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The antigenic mimicry of *Ascaris lumbricoides* and *Toxocara canis* with blood group antigens

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Ascariasis and toxocariasis are among the most common human parasitic infections. The relationship between these pathologies and blood type has been poorly studied, making it an interesting area for research. This study aimed to investigate the incidence of ascariasis and toxocariasis depending on blood group type in patients with coxarthrosis, gonarthrosis, and bone fractures. A total of 82 patients were examined. Hematological and biochemical parameters were determined using a hematological analyzer, spectrophotometer, and commercial kits. The level of IgG antibodies to *Ascaris lumbricoides* and *Toxocara canis* was measured using an ELISA test. A high incidence of T. canis seropositivity (51.2%) and A. lumbricoides seropositivity (25%) was determined in patients with coxarthrosis, gonarthrosis, and bone fractures. Low susceptibility to ascariasis infection was found in individuals with blood group A (13.7%) and group B (15.3%). Persons with weak blood group A and B antigens demonstrated seropositivity to *A. lumbricoides* and the presence of immune group-specific antibodies. The immune anti-A antibodies correlated with antibodies to *A. lumbricoides* and inversely correlated with antibodies to *T. canis*. Persons with blood group B demonstrated a high frequency of seropositivity to *T. canis* (84.6%). Monitoring the level of immune antibodies in persons with seropositivity to *A. lumbricoides* and *T. canis* may help slow down the progression of anemia in this category of patients.

Key words: Ascariasis, toxocariasis, red blood cells, anemia, blood group antigen.

INTRODUCTION

The predisposition of certain blood group types to various diseases has been proven. A high incidence of blood type A was registered in smallpox and *Pseudomonas aeruginosa* infection. Blood type B was associated with gonorrhea, tuberculosis, *Streptococcus pneumoniae, Escherichia coli* and salmonella infections (Abegaz, 2021). Ongoing research is devoted to studying the connection between specific blood group types and parasitic infections. Studies indicate a higher prevalence of helminth infections in individuals with blood types A and B compared to those with blood type O (Degarege et al., 2017). Additionally, individuals with blood group B have shown high susceptibility to *Plasmodium falciparum* compared to those with blood group O (Yeda et al., 2022).

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Research has also investigated parasitic bone and cartilage infections (Rommani and Romdhane, 2024). Furthermore, alveolar echinococcosis has been reported as a cause of osteomyelitis and soft tissue infection with recurrent cutaneous fistula (Schmidt-Hellerau et al., 2022).

Ascaris lumbricoides or *Toxocara canis* infection has been reported to manifest with gastrointestinal and musculoskeletal symptoms. Ascariasis and toxocariasis were reported to coexist with articular symptoms and parasitic infection was considered to be one of the factors that initiate the rheumatoid process (Janicka-Szczepaniak et al., 2016).

Ascaris infection has been shown to have a certain correlation with blood group type A (Chulanetra and Chaicumpa, 2021; Martins et al., 2023). The increased haemagglutinins to A Blood group B antigens due to cross-reactive polysaccharide antigens were found in *Toxocara* larvae. The studies have shown that 56% of patients with toxocariasis have increased antibodies to group A and 96% to group B. The isohaemagglutinins to group A were considered to be more common (Waindok et al., 2021). *T. canis* and *A. lumbricoides* share similar glycoproteins to human blood group antigens (Moulds and Moulds, 2000; Shanawany et al.,2019). The data indicate that group antigens bind with parasitic agents and promote or prevent parasitic invasion. The enzyme-linked immunosorbent assay (ELISA), detecting IgG-specific antibodies against *T. canis* and *A. lumbricoides*, is used for diagnosing toxocariasis and ascariasis (Maizels, 2013). The incidence of ascariasis and toxocariasis in patients with coxarthrosis, gonarthrosis and bone fractures has not been studied. The predisposition of these infections to certain blood groups and their effect on hematological and metabolic parameters has not been elucidated. Therefore, the study of the prevalence of toxocariasis and ascariasis depending on the blood group type is important for elucidating the pathogenesis of the parasitic infection and determining the tactics of the treatment.

The aim of the study was to determine the incidence of ascariasis and toxocariasis in patients with coxarthrosis, gonarthrosis and bone fractures, depending on blood group type, and to identify the characteristic hematological and biochemical parameters associated with these parasitic infections.

