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Research article

Effect of human blood groups *on Leishmania dnovani* growth *in vitro*

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ABSTRACT

Visceral leishmaniasis (VL), a disease caused by *Leishmania donovani*, is still a health problem and a common parasitic infection. Several questions related to this disease have been raised that have still no answer in spite of new scientific developments in recent years. This study was performed to investigate the effect of human blood group types (O-, A+, A^-, B+, B^-, AB+ and AB^-) on *L. donovani* growth rate *in vietro*. The growth rate of *L. donovani* was increased during fourth days in media supplemented with all types of blood groups. In fifth day, growth rate of *L. donovani* was significantly decreased in media supplemented with the blood group type (O-, A+, A^-, B+, B^-, AB+ and AB^-) compare with *L. donovani* growth rate in media supplemented with blood group type (O+). The lowest growth rate of *L. donovani* was seen in media supplemented with the blood group type (AB+). Taking together, there is an association between *L. donovani* growth rate in *vitro* and the supplemented human blood group. These results indicate that it is possible to culture *Leishmania* parasite in axenic culture supplemented with human blood, especially the blood type O +.

Keywords: Growth rate, Human blood group, Leishmania

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INTRODUCTION

Leishmania species are obligate intracellular parasites and they are the causative agents of diseases group called Leishmaniasis [1]. Clinical features of Visceral Leishmaniasis affects many organs including spleen, and liver. According to Marovt *et al*. [2]. The disease is endemic in 88 countries. There are 12 million cases worldwide yearly [3]. *Leishmania* spp. is a digenetic parasite, whose life cycle involves two hosts, a vertebrate and an invertebrate, the sandfly. *Leishmania* infection is transmitted to susceptible mammalian hosts by the bite of a female sand fly (subfamily Phlebotominae) [4, 5].

The ABO human blood types are due to two antigens and two antibodies. For example, the A antigen on the surface of their red cells, people will be with type A blood. So, anti-A antibodies will not be produced by them because they would cause the damage of their own

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blood. If B type blood is injected into their systems, anti-B antibodies will produce in their plasma as foreign and rupture the introduced red cells to clean the blood from the foreign protein. Individuals with type O blood do not have ABO antigens. Thus, their blood usually will not be rejected when it is given to people with different ABO types [6]. On the other hand, the Rh blood group is the most complex blood groups known in humans. It is important to the ABO blood group in transfusion medicine. The polymorphic genes that encode them are two genes, RHD and RHCE that are closely linked. Genetic rearrangements between them produced hybrid Rh genes that encode the Rh antigens. The significance of the Rh blood group is related to the Rh antigens which are highly immunogenic [7].

The association of certain human blood groups with parasites is a medically important subject. In some cases, positive relations have been confirmed as in schistosomiasis [8] and giardiasis [9]. In other cases, no evidence of association appeared to the researches such as filariasis [10]. There is very little, if any, scientific data to support that there are some undeniable associations of blood groups with disease. *Plasmodium falciparum* malaria has effect on the distribution of ABO blood groups. Some erythrocyte mutations associated with a survival advantage in *P. falciparum* infection [11]. Though, the distribution of ABO has also been affected by other events [12] including people migration, population after wars, and other lethal pediatric diseases related with a specific ABO antigen. For instance, it has been proposed that the isohemagglutinins found in group O persons produce broader immunity against several pathogens [13]. In particular case of leishmaniasis, racial differences in the evolution of mucocutaneous leishmaniasis (caused by *L. braziliencesis*) has been noticed [14]. On the other hand, a study in Brazil, patients with *L. donovani chagasi* did not show any significant relation between ABO blood groups and the development of the disease [15].

In this study, *L. donovani* were grown in axenic cultures supplemented with different human blood groups (O+, O-, A+, A-, B+, B-, AB+ and AB-) to find out the ability of *Leishmania* growing in media supplemented with different human blood group. The results have been shown that there is an association between *L. donovani* growth rate in *vitro* and the supplemented human blood group.

