

Effect of *Toxoplasma Gondii* Infection on Haematological and Liver Function Parameters among Abortive Women in El-Beida City

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Abstract

This work was carried out to investigate the changes in haematological and some of liver function parameters among abortive females after diagnosing toxoplasmosis-related symptoms in El-Beida City, Libya. Eighty seven abortive women during the period from October 2018 to March 2019 were chosen as a study group. These women were split into two groups when the antibodies of *Toxoplasma* was present. (IgG and/or IgM) (44 cases who positive with toxoplasmosis). The largest incidence was observed in age group 21-25 years followed by age groups 31-35 and 26-30. While the smallest incidence was observed in the age group, more than 46 years followed with age groups of less than 20 years. At age group 41-45, percentage of positive toxoplasmosis was found higher than negative toxoplasmosis. All positive cases, 50.6% of them was found to have IgG anti-toxoplasma antibodies sero-positive. 13.6% of total cases was mixed seropositive for IgG and IgM. These value of antibodies IgG/IgM were found statistically difference between positive and negative toxoplasmosis. Only, the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and white blood cell count were decreased significantly in positive toxoplasmosis. Mean values of RBC count, HGB concentrations, percent of hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) were not statistically different in positive compared to negative cases. Lymphocytes (Lymph %) and mid cells total count (MID %) were increased and granocytes (Gran %) decreased slightly in positive toxoplasmosis without significantly deference. Meanwhile the mean platelets number (PLT) the volume occupied by platelets in the blood (PCT %), the mean platelet volume (MPV %) and platelets distribution width (PDW %) were changed without significant deference. In terms of blood group, the smallest incidence was discovered with blood group O and the largest incidence with blood group AB. The highest prevalence among individuals with blood group who have Rh-positive at same blood group. The mean levels of AST, ALT and ALP in the serum of the positive toxoplasmosis was decreased without significantly deference. These results were might give a better understanding about pathogenesis of toxoplasmosis.

Keywords: Clinical, laboratory characteristics, toxoplasmosis, El-Beida City and Libya.

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INTRODUCTION

T. gondii is considered the second leading cause of foodborne-related deaths [1]. It is a zoonotic disease that causes abort and fetal destruction due to placental transmission [2]. Infection with *T. gondii* before pregnancy confers little or no risk to the fetus except in women who become infected up to three months before conception [3]. In the neonate, manifestations of congenital toxoplasmosis might include hydrocephalus, microcephaly, intracranial calcifications, retinochoroiditis, strabismus, blindness, epilepsy, psychomotor and mental retardation, petechiae due to thrombocytopenia, and anemia [4, 5]. The infection is carried to the infant through the mother's placenta, and can cause infections of the eyes

or central nervous system. It has a worldwide distribution and is one of the most prevalent infectious agents in human, serologic studies which infests nearly one – third of the world human population [6]. *T. gondii* can be located in every vital organ, and especially in acute stage it can be seen in blood, cerebrospinal fluid, semen, tears, saliva and urine. Many research works have been done on different clinical forms of the disease, an association between toxoplasmosis with hepatomegally and some abnormal liver function tests was found [7]. *T. gondii* infection induces several immunological changes in the body which are characterized by the production of the immunoglobulins IgM, IgG and IgA [8, 9]. Identification of positive titer of immunoglobulin G (IgG) and immunoglobulin M

(IgM) during pregnancy in women with previous negative titers of anti-toxoplasma IgG antibodies suggests a proliferative disease condition dangerous to the fetus and is more likely to cause a miscarriage or serious birth defects [10]. Most of the previous studies in Libya have concentrated on the prevalence of *T. gondii* infection among pregnant women. These studies appear to be in accordance with the high prevalence rates of toxoplasmosis which reported by most of the previous studies e.g. in Poland [11] in France [12], and in Turkey [13]. This prevalence was high in Arab countries for example in Saudi Arabia [14, 15], in Kuwait [16], in Tunisia [17, 18]. In Libya, many studies done to detect the prevalence of Toxoplasma antibodies and show high prevalence rates in these studies [19-24]. However, the main reasons for this pattern of high prevalence rate of toxoplasmosis in Libya is yet unclear as no adequate studies regarding transmission modes were available. Due to the limited number of studies in El-Beida-Libya about toxoplasmosis in general and physiological effect in particular. This study was designed to perform effect of *T. gondii* infection on hematological and some liver function parameters among women who had abortion in El-Beida City.

