and stored at −80°C until analysis

Amyloid A assay

Serum Amyloid A (SAA) levels were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory , China , Cat. No.E0679Ra) following the manufacturer's instructions. The assay is a sandwich ELISA designed to detect and quantify total SAA in biological samples. **Ethical Approval**

The University of Babylon's Faculty of Science Institutional Ethics Board's regional ethical committee gave its approval to this project (Z240801 on August 28, 2024).

RESULTS

Table 1: Mean ± standard deviation of Serum Amyloid A (SAA) levels measured in the brain of control, Leishmania-infected, diabetic, and co-infected (diabetic + Leishmania) rat groups using ELISA. Units are expressed in ng/mL.

Groups – Brain Tissue	**Mean Amyloid Level (ng/mL)**	**Standard Deviation (SD)**
Control group	3.879	2.655
Diabetes group	5.638	1.522
Leishmania infected group	3.367	0.555
Co-infected group (Leishmania + diabetes)	5.847	1.207

Table 2: Mean ± standard deviation of Serum Amyloid A (SAA) levels measured in the pancreas Tissues of control, Leishmania-infected, diabetic, and co-infected (diabetic + Leishmania) rat groups using ELISA. Units are expressed in ng/mL.

Groups – pancreas Tissue	**Mean Amyloid Level (ng/mL)**	Standard Deviation (SD)
Control group	1.366	0.964
Diabetes group	3.369	3.911
Leishmania infected group	3.384	0.816
Co-infected group (Leishmania + diabetes)	2.351	1.769

Table 3: Mean ± standard deviation of Serum Amyloid A (SAA) levels measured in the serum of control, Leishmania-infected, diabetic, and co-infected (diabetic + Leishmania) rat groups using ELISA. Units are expressed in ng/mL.

Groups – serum	**Mean Amyloid Level (ng/mL)**	**Standard Deviation (SD)**
Control group	4.426	0.872
Diabetes group	3.787	0.346
Leishmania infected group	3.918	0.268
Co-infected group (Leishmania + diabetes)	4.383	0.399

In this study, Amyloid A levels were measured in the serum, brain, and pancreas of control, diabetic, Leishmania-infected, and co-infected (diabetic + Leishmania) rats to evaluate inflammatory responses under each condition. The control group served as the healthy reference for all comparisons.

In the brain tissue, as shown in **table 1**, amyloid A levels increased in all experimental groups compared to the control. The most notable elevation was observed in co-infected rats, demonstrated the highest mean amyloid levels (5.847± 1.207 ng/mL) followed by the diabetic group (5.638 ± 1.52 ng/mL) , suggesting that both diabetes and combined pathology may enhance neuroinflammatory processes and amyloid accumulation. Leishmania-infected rats also showed elevated brain levels (4.367 ± 0.55 ng/mL), but to a lesser extent than diabetic and co-infected animals.

In the pancreas, as shown in **table 2** amyloid A accumulation was more prominent in the diabetic (3.369 ± 3.91 ng/mL) and Leishmania-infected groups (3.38 ± 0.816 ng/mL), both showing clear increases relative to the control. This elevation may reflect localized inflammatory or stress responses in pancreatic tissue. Interestingly, co-infected rats exhibited lower pancreatic amyloid levels than either single-condition group (2.35 ± 1.769 ng/mL), which could indicate a complex interplay where Leishmania infection potentially modulates the inflammatory impact of diabetes in this organ.

In the serum, as shown in **table 3**, amyloid A levels were highest in the control group, suggesting a stable baseline in healthy animals. Leishmania-infected (3.918 ± 0.268 ng/mL) and diabetic rats (3.787 ± 0.346 ng/mL) showed moderately reduced serum levels , indicating a possible alteration in the systemic acutephase response under infection and metabolic stress. Interestingly, co-infected rats exhibited serum amyloid levels (4.383 ± 0.399 ng/mL) that were nearly equal to those of controls, implying a potential compensatory or synergistic inflammatory response in the presence of both diabetes and Leishmania infection.

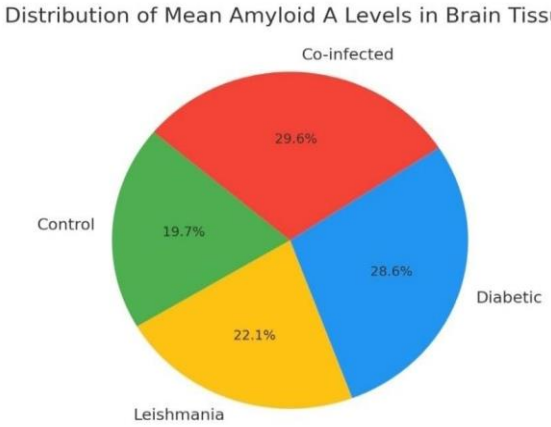

Figure 1. Distribution of mean amyloid A levels in brain tissue across experimental groups. The highest proportion of amyloid accumulation was observed in the co-infected group, followed by diabetic, Leishmania-infected, and control rats. This trend reflects enhanced neuroinflammatory activity, particularly under combined metabolic and parasitic stress.

