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ADVANCED STUDIES ON TRYPANOSOMA EVANSI

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ABSTRACT

Background: Cancer is an uncontrolled growth of abnormal cells found in animals and humans. This study aimed to discuss whether *Trypanosoma evansi* causes cancer or behaves like a cancerous cell and further assess the potential effects of sex on the measured parameters. **Materials:** Advanced tumour biomarkers and accompanied clinicopathological criteria were used to detect the progression of *T. evansi* in experimentally infected Albino Wistar rats. **Results:** Death appears highly correlated with the length of fulmination of parasitemia. The clinicopathological changes were associated with significant increases ($P<0.05$) in antitrypanosomal IgM, IgG and CEA, and highly significant differences ($P<.0001$) in the levels of malignant and tumour biomarkers IL-2, α-TNT, B2-M, AFP. In comparison, levels of CRP, CA-125, and CA-15.3 in females and CRP and serum PSA in males showed a non-significant increase ($P>0.05$). Similarly, CA-19.9 classes despite being elevated in infected males by the end of the experiment. Blood picture analysis showed leucopenia and anaemia rose significantly more in males than females. Biochemical analysis showed hypoglycemia, hypoproteinemia, hypolipidemia, and fluctuations in enzymes in both experimentally infected groups. Despite the examined tissues not being severely changeable by the infection in the short time of the experiment, *T. evansi* infection leads to further adverse pathogenesis for the host, causing cell and tissue destruction as generated by invasive cancer cells. Sex was a risk factor following the trypanosomal infection. **Conclusions:** The uncontrollable parasitaemia and the spread of *T. evansi* infection from the organ to others correspond with the style of malignancy, and *T. evansi* is possibly carcinogenic to rats.

KEYWORDS: Cancer, Clinicopathological, Rats, *Trypanosoma evansi*, Tumour markers.

INTRODUCTION

Cancer is a malignant disease characterized by uncontrolled growth of abnormal and transformed cells which can invade adjacent tissues in animals and humans.[1,2] Many bacterial and viral pathogens are retrieved in association with cancer, such as *Salmonella Typhi*, *Chlamydia pneumoniae*, *Mycobacterium tuberculosis*.[3-5] The involvement of intestinal microflora in the pathogenesis of colon cancer has been hypothesized, whereas many cancers arise from sites of infection, chronic irritation, and inflammation.[6] *Escherichia coli* has been shown to increase the risk of colon cancer.[7] Hepatitis C is not only characterized by inflammation of the liver resulting from infection with the *hepatitis C virus* (HCV), but also by the increased risk of liver cancer and developing other cancers.[8]

Helminth diseases like schistosomiasis, opisthorchiasis, and clonorchiasis are highly carcinogenic.[9-11] *Trypanosoma cruzi* has a dual role in the development of cancer, including both carcinogenic and anticancer properties.[12] Although malaria does not appear to be causative in carcinogenesis, it is strongly associated with the occurrence of endemic Burkitt's lymphoma in areas holoendemic for malaria.[13] Cancer occurs in parasitic protozoans such as *Trypanosoma brucei*, a blood parasite, and *Toxoplasma gondii*, an obligate intracellular pathogen,[2] and may cause severe diseases and death in animals and humans if not treated.[14]

The origins of cancer are not well understood because of the complexities of the disease.[15] It may induce by many environmental and physiological conditions.[16] The infection can start or promote carcinogenesis by one of three techniques[17] (a) chronic inflammation because of prolonged persistence of infectious agent in the host with the release of radicals having the potential to damage DNA as in *Helicobacter pylori* and *Chlamydia* that causes adenocarcinoma of the stomach and cervical carcinoma, respectively[3,18] (b) insertion of an active oncogene in the host genomes causes cellular carcinoma-like in herpes virus[19] (c) reduced immunosurveillance

because of immunosuppression as in human immunodeficiency virus (HIV).[20]

Remarkably, tumour cells used a variety of mechanisms like those used by some parasites to escape the host's immune system. However, both parasites and tumours have developed strategies like the production of specific inhibitory cytokines and altering the function of antigen-presenting cells (APCs) to escape the immune system by expanding T regulatory cells[21-23] In some types of cancer, the levels of tumour markers reflect the stage of the disease and or the host prognosis and may measure before treatment to help in planning the therapy.[24]

Trypanosoma evansi, the agent of trypanosomiasis-commonly named Surra disease may be acute in camels and horses with a high death in a few weeks, or chronic and fatal sometimes within months and extends for years in other hosts.[25] It has developed particular strategies such as the rapid multiplication and an escape mechanism used by the development of variant surface glycoproteins (antigenic variation) as immunosuppression before the humoral response is effective.[26] The current study aimed to evaluate the tumor's markers associated with the clinicopathological changes in experimentally infected rats, and discuss the potential effects of sex on the measured parameters.