METHODOLOGY

The study protocol was reviewed and approved by Bioethics Committee at Biotechnology Research Center (BEC-BTRC) with Ref No: BEC-BTRC 06-2018. Inclusion criteria involve: patients agreement participation in the study. Eighty seven pregnant women who had abortion seeking medical attention from October 2018 to March 2019 at Al-Thawra Hospital in El-Beida City were taken as a study group. This Hospital was only Center that received the majority of patients in the littoral and outskirt regions which have a population of more than 500,000 inhabitants. The study was included blood samples taken from aborted females to detect presence of Toxoplasma (IgG and IgM). Their ages were ranging from less than 19 to more than 45 years old. Full information was obtained from each pregnant woman in special questionnaire form, including age, occupation, and number of abortion. Then these women were divided to couple groups upon presence and absence of Toxoplasma’s antibodies. All blood tests were performed by automatic blood cell analyzer (XP-300 Automated Hematology Analyzer, Sysmex American,

Inc [25, 26]. Liver function parameters were estimated in all subjects by a commercially available test kits method on automatic analyser from Biotechnologies, Germany with the manufacturer’s instructions strictly adhered to using spectrophotometers (Humalyzer Junior).

Statistical Analysis

Data from patient was compared with data of normal subjects. Statistical analysis was carried out in Minitab software; statistical significance was assessed using two samples T- test analysis. After detection normal distribution to the data and appropriate P < 0.05 consider significant [27].

RESULTS

During the 6 months period of the study which include 87 Abortive women attending to Al-Thawra Hospital from different location around El Beida City. The study was included blood samples of women to detect presence of Toxoplasma antibodies (IgG and/or IgM). These women were divided to couple groups upon presence and absence of Toxoplasma’s antibodies (44 Abortive women who positive with toxoplasmosis and 43 was negative with toxoplasmosis). Upon study 44 Abortive women who positive with toxoplasmosis compared to negative subjects with toxoplasmosis. The prevalence Abortive women was high in El-Beida City with 43.18% compared to fourteen different location. Overall 87 women, the age groups, (as each group consist of 5 years intervals). Highest prevalence was noted in age group 21-25 years 27.27% followed with age groups 31-35 and 26-30 with 23.86, 20.45% respectively. In subject who positive with toxoplasmosis, the highest prevalence was noted in age group 21-25 years 27.27% followed with age groups 31-35 and 26-30 with 23.86, 20.45% respectively (Figure-1). While, lowest prevalence was noted in age group more than 46 years 1.14% followed with age groups less than 20 years with 5.68%. All subjects (87) were subdivided into three groups according to number of miscarriages (1, 2 and more than 3 miscarriages) as shown in Figure-2. The rate of one miscarriage in abortive women was 50.6% (44/87) higher than two and three miscarriages 29.9% and 11.5% respectively. On other hand in positive cases were shown increased in percentage number of miscarriages to more than three times compare to negative cases.