MATERIALS AND METHODS

Participants and study area

Eighty two patients with coxarthrosis, gonarthrosis and bone fractures aged 50-72 years old were enrolled during the study and their blood samples were screened for IgG antibodies against *A. lumbricoides* and *T. canis* using ELISA test. The exclusion criteria for the study: autoimmune diseases, rheumatoid arthritis, oncological diseases, acute infectious diseases. The participants were informed and enlightened about the study. The study was carried out in Kharkiv, Ukraine. Kharkiv is a city located on Latitude 50° 0' 5.6160" N and Longitude 36° 13' 53.5332" E and is 152 m above sea level. The temperature typically varies from 18 to 80°F and is rarely below -2°F or above 91°F (Reshetchenko et al., 2016).

Collection of blood samples

Three mililiters of blood were collected from every person by venipuncture into EDTA tube using 5 ml capacity syringe. The tubes were prelabeled with the subject's names. Plasma was separated by centrifugation at 2000 rpm for 10 min. The plasma were transferred into vials using Pasteur pipette and stored at -20°C until serological study was carried out.

Laboratory analysis

Plasma anti-*T. canis* and anti-*A. lumbricoides* IgG antibodies were detected using commercial (ELISA) kits (Abcam, London, UK). Thaw plasma samples and reagents were brought to room temperature and tests were performed according to the manufacturer's instructions. The results were interpreted as follows: >1.0: positive; 0.91-0.99: equivocal result (should be retested); <0.9: negative. Blood group typing has been performed according to the protocols (World Health Organisation [WHO], 2009). The patients were divided into four groups according to the blood group type: blood group B and presence of weak A antigen (Group I, n= 10), blood group B (Group II, n= 13), blood group A and presence of weak B antigen (Group III, n= 8), blood group A (Group IV, n= 51). Control group consisted of the persons with coxarthrosis, gonarthrosis and bone fractures and seronegativity to *A. lumbricoides* and *T. canis* without weak A and B antigens.

Analysis of hematological parameters

Red blood cells (RBCs), hemoglobin, leukocytes, neutrophils, lymphocytes, eosinophils, monocytes, erythrocyte sedimentation rate (ESR) and platelets were measured using a complete blood count (CBC) by the autohematological analyzer MYTHIC 3CRP - 3 DIFF (Cormay Diagnostics, Poland).

Biochemical parameters

The study used diacetyl monoxime to determine the concentration of urea in plasma (Langenfeld et al., 2021) with a spectrophotometer. The reaction between diacetyl monoxime and urea in the presence of sulfuric acid, phosphoric acid, thiosemicarbazide, and ferric chloride produced a chromophore and a peak absorbance was measured at 520 nm. Plasma creatinine concentrations were measured using commercial kit (Felicit diagnostics) by color Yaffe reaction (Toora and Rajagopal, 2002).

Aspartate aminotransferase (Ast) and alanine aminotransferase (Alt) activities were determined through the colorimetric method using multiparametric Photocolorimetric biochemical analyzer ALIZE (Lisabio, France) (Huang et al., 2006). The optical density of the test sample for Ast and Alt was measured against a control sample on a photometer at a wavelength of 500 and 340 nm,

Table 1. Distribution of blood groupsre according to seropositivity and seronegativity to *Ascaris lumbricoides* and *Toxocara canis.*

Group	Seropositivity to *A. lumbricoides* (%)	Seronegativity to *A. lumbricoides* (%)	P-value	Seropositivity to *Toxocara canis* (%)	Seronegativit to *Toxocara canis* (%)	P-value
Group I: BA+ (n=10)	4 (40)	6 (60)	0.047	3 (30)	7 (70)	0.004
Group II: B (n=13)	2 (15.3)	11 (84.6)	0.003	11 (84.8)	2 (15)	0.002
Group III: AB+ (n=8)	2 (25)	6 (75)	0.007	4 (50)	4 (50)	0.785
Group IV: A (n=51)	7 (13.7)	44 (86.2)	0.003	20 (39.3)	31 (60.7)	0.044

accordingly.