MATERIALS AND METHODS

Trypanosome strain: *T. evansi* strain was gained from Parasitology Lab. in Desert Research Center, Cairo, Egypt. It is recovered from a naturally infected camel reared on the northwest coast of Egypt and identified in GenBank.[27] It was maintained in the laboratory by the continuous passage in white Swiss mice of mixed sexes (20–25 g) to give parasitemia of approximately 10^5 parasites/ ml for the experimental infection in rats.

Host-parasite model: Male and female Swiss Albino Wister rats weighing approximately 150 gm were purchased from the Faculty of Science, Ain Shams University in Cairo, Egypt, and kept in well-ventilated plastic cages. They were exposed to 12 hours of light and dark cycles fed with pellets and fresh vegetables and watered ad libitum throughout the experimental period. The rats were allowed 10-days of acclimatization before they were divided into groups of ten rats per cage.

The experimental infection: Sixty rats of both sexes were distributed into 6 cages (n=10): three groups for males (CM, TM & TM-DA) and three for females (CF, TF & TF-DA). Of which, two healthy control groups were neither infected nor treated (CM, CF), and two infected untreated groups (TM, TF) were IP administered 100 µl of the infected blood/rat. The others (TM-DA, TF-DA) were infected with 100 µl of the infected blood/ rat and IP was treated with two doses of 50 µl of diluted diminazene aceturate (3.5 mg/kg) /rat, one-day post-infection, and the day-15 after trypanosomes reappeared according to.[28] Every seven days, 5 ml of blood samples were got from each group by heart puncture in two parts, with and without anticoagulant, on days 7, 14, 21, 28, and 35 post-infection, and all sera samples were stored at 4° C until used. The numbers of parasites were determined microscopically at a magnification of ×400 by using a wet blood film method to detect any motile trypanosomes.[29] Different immunological and tumor biomarkers, serum biochemical, and haematological parameters comprise important indices for disease diagnosis and the prognosis recorded at the same time for comparison. Also, the macroscopic and microscopically histopathological changes were observed.

Immunological and Tumour biomedical analyses: The list of tumour markers used in the current study to assess stage, prognosis, look for recurrence and follow to infection and treatment with DA was: Tumour-derived markers produced by cancer cell includes (1) Oncofetal antigen such as Alpha-fetoprotein (AFP) for liver and germ cell tumours, and carcinoembryonic antigen (CEA) for colorectal cancer (2) Isoenzymes like Beta-2-microglobulin (B2M) for multiple myeloma and chronic lymphocytic leukaemia, and C-reactive protein (CRP) (3) New tumour markers associated with host-response include Interleukin-2 (IL-2) as immune cytochemical identification and Alfa tumour necrosis factor (TNF- α) produced by γ interferon activated macrophage (4) Mucin-glycoproteins such as CA19-9 for pancreatic, gallbladder, bile duct, and gastric cancers, and CA-15.3 for breast cancer (5) Creatine-kinase like CA-125 for ovarian cancer and Prostate-specific antigen (PSA) for prostate cancer (6) Immunoglobulins (IgM, IgG) for multiple myeloma and macroglobulinaemia. They measured using the ADVIA Centaur CP Immunoassay System (SIEMENS, Germany) and commercially available kits (BioSystems, Egypt), following the manufacturer's protocols.

Accompanied clinicopathological changes: For the hematology analysis: the micro-hematocrit technique (HCT), the red blood cells count (RBCs), the total white blood cells count (WBC), hematocrit, lymph, platelets (PLT), haemoglobin (Hb), besides mean corpuscular haemoglobin (MCH), packed cell volume (PCV), mean cell volume (MCV), mean corpuscular haemoglobin (MCH) mean corpuscular haemoglobin concentration (MCHC) evaluated. For biochemical analysis: Liver enzyme (alanine) aminotransferase (ALT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), total protein, albumin, globulin, cholesterol, calcium and glucose levels, triglyceride, serum creatinine and urea estimated according to the manufacturer's instructions and commercial kits (BioSystems, Egypt). All biochemical analyses automatically were done using (BA200, A25 BioSystems). The pathology of *T. evansi* infection in experimentally infected rats by macroscopical and microscopical examination. The fresh tissue samples have been collected in 10% neutral buffered formal saline to prepare paraffin tissue sections

at a 4-6 µ thickness that were stained with hematoxylin and eosin.[30]

Statistical analysis: Data was analyzed using the General Linear Model (GLM) procedure. [31] The significance of differences between groups was determined by analysis of variance (ANOVA). Statistical analysis was determined by the Least Significant Difference (LSD) test ($p \leq 0.05$) and a highly significant when $p<0.001$.