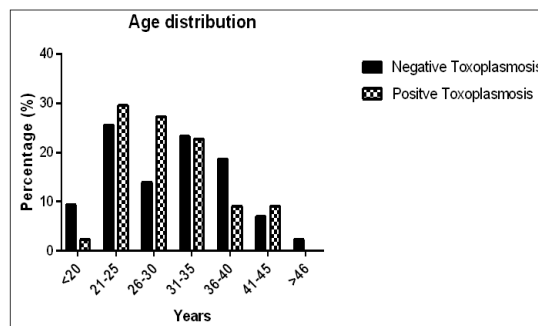


Fig-1: Age distribution for positive and negative cases

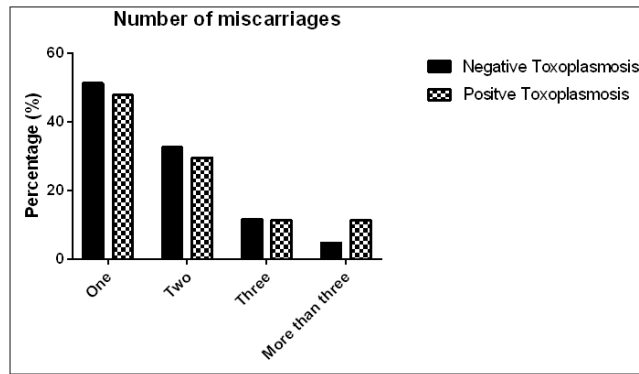


Fig-2: Number of miscarriage in positive and negative toxoplasmosis

The sero-prevalence of IgG and IgM anti-Toxoplasma antibodies were described in Table-1. All positive subjects 44/87 (50.6) were IgG anti-toxoplasma antibodies sero-positive (titre >1 IU/ml), 43/87(49.4%) were sero-negative for IgG anti-toxoplasma antibodies

(titre <1 IU/ml). Sixteen of total cases was mixed seropositive for IgG and IgM with 13.6%. These value of antibodies IgG/IgM were found statistically difference between positive and negative subjects.

Table-1: Levels of anti-toxoplasma antibodies in positive/negative cases

Type of Antibody against Toxoplasma		Number of case (%)	Level of antibody (U/ml) Mean ±SEM
Positive toxoplasmosis	IgG	43 (49.4)	5.79 ± 0.94 ***
Negative toxoplasmosis	IgG	44 (50.6)	0.581± 0.03
Positive toxoplasmosis	IgM	6 (13.6)	0.706 ± 0.14*
Negative toxoplasmosis	IgM	44 (50.6)	0.392 ± 0.03

Data are expressed as mean ± SEM. Means with different superscript (*, ** or ***) were significantly different at p<0.05 within same raw. Where means without superscripts mean that there is no significant difference (p>0.05).

Values derived from complete blood counts (CBC), including differential cell counts were recorded for each positive case with toxoplasmosis and analyzed comparison to negative case subjects. Red blood corpuscles (RBC) count was studied, and the results are shown in Table-2. The positive and negative toxoplasmosis had a mean count of 4.144 ± 0.11 , $3.930 \pm 0.10 \times 10^6/\mu\text{l}$ RBC respectively. The result was not statistically different in positive subjects compared to negative subjects. The HGB concentrations in the positive female and negative female subjects were 11.36 ± 0.27 and 11.51 ± 0.30 g/dl. These values also are not statistically different. Toxoplasmosis had no significant effect on the percent hematocrit (HCT for positive subjects 33.38 ± 0.81 and negative subject's $32.81 \pm 0.80\%$). However the mean corpuscular volume (MCV for couple group's 80.89 ± 1.1 and $84.00 \pm 1.11 \mu\text{m}^3$) was found statistically significant deference in negative comparison to positive subjects. Mean corpuscular hemoglobin (MCH) was also significantly ($p>0.05$) decrease in positive toxoplasmosis (27.77 ± 0.63 pg) comparison to negative toxoplasmosis (29.47 ± 0.48 pg). Mean corpuscular hemoglobin concentration (MCHC) were decrease in negative subjects compared to positive subjects with 34.24 ± 0.48 and 35.03 ± 0.32 g/dl respectively. Similar result in term of decreasing were obtained with red blood cell distribution width (RDW %) with 13.95 ± 0.40 and $13.37 \pm 0.41\%$ in positive and negative toxoplasmosis

respectively. Table-2 was shown results of white blood cells (WBC). With regard to these cells ($WBC \times 10^3/\mu\text{l}$) were significantly decreased in positive toxoplasmosis to 7.37 ± 0.34 compared to negative toxoplasmosis 9.48 ± 0.58 . Lymphocytes (Lymph %) and mid cells total count (MID %) were increased and granocytes (Gran %) decreased slightly in positive toxoplasmosis without significantly deference compared to negative subjects.

Results of platelets that derived from complete blood counts (CBC) were shown in Table-3. The mean platelets number ($PLT \times 10^3/\mu\text{l}$) was decreased in positive toxoplasmosis. Meanwhile the volume occupied by platelets in the blood (PCT %), the mean platelet volume (MPV %) and platelets distribution width (PDW %) were found high without significant deference compared to negative subjects.