Immune antibodies

Immune antibodies were detected by haemagglutination method at 4 and 37°C (Issitt and Anstee, 1998). The plasme samples were diluted with 0.9% saline (1:4) and heated at 56°C for 30 min. The heated plasma (100 µl) was added to 50 µl of 2% suspension of washed in 0.9% saline RBCs and incubated at 37°C for 1 h. The agglutination was estimated under microscope MicroMed XS-3330 and graded according to the degree, from strongly positive (4+) to negative (-).

Consent and ethical issues

The study was approved by the Ethical committee of Kharkiv National Medical University (Institutional approval N 5, 26. 01. 23). Informed consent was obtained from all the participants. The study adhered to the ethical guidelines of the Declaration of Helsinki.

Statistical analysis

The statistical analysis was carried out using Statistica 10.0 software (StatSoft, Kraków, Poland). The mean and standard deviation were used to describe quantitative data. The statistical difference was assigned at P<0.05. The hematological parameters were compared using Student's t test. The frequency data were comparison using Fisher criterion. Correlation analysis was performed using the Spearman's correlation test.

RESULTS

The patients with coxarthrosis, gonarthrosis and bone fractures demonstrated high frequency of seropositivity to *T. canis* (51.2%, n=42) and A*. lumbricoides* (25%, n=21). Persons with blood group B (n=11 of 13; 84.6%) showed higher frequency of seropositivity to *T. canis* compared to the persons with other blood group types (P<0.05) (Table 1). Persons with Blood group A with weak B antigen (n=2 of 8; 25%) and Blood group B with weak A antigen (n=4 of 10; 40%) showed higher frequency of seropositivity to *A. lumbricoides* compared to persons with Blood group B (n=2 of 13; 13%) and persons with Blood group A (n=7 of 51; 13. 7%).

The hematological parameters of the individuals with weak blood group antigens are represented in Table 2. Eosinophilia and increased immune antibodies were revealed in persons with group B and weak antigen A (Group I) compared to the persons with blood group B (Group II) (P<0.05). Decreased leukocytes, eosinophilia, increased immune antibodies and antibodies to *A. lumbricoides* were revealed in persons with group A and weak antigen B (Group III) compared to persons with group A (Group IV) (P<0.05).

The persons with seropositivity to *T. canis* demonstrated decreased monocytes, eosinophilia, increased thymol probe and C-reactive protein compared to the persons with seronegativity to *T. canis* (P<0.05) (Table 3). The persons with seropositivity to *A. lumbricoides* demonstrated decreased platelets and leukocytes and increased serum urea, thymol probe and natural antibodies compared to the persons with seronegativity to *A. lumbricoides* (P<0.05). The immune antibodies were inversely correlated with RBCs (r=-0.6) and hemoglobin (r=-0.38), (P<0.05), and positively correlated with ESR (r=0.4), eosinophils (r=0.27), creatinine (r=0.6), thymol probe (r=0.4), antibodies to *A. lumbricoides* (r=0.4) and antibodies to *T. canis* (r=0.3) (P<0.05). Seropositivity to *A. lumbricoides* and *T. canis* was inversely associated with RBCs (r=-0.2; r=-0.3), hemoglobin (r=-0.38; r=-0.3) and directly associated with ESR (r=0.3; r=0.62, respectively). The antibodies to *A. lumbricoides* were correlated with eosinophils (r=0.15), thymol probe (r=0.47) and natural antibodies (r=0.23) (P<0.05).

In the group with weak blood group B antigen the antibodies to *A. lumbricoides* were associated with natural and immune antibodies (r=0.4) (P<0.05). Antibodies to *A. lumbricoides* in the group without weak blood group B antigen were associated only with natural antibodies (r=0.2) (P<0.05). In the group with weak A antigen the antibodies to *A. lumbricoides* were directly correlated with immune anti-A antibodies (Table 4). Importantly, in group of the persins with weak A and B antigens anti-A and anti-B immune antibodies were

Table 2. Hematological parameters of the patients with coxarthrosis, gonarthrosis and bone fractures.