RESULTS

Parasitemia course: The course of *T. evansi* in rats characterized by relapsing crises usually ended in death within 17-35 days after infection with blood contains 5 x 10^5 motile trypanosome/ ml. The acute phase but not the chronic phase of the disease is progressive as a period in which rats present higher parasitemia in males than females. Death appears highly correlated with the length of fulmination of parasitemia in males than females, despite the dose of infection and the control diet being, the same. No rats from health groups have died along the course of the experiment (Table 1)**.**

Table 1: Parasitaemia progress in the infected and treated groups of rats compared to healthy control groups

Groups	Infective dose	Treat	1st week	2nd week	Day 15	3rd week	4th week	5th week
	Day 0	Day 1	Day 7	Day 14	Dose	Day 21	Day 28	Day 35
CM	100 µl saline	No treat.	0	0	No treat.	0	0	0
CF	100 µl saline	No treat.	0	0	No treat.	0	0	0
TM	100 µl IB/Rat	No treat.	2.5×10^4	5.3×10^4	No treat.	1.17×10^6	2.12×10^7	2.71×10^8
TF	100 µl IB/Rat	No treat.	1.7×10^4	2.8×10^4	No treat.	93×10^6	1.12×10^7	1.67×10^8
TM-DA	100 µl IB/Rat	50 µl DA	Free	5×10^3	50 µl DA	Free	Free	12×10^4
TF-DA	100 µl IB/Rat	50 µl DA	Free	3×10^3	50 µl DA	Free	Free	7×10^4

IB = Infected blood contains approximately 5×10^5 Trypanosoma/ml.

Immunological and tumour markers studies: Results in Table 2 revealed that the antitrypanosomal IgM and IgG were significantly higher in infected and treated groups than in healthy ones ($P<0.05$), and IgG remained significant despite relatively low levels by the end of the experiment. The infection caused an increase in the pro-inflammatory cytokines associated with regulating immune responses against the parasite. However, IL-2 combination with TNF-α showed a highly significant increase ($P<0.0001$) observed between infected and treated groups that increased within the normal range above healthy ones**.** Oncofetal antigen levels by AFP showed a progressive and fluctuating decrease in infected and treated groups at the start of the infection but progressively in the TF group with a highly significant difference ($P<0.0001$). Also, CEA levels showed fluctuated decreases and increases with significant differences ($P<0.05$). Isoenzymes responsible for multiple myeloma and chronic lymphocytic leukaemia showed a highly significant increase in Beta2-M levels ($P<0.0001$) maintained during the infection in infected and treated groups rather than healthy ones.

Following experimental infection, plasma concentrations of the CRP increased then decreased with no significant differences ($P>0.05$) in infected females than males whereas, diminazene aceturate caused a slight increase in CRP levels in the treated groups. Results detected also increased with no significant differences in CA-125 and CA-15.3 levels (the ovarian and breast tumour markers, respectively) in TF rather than CF. Curative treatment with DA caused a gradual but slow decrease in CA-15.3 and CA-125 in weeks 3, and 4 with no significant changes ($P>0.05$). Despite CA-19.9 concentrations remaining within natural levels of the TM group at the beginning, it increased progressively, with no significant difference by the end of the experiment. In contrast, the TF group rapidly increased, and then sharply decreased, whereas the difference in treated groups was at a constant value within normal ranges. Also, serum PSA was slightly elevated to TM rather than that treated and CM groups with no significant difference ($P>0.05$).

Table 2: Immunological and tumor biomedical values in healthy and experimentally infected Wistar rats with *Trypanosoma evansi*, and after treatment.

Group	IgM	IgG	CRP	TNT-α	IL-2	B2-M	AFP	CEA	CA-19.9	CA-125	CA-15.3	PSA
	ng/ml	ng/ml	ng/ml	pg/ml	pg/ml	ng/ml	ng/ml	ng/ml	U/ml	U/ml	ng/ml	pg/ml
CM	209^{a}	685^{c}	3.75^{a}	$64.0\ 25^{c}$	$57.\ 2^{cd}$	$0.0\ 75^{d}$	$3.2\ 03^{a}$	$0.0\ 48^{b}$	$0.04\ 73^{a}$	-	-	$0.0\ 643^{ab}$
CF	$124.\ 5^{b}$	$630.\ 5^{c}$	$3.9\ 25^{a}$	$29.4\ 25^{c}$	$22.\ 28^{d}$	$0.07\ 93^{bcd}$	$3.3\ 98^{a}$	$0.3\ 13^{a}$	0.11^{a}	$0.11\ 33^{a}$	$0.06\ 18^{ab}$	-
TM	229.	104	4.8^{a}	194.	164.	0.07	3.1	0.0	7.4	-	-	0.0