Results of different blood groups were shown in Figure-3. Type of blood groups was tested for positive and negative subjects. Overall subjects, with the lowest prevalence of being among subjects with blood group O, and the highest prevalence among individuals with blood group AB. This results was similar with positive cases with toxoplasma. Rh-positive and Rh-negative subjects responded differently to toxoplasmosis, the highest prevalence among individuals with blood group with Rh-positive compared to Rh-positive negative at same blood group.

Table-2: Mean values of RBC, HGB, HCT, MCV, MCH, MCHC and RDW in positive/negative Toxoplasmosis

Parameters	Negative Toxoplasmosis Mean ±SEM	Positive Toxoplasmosis Mean ±SEM
RBC (×10 ⁶ /μl)	3.930 ± 0.10	4.144 ± 0.11
HGB (g/dl)	11.51 ± 0.30	11.36 ± 0.27
HCT %	32.81 ± 0.80	33.38 ± 0.81
MCV (fL)	84.00 ± 1.11	80.89 ± 1.1*
MCH (pg)	29.47 ± 0.48	27.77± 0.63*
MCHC (g/dL)	35.03 ± 0.32	34.24 ± 0.48
RDW (%)	13.95 ± 0.40	13.37 ± 0.41

Data are expressed as mean ± SEM. Means with different superscript (*, ** or ***) were significantly different at p<0.05 within same raw. Where means without superscripts mean that there is no significant difference (p>0.05).

Table-3: Mean values of WBC, Lymph, Mid and Gran in positive/negative Toxoplasmosis

Parameters	Negative Toxoplasmosis Mean ± SEM	Positive Toxoplasmosis Mean ±SEM
WBC (×10 ³ /μl)	9.48 ± 0.58	7.37± 0.34**
Lymph %	2.207 ± 0.11	2.78± 0.58
Mid %	0.643 ± 0.04	0.87± 0.30
Gran %	6.23 ± 0.36	5.62± 1.04

Data are expressed as mean ± SEM. Means with different superscript (*, ** or ***) were significantly different at p<0.05 within same raw. Where means without superscripts mean that there is no significant difference (p>0.05).

Table-4: Mean values of PLT, MPV, PDW and PCT in positive/negative Toxoplasmosis

Parameters	Negative Toxoplasmosis Mean ±SEM	Positive Toxoplasmosis Mean ±SEM
PLT (10 ³ /μl)	234.7 ± 14.4	215 ± 16.7
MPV (fL)	9.95 ± 0.20	9.99 ± 0.30
PDW (%)	13.65 ± 0.40	14.82 ± 0.52
PCT %	27.65 ± 1.16	31.90 ± 1.47

Data are expressed as mean ± SEM. Means with different superscript (*, ** or ***) were significantly different at p<0.05 within same raw. Where means without superscripts mean that there is no significant difference (p>0.05).

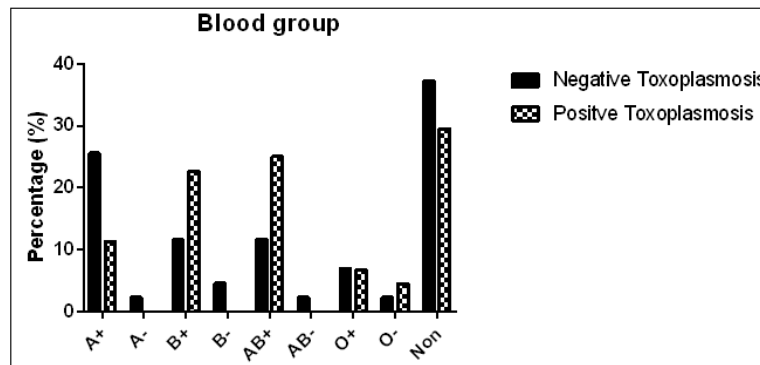


Fig-3: Percentage values for different blood groups in positive/negative Toxoplasmosis

The liver function enzymes were also measured in all subjects. The effect of the presence of toxoplasma on serum AST, ALT and ALP are shown in Table-5. The mean level of AST, ALT and ALP in

the serum of the positive toxoplasmosis was decreased from (31.2 ± 1.58, 22.50 ±8.95 and 163.2 ±48.5 U/L) to (24.2 ±10.5, 21.23 ± 1.40 and 159.1 ± 8.50 U/L) respectively.