MATERIALS AND METHODS

L. donovani culture conditions

L. donovani promastigotes were provided from the parasitology lab/ Biology Department/ College of Science/ University of Baghdad, Iraq. *L. donovani* promastigotes were cultivated in NNN culture medium. For all experiments, *L. donovani* promastigotes were grown axenically at 26 ^{0}C in NNN medium (Nicolle–Novy–MacNeal; Brain Heart infusion Agar, Blood Agar, D-Glucose, supplemented with 100 mg/ml of streptomycin).

Leishmania growth rate

Blood groups were obtained from Blood bank. The experiments were done in triplicate. *Leishmania* parasites were counted under a microscope using a heamocytometer. For each blood group, the counting was done in triplicate for 5 days at the same time for each day.

Statistical analysis

The Statistical Analysis System- SAS [16] was used to effect of different factors (Blood group and days) in growth rate. Least significant difference –LSD test was used to significant compare between means and Chi-square test was used to compare between percentages in this study.

RESULTS

Leishmania growth in different blood groups was determined and compared with the O+ blood group. The current study revealed that media supplemented with blood group O+ characterized by increase ($p<0.05$) the parasite growth rate during the first three days of cultivation and reached its maximum at day four with concentration of $(268.80\pm16.54)*10^5$ parasite/ml. **Fig 1** and **table 1**.

The parasite growth rate on media supplemented with O^- blood group reached to its high value $(245.33\pm 16.42)*10^5$ parasite/ml in day four, while in day five the growth rate start to decline $(96.00\pm14.84)*10^5$ parasite/ml. Figure (1) table (1). These result also showed that there was an increase of *Leishmania* parasite growth rate in media supplemented with (O-, A+, A^-, B+, B^-, AB+ and AB^-) blood groups in day two and three compare with its growth in media with O+ blood group. In day four and five, there was a significant decrease ($p<0.05$) of *Leishmania* parasite growth rate in media supplemented with (A+, A^-, B+, B^-, AB+ and AB^-) blood group compare with its growth in media with O+ blood group. This result means that O+ more potent in growth of *L. donovani* parasite than the other blood groups (**Fig. 1** and **table 1**).

The lowest growth rate reached to its low values was in media supplemented with blood group (AB-) in day four $(117.87 \pm 3.39)*10^5$ parasite/ml. In day five, the growth rate was starts severely declined in media supplemented with blood group (B+) $(62.00 \pm 3.26)*10^5$ parasite/ml. This result showed that there is in fact an average decrease of relative growth of media supplemented with blood group (O-, A+, A^-, B+, B^-, AB+) and specifically in blood group (AB^-) as compared with the parasites growth in media supplemented with blood group (O+) (**Fig. 2** and **table 1**). These results showed clearly significant difference when analyzed statically by using LDS value at probability $P\leq0.05$.

Table 1. Least significant difference –LSD test compare between means and Chi-square test was used to compare between percentages in this study. *, significant difference (P<0.05), the data represented by mean ± sd (standard deviation).

Day	O^+	O^-	A^+	A^-	B^+	B^-	AB^+	AB^-	LSD value
1	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.00 NS
2	9.77 ± 0.82	41.07 ± 1.05	40.00 ± 2.37	38.57 ± 2.04	28.80 ± 2.68	26.67 ± 2.09	27.20 ± 1.77	20.80 ± 1.69	8.15 *
3	31.95 ± 1.94	214.40 ± 11.79	174.40 ± 4.63	216.53 ± 5.77	99.47 ± 2.74	103.20 ± 1.25	110.67 ± 4.51	94.93 ± 5.03	27.94 *
4	249.00 ± 7.62	245.33 ± 16.42	216.00 ± 9.71	226.67 ± 7.84	218.67 ± 15.83	144.53 ± 7.56	153.07 ± 7.58	117.87 ± 3.39	30.66 *
5	268.80 ± 16.54	96.00 ± 14.84	125.33 ± 14.95	122.67 ± 3.56	62.00 ± 3.26	77.33 ± 2.86	77.76 ± 4.64	70.93 ± 2.81	28.72 *
LSD value	37.05 *	26.42 *	28.77 *	26.38*	23.69 *	31.25*	13.86*	10.62*	---