	75[a]	9.75[a]		875[a]	43[a]	75[cd]	63[a]	633[b]	34[a]			72[a]
TF	189.75[ab]	911.5[ab]	5.375[a]	140.35[b]	130.03[ab]	0.0875[a]	0.29[d]	0.0398[b]	3.241[a]	0.05 75[a]	0.233[a]	-
TM-DA	172.25[ab]	897.75[ab]	4.725[a]	121.35[b]	102.43[b]	0.084[ab]	2.468[b]	0.0525[b]	0.0265[a]	-	-	0.043[b]
TF-DA	161.75[ab]	737.25[bc]	4.225[a]	110.75[b]	93.75b[c]	0.081[bc]	2.04[c]	0.0495[b]	0.0245[a]	0.019[a]	0.0103[b]	-
LSE	21.66	62.02	0.923	14.95	12.83	0.016	0.109	0.0395	3.375	0.034476	0.055989	0.00699
F test	0.04	0.0069	0.7807	<.0001	<.0001	<.0001	<.0001	0.0052	0.6328	0.6071	0.2279	0.1671

Values are expressed as mean ± LSD; Significant difference at $P < 0.05$; highly significant difference at $P < 0.001$.

Clinicopathological progression associated with *T. evansi* infection: Blood picture analysis in Table 3 revealed leucopenia observed from second week post-infection. Mean absolute values for WBC and lymph in the TM group differed significantly from other groups (P<0.05). In contrast, the infected groups showed fluctuating increases and decreases in RBC count and HGB concentration, leading to anaemia, which persisted from the second week after infection until the end of the study. MCHC values in infected animals remained within normal ranges as in treated groups, despite a slight gradual increase in TF compared to TM by weeks. MCV means became above normal levels from week two till the end, at the same period, the lowest RBC values were registered in infected animals, denoting macrocytic normochromic anaemia. MCH means increased irregularly above normal levels throughout the experimental period and no significant differences were detected between groups. Also, statistical analysis showed no significant difference (P>0.05) in PLT levels between the six groups.

Table 3: Haematology out values based on comparison between healthy and experimentally infected Wistar rats with *Trypanosoma evansi*, and after treatment.

Group	WBC	Lymph	RBC	HGB	MCV	MCH	MCHC	HCT	PLT
	10^3/ µl	%	10^6/ µl	g/dL	fl	pg	g/dl	%	10^3/µl
CM	6.725[b]	5.85[b]	5.34[a]	7.1b[c]	39.93[d]	13.35[c]	33.53[ab]	21.55[b]	604.3[a]
CF	10.6[b]	9.15[b]	4.88[a]	8.65[b]	53.95[b]	17.55[b]	32.33[bc]	26.95[a]	402[a]
TM	20.08[a]	17.45[a]	2.89[b]	5.75[c]	56.78[c]	19.93[ab]	34.75[ab]	16.13[c]	468.3[a]
TF	8.95[b]	6.95[b]	2.52[b]	5.05[c]	63.4[c]	23.25[a]	35.78[a]	15.08[c]	356.5[a]
TM-DA	6.85[b]	5.78[b]	5.43[a]	11.13[a]	46.78[a]	18.65[b]	29.9[c]	27.33[a]	525.3[a]
TF-DA	6.63[b]	5.11[b]	4.97[a]	10.08[a]	45.89[a]	17.69[b]	28.7[c]	26.42[a]	385.7[a]
LSE	2.519	2.175	0.335	0.736	2.38	1.15	0.906	1.6493	85.63
F test	0.0465	0.0387	0.0002	0.0027	0.0008	0.0026	0.0157	0.0007	0.2506

Biochemical analyses in Table 4 showed hypoglycemia, hypolipidemia, and hypoproteinemia, followed the experimental infection with *T. evansi*. Glucose levels decreased with highly significant differences (P<0.001) in both infected and treated groups. Whereas calcium levels slightly decreased in infected animals than in treated and healthy ones, it maintained at a near-constant value during the infection. Triglycerides, creatinine, and urea concentrations decreased with significant differences (P<0.05). Cholesterol levels decreased with a highly significant difference (P<0.0001) observed within and between six groups. For enzymes: Whereas mean values of AST activity increased, LDH levels decreased with highly significant differences (P<0.0001). ALT and ALP levels decreased with significant differences (P<0.05) in infected and treated animals, rather than in healthy groups during the experimental period. Total protein and albumin concentrations decreased with highly significant differences (P<0.0001) in infected groups than in others, and the A/G ratio also reduced with a significant difference (P<0.05). By the end of the experiment, despite total bilirubin concentrations remaining within normal levels in TF and treated groups, it was above normal levels in the TM group from second week with a significant difference (P<0.05).