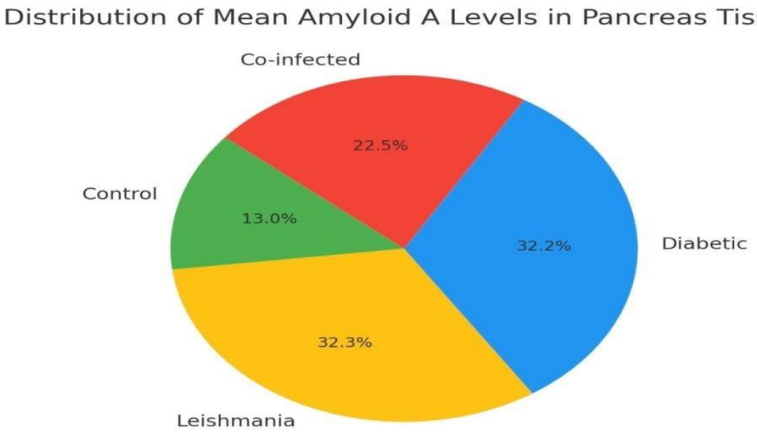

Figure 2. Distribution of mean amyloid A levels in pancreas tissue across experimental groups. Diabetic and Leishmania-infected rats contributed the largest proportions of amyloid accumulation, while the coinfected group showed a moderate level and the control group the lowest. These findings suggest localized pancreatic inflammatory responses under single pathological conditions.

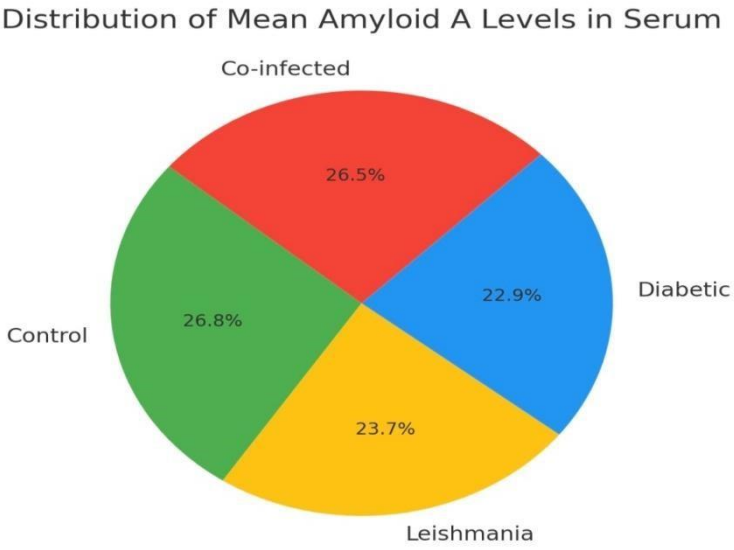

Figure 3. Distribution of mean amyloid A levels in serum across experimental groups. The control and co-infected groups showed the highest proportions of serum amyloid A, while Leishmania-infected and diabetic rats exhibited lower levels. This pattern suggests a partial restoration or enhancement of systemic inflammatory response under co-infection compared to single pathological conditions.

CONCLUSION

Diabetes and Leishmania infection, individually and in combination, influenced tissue-specific amyloid A accumulation, with the most notable elevation occurring in the brain during co-infection. These findings suggest differential inflammatory responses in peripheral and central tissues under metabolic and parasitic stress.

DISCUSSION

In this study, the control group, representing healthy and untreated rats, served as the baseline for evaluating the effects of Leishmania donovani infection, diabetes mellitus, and their combination on Serum Amyloid A (SAA) levels in brain, pancreas, and serum tissues. As observed, SAA concentrations in control rats were relatively low across all tissues. This finding is consistent with previous reports indicating that SAA is a liver-derived acute-phase protein typically expressed at minimal levels under noninflammatory conditions (7). Leishmania-infected rats showed an increase in brain SAA levels and a marked rise in pancreatic SAA compared to controls, reflecting heightened neuro- and pancreatoinflammation in response to infection (8,9), he increase in serum SAA, though modest, points to a systemic acute-phase reaction, as SAA is a sensitive biomarker for inflammation and infection (9,10)SAA levels increased across all examined tissues, with particularly elevated concentrations in the brain an observation consistent with studies demonstrating that Leishmania infantum can cross the blood–brain barrier and be detected in the cerebrospinal fluid, brain, and spinal cord of naturally infected dogs, even in the absence of neurological signs (11). Furthermore, experimental infections in mice have confirmed the presence of L. donovani in the brain and described a dual-phase inflammatory response characterized by mononuclear infiltrates and upregulation of pro-inflammatory mediators, supporting the notion of parasite-induced neuroinflammation (5). In the diabetic rat group,serum amyloid A (SAA) concentrations were markedly elevated across all examined tissues. The notably high pancreatic SAA levels may reflect localized inflammation or stress-induced β-cell dysfunction. This finding aligns with previous reports that chronic hyperglycemia promotes oxidative stress, cytokine production, and low-grade systemic inflammation, all of which are known inducers of acute-phase proteins like SAA ((7,12). Chronic hyperglycemia, a hallmark of diabetes, induces systemic low-grade inflammation, contributing to

increased hepatic synthesis of serum amyloid A (SAA) in response to interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α)(7,4) Co-infected (diabetic + Leishmania) rats displayed the highest SAA accumulation in the brain and serum, suggesting a synergistic activation of systemic and neuroinflammatory pathways. This observation is in line with evidence that coexisting metabolic and infectious diseases, such as diabetes and visceral leishmaniasis, can amplify inflammatory responses through overlapping mechanisms involving cytokine production, Toll-like receptor activation, and acutephase protein synthesis (8,13,14). Interestingly, pancreatic SAA in co-infected rats was lower than in diabetic-only rats. This may reflect immunomodulatory properties of Leishmania infection, which can downregulate host inflammatory responses to promote parasite survival (15), potentially dampening local SAA expression in the pancreas. Overall, these findings reinforce the role of SAA as both a systemic inflammatory marker and a possible mediator of tissue-specific inflammation under metabolic and infectious stress.

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