DISCUSSION

Decker-Jackson and Honigberg [15] showed, through sensitive immounological techniques, the existence of cross reaction between the antigens of the ABO blood group system and some surface antigens of *Leishmania*. On the basis of these results, a mechanism employed by the parasite to evade the host's immune response was suggested [17]. In Sri Lanka, 243 adult cases (mean age, 29.8 years) of *P. falciparum* malaria (163 mild, 80 severe) were assessed compared with 65 control patients with other infections. The proportion of group O in mild malaria cases was 48%, but was only 24% in severe malaria cases [18].

A blood group type is classification of blood based on the presence or absence of inherited antigen substance on the surface of red blood cells (R.B.Cs). These antigens may be protein, carbohydrate, glycoprotien or

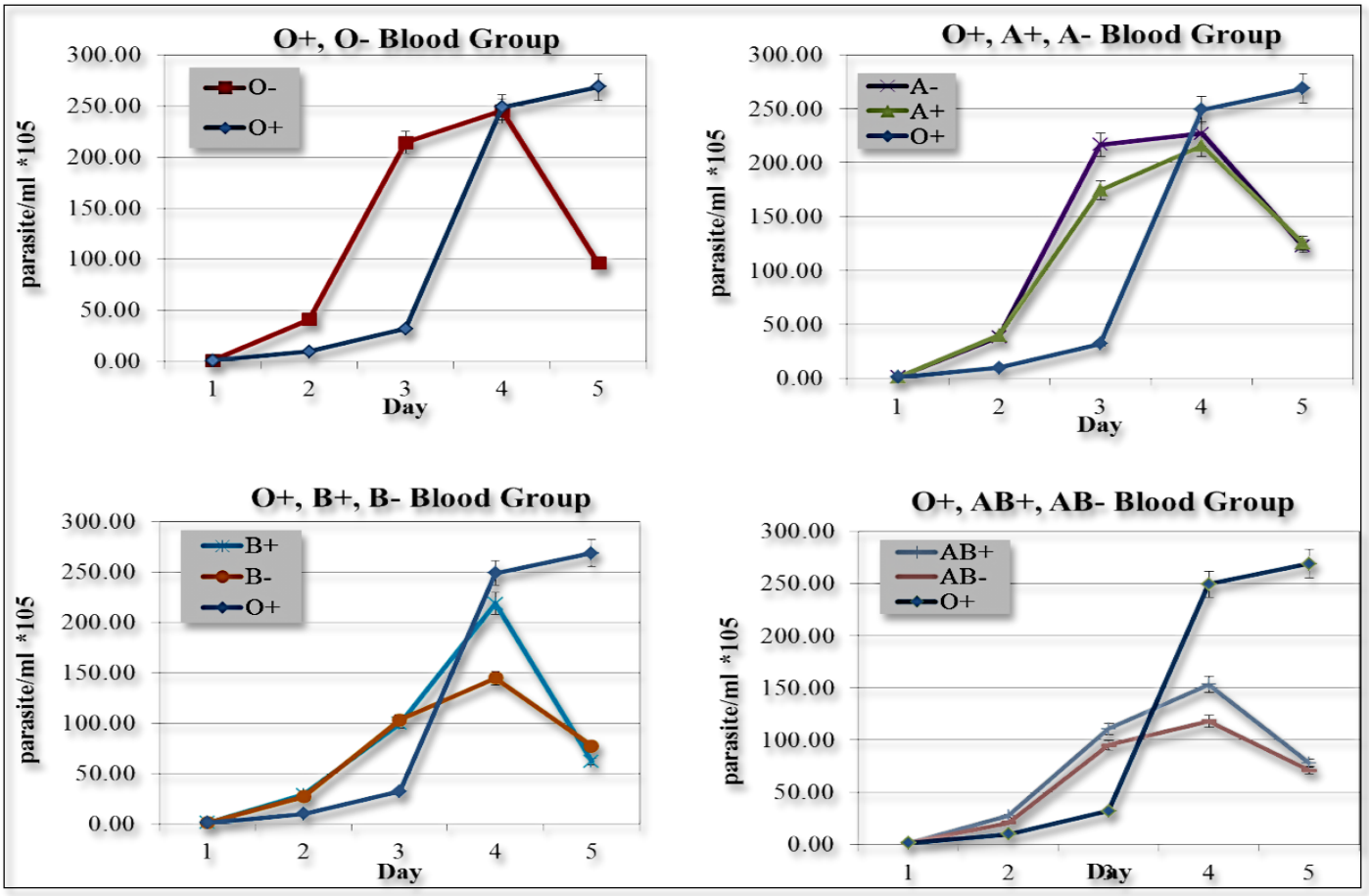

Fig 1. *Leishmania* growth rate in NNN media supplemented with O+, O-, A+, A^-, B+, B^-, AB+ and AB^-) blood groups.

glycolipid, depending on the blood system. Black Well *et al*. 2002 [19] showed that Scotland patients of group O were more susceptible to gastrointetinal infection caused by *Escherichia coli* and 87.5% of them dead. Another study by AL-Shikhly *et al*., 2013 [20] showed that the highest prevalence of toxoplasmosis begin among Iraqi pre-marital female with blood group of O+ and the lowest with A+ blood group.

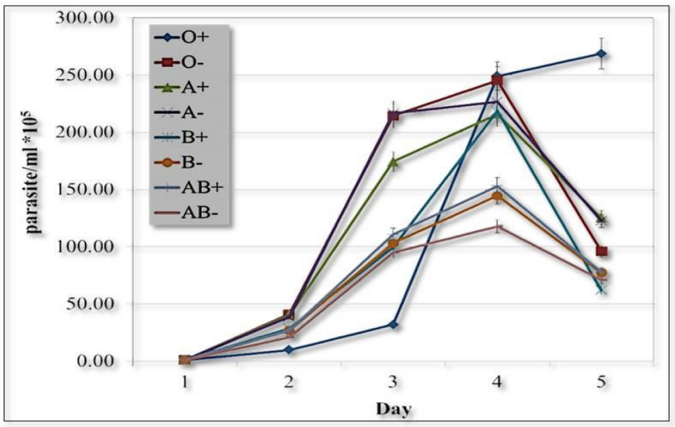

Fig 2. *Leishmania* growth rate increased at day three in NNN media supplemented with (O-, A+, A$^-$, B+, B$^-$, AB+ and AB$^-$) blood groups compare with blood group O+. In day four and five, *Leishmania* growth rate significantly decreased in media supplemented with (O-, A+, A$^-$, B+, B$^-$, AB+ and AB$^-$) blood groups compare with blood group O+.