Table 4: Biochemical values based on comparison between healthy and infected rats, and after treatment

Groups	Chol.	Trigly.	Urea	Creat	AST	ALT	ALP	LDH	T. Bili.	T. Pro	Alb.	Glob	A/G	Calc.	Glu.
	mg/dL	mg/dL	mg/dL	mg/dL	Pg/ml	ng/ml	u/l	u/l	mg/dL	g/dL	g/dL	g/dL	Ratio	mg/dL	mg/dL
CM	114.8[a]	176.0[a]	102.8[a]	1.8[a]	49.0[bc]	47.0[b]	169.5[a]	475.0[a]	0.58[b]	6.53[a]	3.50[ab]	3.03[a]	1.18[bc]	8.53[ab]	146.3[a]
CF	49.	123.	90.	0.8[bc]	37.	51.	127.	158.	0.	5.	3.	2.	1.	5.	115.

	8b^{c}	6^{ab}	08^{ab}		3^{c}	0^{b}	3^{ab}	8^{c}	50^{b}	80^{b}	25^{b}	53^{ab}	39^{b}	85^{c}	5^{b}
TM	64. 1^{b}	76. 8^{bc}	97. 8^{ab}	1.2^{b}	22 3.5^{a}	90. 3^{a}	103. 3^{b}	48 0.5^{a}	1. 83^{a}	3. 93^{c}	1.8 3^{d}	2. 10^{bc}	0. 93^{c}	6. 78^{bc}	37. 0^{d}
TF	38. 6b^{c}	12. 3^{c}	53. 5^{c}	0.6^{bc}	10 5.0^{b}	64. 3^{b}	80. 3^{bc}	62. 0^{d}	0. 52^{b}	3. 95^{c}	2.4 3^{c}	1. 53^{c}	1. 60^{b}	6. 50^{c}	33. 3^{d}
TM- DA	36. 8^{c}	25. 3^{c}	75. 3^{bc}	0.6^{c}	18 2.0^{a}	97. 8^{a}	45. 0^{c}	36 5.5^{b}	0. 27^{b}	5. 43^{b}	3.6 3^{a}	1. 80^{c}	2. 05^{a}	9. 58^{a}	66. 8^{c}
TF-DA	35. 1^{c}	23. 9^{c}	73. 7^{bc}	0.5 7^{c}	17 7.8^{a}	92. 9^{a}	42. 8^{c}	35 7.3^{b}	0. 26^{b}	4. 47^{b}	3.5 2^{a}	1.6 9^{c}	2.0 89^{a}	9. 23^{a}	68. 3^{c}
LSE	7.9 08	20. 567	7.4 99	0.1 67	18. 29	6.8 54	14. 873	20. 55	0.1 70	0.2 28	0.1 11	0.2 19	0.1 40	0.6 06	1.9 53
F test	<.00 01	0.0 02	0.0 058	0.0 019	0.0 001	0.0 014	0.0 034	<.0 001	0.0 011	<.00 01	<.00 01	0.0 07	0.0 035	0.0 184	<.00 01

Values are expressed as mean ± LSD; Significant difference at $P < 0.05$; highly significant difference at $P < 0.001$.

Macroscopically, spleen and liver became enlarged and dark, followed by petechial haemorrhages in the liver and lung in the terminal stages consistent with trypanosome infection, starting from the second week. Gross examination of the other tissues by the end of the experiment revealed testicular enlargement and anaemic signs were more visible at peak parasitemia in the TM group than others were. These changes started on the 7th day and increased gradually until reaching the maximum severity by the end of the experiment. The presence of trypanosomes in the blood confirmed all changes in five weeks from the infection.

Microscopically, the histopathological changes represented in Fig. 1, 2 showed multiple focal areas of haemorrhage in the brain of females (TF) and perineural and perivascular oedema in males (TM). It also showed dilatation in the myocardial blood vessel with a thickened muscular wall (TF) while a thickness of pericardial layer together with dilated and congested blood vessels (TM). Lungs showed bronchopneumonia, the bronchial hyperplasia, peribronchial and interstitial leucocytic cells infiltration, and dilated blood vessels in female and male groups of rats. Livers in infected groups in both sexes showed portal tracked changes, note dilated and congested hepatoportal blood vessels and mononuclear cell infiltration. Also, pancreas shows interlobular hyperplastic pancreatic islets (F) and interlobular dilated blood vessels (TM). Spleen revealed healthy lymphoid follicles (TF), whereas the kidneys showed congestion in the interstitial blood vessels with a thick dilated wall in infected rats.