Table-5: Results of AST, ALT and ALP in positive/negative toxoplasmosis

Parameters	Negative Toxoplasmosis Mean ±SEM	Positive Toxoplasmosis Mean ±SEM
AST (U/L)	31.2 ± 1.58	24.2 ±10.5
ALT(U/L)	22.50 ±8.95	21.23 ± 1.40
ALP (U/L)	163.2 ±48.5	159.1 ± 8.50

Data are expressed as mean ± SEM. Means with different superscript (*, ** or ***) were significantly different at p<0.05 within same raw. Where means without superscripts mean that there is no significant difference (p>0.05).

DISCUSSION

Diagnosis of toxoplasmosis based on clinical sign and supporting laboratory analysis including blood and urine tests and visualization of the organism in body tissues [28]. The diagnosis of infection can be made directly by identifying the parasite in tissue sections or in body fluid or indirectly by serological and biochemical techniques [29]. The clinical characteristics of the 87 abortive women (positive and negative with toxoplasmosis) were enrolled in this study. This study found that age group from 21 to 40 years were highest prevalence for both positive/negative toxoplasmosis and this is pointing to rate of infertility among this age group. In positive cases with toxoplasmosis, the highest prevalence was noted in age group 21-25 years followed with age groups 31-35 years. This result agree with study reported the highest rate of seroconversion among pregnant women aged 35-45 years [30], and also agrees with the data recently collected from Libya [31, 32]. This high rate of seroprevalence in mid age group may belong to higher contact with cats or infected things and vegetables than other age groups. This finding relatively analogous with study in Iran [33] and in Iraq [34]. Our results shown that at age group 41-45, percentage of positive subjects was higher than negative subjects. This observation was similar to study in Tripoli – Libya, that patients age group of ≥ 41 years old was more affected than other age groups (the prevalence of anti-*T. gondii* antibodies are increases with age [31]. Result from number of miscarriages was found that over all subjects the rate of one miscarriage in abortive women was higher than two and three miscarriages. However, in positive cases were shown increased in percentage number of miscarriages to more than three times compare to negative cases. The current study agreed with studies where they found the highest rate of infection among women who have suffered single abortion [34, 35]. The reason for the high incidence among aborted women may return to the type of acute injury or reactivate chronic injury due to decrease immunity of pregnant mother's body as the time of the injury during pregnancy has an important role to determine the fate of the fetus [34, 35]. A high prevalence of chronic toxoplasmosis (IgG positive) in abortive women was found in this study (50.6 %). In our study, the prevalence of recently acquired infections (IgM positive) was (13.6 %), while those with both IgG and IgM was (17.9 %). These results were significantly difference compared to negative subjects. In Iraq, similar results were obtained [36]. There are similar reports in Turkey and Iran [33, 37]. This is similar study in Palestine [38]. Our result is comparable to results previously reported in Abha [39] and in Makkah [30, 40]. However, this result was high compared to previous study in Northwest of Iran that found the prevalence of IgM (1.4%) [41]. This results may refer to increase contact with cats in recent years in this area. Values derived from CBC, including differential cell