The surface glycoproteins of *L. tropica* and *L. donovani* were similar to certain ABO blood groups, thus this will provide a possible escape mechanism for the parasites that are similar to certain ABO blood groups of the patient [15]. Since type O blood does not produce ABO antigens thus, the parasites growth in media supplemented with this blood group normally will not be rejected compare with the other ABO types. Previous study conducted on the relationship between *Toxoplasma gondii* and ABO blood group showed that there is a possibility that parasite utilize glycoconjugates, which characterize the blood phenotype of the ABO system as a potential receptor [21; 22; 23; 24]. In Zimbabwe, outcomes for group O individuals were reported compared with group A among 489 patients with *P. falciparum* malaria. The study included 209 outpatients and 280 severely ill patients [17]. Among 482 patients in Isfahan- Iran, there was no association between blood groups and cutaneous leishmaniasis infection [25, 26]. Group O individuals have a survival advantage in severe malaria which means that there is an association between ABO and disease severity in *P. falciparum* infection. The binding of parasitized RBCs to groups A and B determinants on endothelial cells would be expected to contribute to cytoadherence [27]. Recently, many studies suggest that group O hosts of *P. falciparum* malaria tend to have fewer severe clinical consequences than group A hosts. These reports provide evidence for a survival advantage for group O individuals with *P. falciparum* infection [28].

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Conflict of interest

The authors declare that they have no conflict of interests.

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