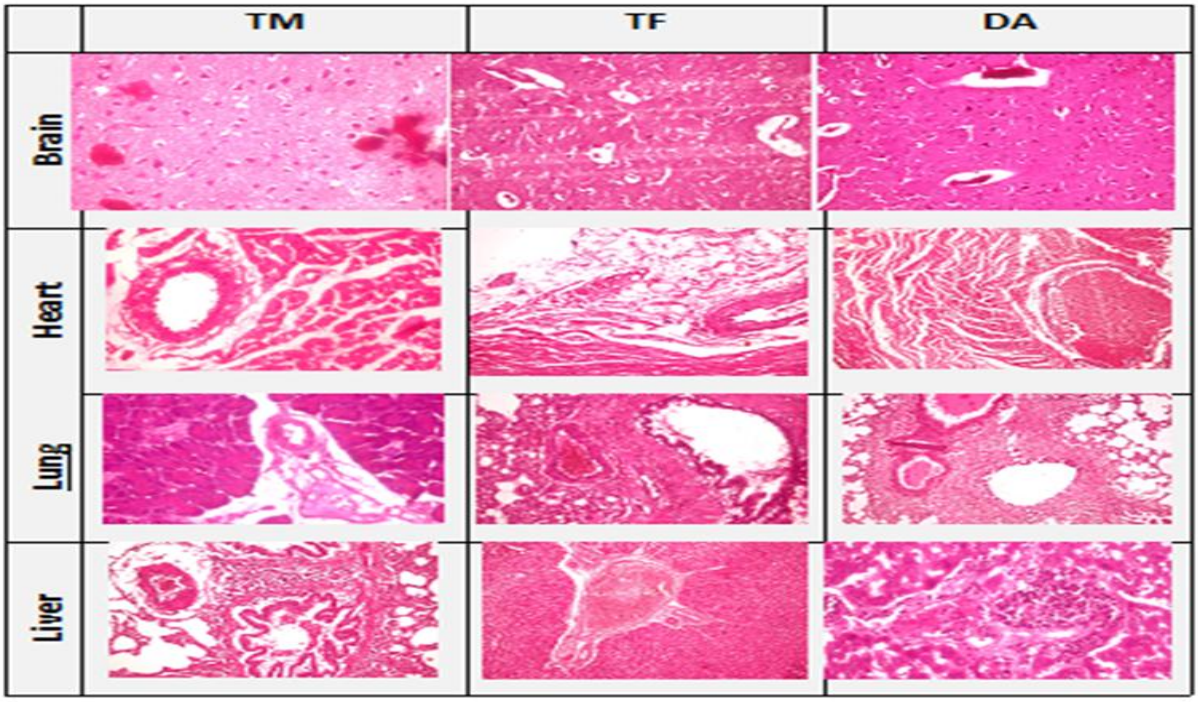

Figure 1: Microphotograph for brain, heart, lung, and liver in infected groups (TF, TM), and treated (DA), showing histopathological findings associated with *T. evansi* infection.

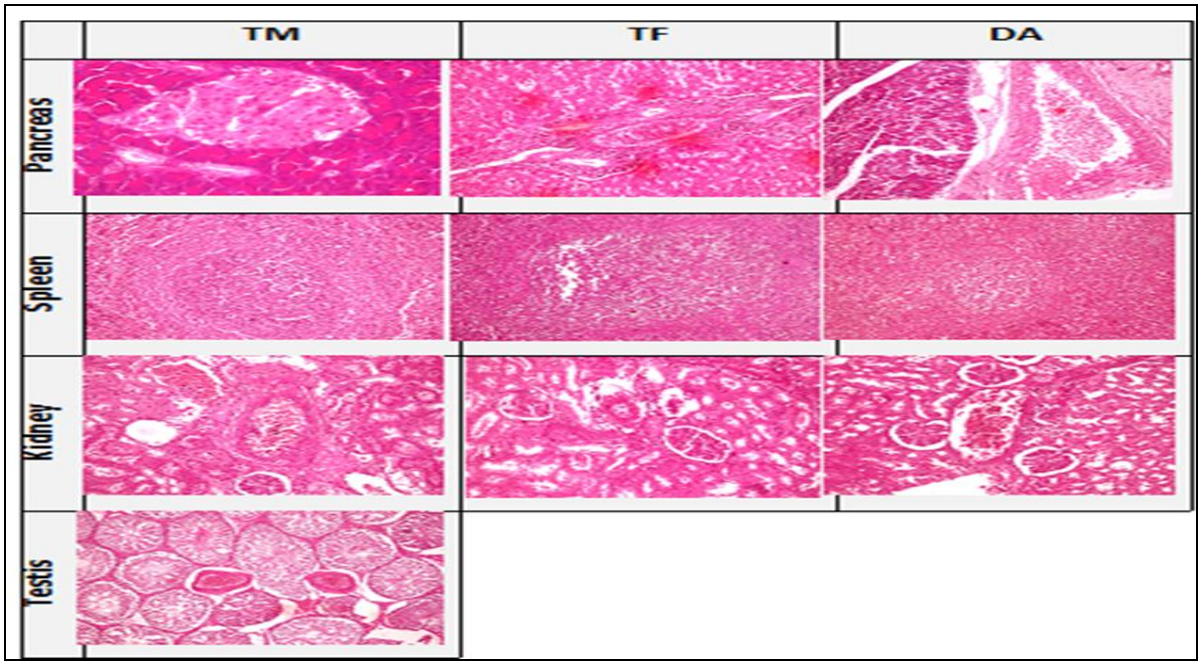

Figure 2: Microphotograph for pancreas, spleen, kidney, and testis in infected male (TM), female (TF), and treated rats (DA), showing histopathological findings associated with *Trypanosoma evansi.*