counts were recorded for each positive toxoplasmosis and analyzed comparison to negative toxoplasmosis subjects. Generally, the results were shown a lot of similarity between positive and negative toxoplasmosis. Couple groups had a similar values in mean count of RBC, level of HGB, percent of HCT, level of MCHC and percent of RDW. Other RBC parameters were recorded statistically decrease in levels of MCV and MCH in positive subjects compared to negative subjects. Our results are comparable to other studies found that the blood parameters some time remains stable and not changeable during period of infection [42, 43]. Similar results were obtained experimentally from evaluation haematological parameters during *T. gondii* infection in gerbils [44] in cats [45], in mice and rats [46]. However, our results conflict with study that found significantly affected hematologic parameters noteworthy implication of active toxoplasmosis in seropositive cats [47]. In totality, the presence of WBC in the blood reflects immunologic condition of seropositive patients [42, 48]. Any changes in the WBC may reflect serious abnormalities on the health condition of the positive subjects with toxoplasmosis. In current study, with regard to the WBC, Lymph (%), MID (%) and Gran (%) were decreased with significantly difference in term of total number of WBCs. The results show decrease in WBCs in infected women which might because these cells had been affected by toxoplasmosis. Which it considered as one of the important factors that control the natural and acquired immunity response in the body of pregnant infected women [49]. Our results were similar to another studies among abortion women in Iraq [43]. Previous studies have suggested that neutrophils are important for controlling toxoplasmosis in mice [50, 51] and humans [52]. With the *T. gondii*-infected group, our data showed that Mean Lymph and MID were increased compared to the negative group. In contrast, total WBC count and the Gran percentage were decreased in positive subjects. The reason of the high percentage of lymphocytes and Gran are probably an inflammatory response to the tachyzoite proliferation during infection. Other studies reported that the numbers of circulating lymphocytes were increased in *T. gondii* infected rats [53] and gerbils [44]. The mean PLT number in patient subjects was decreased, other parameters of platelets MPV, PDW and PCT were slightly increased compared to negative subjects. Similar results were obtained following infection with tachyzoites of *T. gondii*, a thrombocytopenia observed in experimentally infected rats [54]. Toxoplasma are among the infectious agents with protean clinical manifestations which may induce immune thrombocytopenia [55]. Thrombocytopenia that is caused by mechanisms such as increased clearance of damaged platelets with endotoxins, exotoxins, or platelet-activating factor or direct platelet toxicity caused by the microorganism, immune-mediated destruction of the platelets, and platelet adherence to

damaged vascular surfaces [54, 56, 57]. In positive toxoplasma infection, the lowest prevalence of Toxoplasma infection being among individuals with blood group O (positive and negative Rh), and the highest prevalence among individuals with blood group AB (positive and negative Rh). Different studies in Czech and in Russia have reported similar findings, with Toxoplasma seroprevalence being twice as high among subjects with blood group AB than among subjects with blood group O [58, 59]. It is known that natural resistance to many infectious diseases may depend, to a certain extent, on the blood group of an individual [60, 61]. The A, B and O blood group phenotypes are determined by the presence or absence of A and/or B carbohydrate antigens on the surface of red blood cells [62]. This determines natural resistance in humans to many infectious disease agents that have cell surface antigens similar to the antigens of different blood group types. This mechanism may, in part, explain the higher susceptibility of individuals with blood type AB to several infectious diseases, since the blood of these individuals does not contain the corresponding natural antibodies [58]. In current study in the influence of RhD, that found Rh-positive and Rh-negative subjects responded differently to toxoplasmosis, the highest prevalence among individuals with blood group with Rh-positive compared to Rh-positive negative. This observation was similar to study indicate that RhD phenotype might influence the effect of toxoplasmosis [63]. The mean level of AST, ALT and ALP in the serum of the positive toxoplasmosis was decreased. Toxoplasmosis causes extensive and progressive damage to the liver, remarkable proliferations of organisms such damage in the liver brought about changes in the liver metabolism [64]. Our results are similar to other studies [65-67]. These results also agree with the studies performed on experimental animals [68-70]. These elevations suggest the involvement of liver cells. Hepatic necrosis is a well-established complication of toxoplasmosis [66], where this infection can cause round cell infiltration in the portal areas, cholestasis, swollen endothelial cells and focal necrosis of liver cells [71]. Despite the decrease of AST and ALT activities compared with the negative subjects, the levels are still within normal ranges suggesting a mild effect on the liver. Remarkable changes of enzymes in sera showed a tendency to increase after infection which might reflect the degree of damage of liver [72]. The liver enzymes activities are statistically elevated but they are still within normal acceptable ranges suggesting that toxoplasmosis may affect the liver in a way that this effect is not sufficient to produce clinical signs and symptoms.

CONCLUSION

Infections with *T. gondii* can cause harmful effects on healthy hosts and stimulate the infected host's immune system. The assessment of the severity of disease and the prognosis of recovery of affected

subjects can only be made on the basis of clinical and laboratory data such as haematological and biochemical parameters. Therefore, the main objective of this study was to determine the effects of toxoplasmosis on haematological and some biochemical parameters among abortive women in El-Beida City. Results were compared to those obtained with abortive women but negative toxoplasmosis for a better understanding of toxoplasmosis pathogenesis. Integration of the clinical and experimental data on *T. gondii* should continue to lead to important insights into how pathogens evolve into successful parasites.

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