DISCUSSION

Trypanosoma evansi infecting mammals is a subspecies evolved from *T. brucei*, which cannot further differentiate or develop into the stages in the insect vector. Subsequently, *T. evansi* has only the uncontrolled proliferating stage of the mammalian hosts, suggesting a parallel with the status of acute myeloid leukaemia in humans.[32] The evolution has enabled *T. evansi* to colonize only suitably susceptible hosts.[33] However, host genes can express and take part in several pathological functions and can interfere with the immune system and the inflammation mechanisms.[34] That may explain the higher pathogenicity observed in *T. evansi*[26] than *T. brucei*.[35]

Notably, animal models have provided much insight into the *in vivo* defence reactions against diseases, despite the immune responses observed against parasitic infections differ considerably depending on the species of the animal host used and the variation of the host's response to the same natural parasitic infections.[36] Trypanosomes survive and multiply in the blood and face both innate and adaptive immune defences.[37] Little known about innate immune responses and tumour markers related to *T. evansi* infection. Thus, the use of Wister rats for experimental infection has contributed to our understanding of parasite-host relationships in agreement with.[38]

To date, no tumour marker identified is sufficiently sensitive or specific to be used to screen for cancer despite tumour markers being useful in determining whether the tumour is responding to treatment or assessing whether it has recurred. The current study measured the levels of tumour markers in the serum of rats experimentally infected with *T. evansi* and correlated these levels with haematological and biochemical parameters. It is based on determining serum levels in early detection, prognosis, and prediction of therapeutic response, monitoring disease, and recurrence. These markers include a variety of substances present in, or produced by, a tumour itself or generated by the host in response to a tumour or determine a tumour based on measurements in the blood.[16,21] Although an elevated level of a tumour marker may suggest cancer, no change or an increase in measurements of tumour markers may show that the cancer is not responding. That is not enough to diagnose cancer; therefore, it has to combine with clinic-pathological changes.

In the present study, *T. evansi* developed an immunosuppression strategy to control parasitaemia correlated with high levels of CRP concomitantly with IgM rather than IgG targeting VSG, in agreement with.[39] It seems to be species-dependent; however, it reduced the efficiency of host immune responses in contrast to what happens in *T. brucei* and *T. congolense* infections.[40,41] The increase in the production of pro-inflammatory mediators such as IL-2, TNF-α was also observed. That was showed in the early stages of *T. evansi* infection and during intestinal infection by helminth parasites.[42] Because *T. evansi* kills activated macrophages producing high levels of inflammatory compounds such as TNF-*α*, it was not crucial for parasitaemia control and survival of the infected animals along with the experiment in agreement with.[40,43] No recovery was observed even after treatment with DA.[44] Whereas, *T. evansi* rapidly develop high levels of resistance in agreement with.[45]

While AFP is elevated in hepatocellular carcinoma of the liver, it is also increased with PSA in certain

testicular tumour as observed in infected males of rats in the current study. CA15-3 levels can be higher than normal with cancerous and non-cancerous conditions, but they are most often increased in breast cancer that has spread to the bones, liver, and other parts of the body and rarely with local or primary carcinoma.[46] The gradually elevated levels of CA 15-3 in the TF group compared to the healthy one in our present data showed it could be a reliable prognostic marker as they were directly related to advanced stages and cancer is growing. Whereas, the lower levels of the TF-DA group meant that treatment was working. In contrast, CA 125 tumour marker used to follow the epithelial ovarian and pancreatic cancers[47] was determined without a change in all-female groups. The quick degradation of the effectiveness of the host immune system induced by *T. evansi* in the current study could explain why no vaccine is available until now, despite the identification of several non-variant surface-exposed trypanosome immunogens in agreement with.[48]

In haematological malignancies, such as leukaemia, lymphoma, and multiple myeloma, serum β2M level is found to be elevated, despite preserved renal function. This is independently associated with an unfavourable prognosis for most haematological malignancies.[49,50] The elevation of CA 19-9 in males than females could be following pancreatic cancer, but with colorectal cancer, it is less sensitive than the CEA test. However, it can also be raised in other cancers such as stomach and bile duct cancer and some non-cancerous conditions such as pancreatitis. The main effector mechanism involved in the protective immune response of rats infected with *T. evansi* is the activation of macrophages by IFN-γ and TNF-α to kill the trypomastigotes which were positively correlated with IgG, IgM which was related to parasitic load and clinical progression.

In the current study, the changes in haematological and serum biochemical parameters along with the infection were associated with significant increases in the serum levels of IL-2, and TNF-α predominated in the development of symptomatic infections during the experiment. These pro-inflammatory cytokines can react to different body cells, causing oxidative tissue damage and destroying RBCs, leading to anaemic tissue damage.[51] It was accompanied by enlargement in lymph nodes and spleen, which was a consistent feature reported previously by different hosts infected with *T. evansi*.[25,52] *T. evansi* infection induced significant decreases in HGB, PCV, and RBCs, whereas WBCs and lymphocytes increased, as well as total bilirubin, LDH, AST, and ALT showing a direct link between immune and metabolic disorders associated with infection and disease development as reported in infected water buffaloes.[53] The increase in serum bilirubin levels in infected rats coincides with the lowest RBC values observed; suggesting the occurrence of extravascular destruction of red cells and the hemolytic crisis coincides with.[54] The decrease in the A/G ratio has been frequently reported in *T. evansi* infection[55] because of the prominent hypoalbuminemia resulting from the anorexia and hypoxic hepatic injury related to the *T. evansi* infection. The present leucopenia could have been by immune suppression, which usually co-exists with trypanosomosis**.** It was characterized by lymphocytosis because of lymphoid tissue hyperplasia in the acute phase of the disease in agreement with findings.[56]

In the present study, hypolipidemia results from a decrease in cholesterol and triglycerides levels that may be attributed to the high energy levels required for *T. evansi* in agreement with,[28,57] and in contrast to the experimental infection with *T. b. gambiense* by.[58] Hypoglycemia may be because of excessive utilization of blood glucose by the parasites accompanied by an increased metabolic rate as reported by.[59] It could also be because of the deficiency of adenosine triphosphate (ATP) produced by glycolysis, the tumour cells increase the intake of glucose to boost energy-providing glycolysis. This is in line with[60] who reported that highly consumed glucose levels support tumour progression through a variety of mechanisms, including promoting tumour cell proliferation, invasion, and migration and inducing apoptotic resistance and chemo-resistance. Whilst hypoproteinemia is scheduled, a significant decrease in protein levels, as a concern of albumin levels decrease suggested hepatic damage. The parallel increases in globulin concentrations may result from a decrease in antibody production and the elevated AST levels compared to the rather modest increase in ALT enzyme show that little of the former is derived from the liver, as previously reported.[54] It may also be because of tissue breakdown and inflammation in the host, particularly of the liver, heart, muscle, and kidney, as reported before.[61]

The histopathological alterations were previously reported in *T. evansi* naturally infected camels and experimentally infected rats [28,62] The aetiology of tissue lesions in animals infected with *T. evansi* is unknown. In the current study, despite examined tissues not severely changeable by the beginning of infection, the histopathological findings by the end of the experiment showed dilation, haemorrhage, congestion, hyperplasia, inflammatory cell infiltrations, and loss of structure of organs, besides the mortality of rats in the male and female groups reached 100% in agreement with a previous study by.[28] This may be because of the failure of uncontrolled *T. evansi* growth that could lead to further adverse pathogenesis for the host caused by an increased parasite load.

In addition, toxins liberated by the trypanosomes, hypoproteinemia, hypoglycemia, hypolipidemia, impairment of the blood supply to the affected organs, and the deposition of the immune complexes and the inflammatory cells impair the functions and structures of the organs,[63] caused cell and tissue destruction, a similar outcome to that generated by invasive cancer cells.

Tumours become life-threatening if they spread throughout the body, and metastasis of cancerous cells can compare with the transmission of pathogenic trypanosomes from the original site of ingestion to one or more sites elsewhere in the body by the blood vessels or lymphatics.[2] Thus, the spread to other sites of the body as a defence strategy from the parasite can protect the parasite but in fact, associated with tumour formation.

CONCLUSION

The current study measured the levels of tumour markers in the serum of experimentally infected rats with *T. evansi* and correlated these levels with haematological and pathophysiological abnormalities. The gathered clinical results considered *T. evansi* as a typical cancer cell wherever the subsequent uncontrolled growth and the spread of infection ended by death coincide with the description of cancer. The correlation between all studied parameters in the present study pointed to *T. evansi* may be carcinogenic for rats.

Ethical approval

The experiment was conducted in compliance with internationally accepted principles of the European (EU) Directive 2010/63/EU for animal experiments and the National Institutes of Health guide (NIH Publications No. 8023 revised 1978). All the methods for the use and care of laboratory animals were adopted in agreement with the ethics guidelines of the Ministry of Agriculture and Land reclamation, and Desert Research Center in Egypt.

Author contributions

The author has the idea, planned, designed the work, analyzed the results, and has contributed to writing and approved the manuscript.

Conflict of interest

The author declares that there is no conflict of interest.

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