Parameter	Group I: BA+ (n=10) (mean±SD)	Group II: B (n=13) (mean±SD)	Group III: AB+ (n=8) (mean±SD)	Group IV: A (n=51) (mean±SD)	P-value
Leukocytes (10^9 L^{-1})	5.7±0.31	5.72±0.34	6.2±0.31**	7.3±0.31	PIII, IV=0.031
Eosinophils (%)	2.7±0.5*	1.65±0.31	3.2±0.41**	2.0±0.34	PI, II=0.023,PIII, IVV=0.004
Toxocara canis abs	0.91±0.02*	1.41±0.04	1.1±0.03	1.13±0.02	PI, II=0.052
Ascaris lumbricoides abs	0.9±0.03	0.91±0.03	0.93±0.02**	0.78±0.02	PIII, IV=0.021
Immune abs at 4°C	1.5±0.13*	2.2±0.16	2.53±0.2**	1.62±0.16	PI, II=0.023, PIII, IV=0.005
Immune abs at 37°C	2.45±0.21*	1.67±0.15	3.1±0.35**	1.93±0.22	PI, II=0.023, PIII, IV=0.031

ESR: Erythrocyte sedimentation rate, abs: antibodies, *: P<0.05 compared to Group II; **: P<0.05 compared to Group IV.

Table 3. Hematological and bbiochemical parametersaccording to the seropositivity to *T. canis* and *A. lumbricoides.*

Parameter	Seropositivity to *Toxocara canis* (mean±SD) N=42	Seronegativity to *Toxocara canis* (mean±SD) N=40	Seropositivity to *Ascaris lumbricoides* (mean±SD) N=21	Seronegativity to *Ascaris lumbricoides* (mean±SD) N=61	P-value
Monocytes (%)	5.1±0.23*	6.7±0.42	-	-	P1,2=0.032
Eosinophils (%)	2.6±0.17*	2.0±0.1	-	-	P1,2=0.021
Thymol probe (units)	3.4±0.22*	2.1±0.13	2.83±0.2**	2.3±0.19	-
CRP (units)	8.34±0.67*	2.4±0.19	-	-	P1,2=0.015
Platelets (10^9)	-	-	243.51±17.2**	284.0±19.2	P3,4=0.034
Leukocytes (10^9)	-	-	6.61±0.4**	7.35±0.43	P3,4=0.042
Urea (mmol/l)	-	-	5.72±0.32**	4.1±0.24	P3,4=0.031
Natural abs	-	-	3.6±0.25**	2.5±0.16	P3,4=0.044

Abs: Antibodies, CRP: C-reactive protein, *: P<0.05 compared to the group with seronegativity to *Toxocara canis*, **: P<0.05 compared to the group with seronegativity to *Ascaris lumbricoides*.

Table 4. Result of correlation coefficient calculations between immune anti-A antibodies and antibodies to *Ascaris lumbricoides* and *Toxocara canis* in persons with Blood group B type and Blood group B with a weak A antigen.

Parameter	Immune Abs anti-A BA+ group (n=10)	Immune Abs anti-A BA-group (n=13)
Abs to *Ascaris lumbricoides*	0.69*	0.1
Abs to *Toxocara canis*	-0.2	-0.3

Statistically significant results are marked with * P<0.05. Abs: Antibodies.

inversely correlated with the level of RBCs in contrast to the group without weak A and B antigens, demonstrating absence of the association of immune antibodies with anemia (Table 5).

DISCUSSION

This study investigated the seroprevalence of anti-*Toxocara* and anti-*Ascaris* IgG antibodies and hematological and biochemical parameters in persons with coxarthrosis, gonarthrosis and bone fractures. The high frequency of seropositivity to *T. canis* (60.8%) and *A. lumbricoides* (40.6%) revealed in this category of patients reflects its importance and deserves the need to control the level of these antibodies. The persons with Blood group B type (Group II) demonstrated high level of IgG antibodies to *T. canis*. The persons with weak A and B

Table 5. Correlation coefficients between red blood cells count and immune antibodies in groups with and without weak blood group A and B antigens (r-values).

Parameter	Group with weak A and B antigens (n=18)		Group without weak A and B antigens (n=64)	
	Immune anti-A	Immune anti-B	Immune anti-A	Immune anti-B
RBCs	-0.64*	-0.86*	0.64*	0.2

Statistically significant results are marked with *P<0.05.

antigens (Groups I & III) demonstrated increased IgG antibodies to *A. lumbricoides.* The experiments carried out suggested that *A. lumbricoides* absorblood group B A and B antigens from the host, and modify the cuticular carbohydrate expression as a kind of antigenic mimicry (Ahumada et al., 2023). Blood group H antigen was revealed in *A. lumbricoides* isolated from blood group O persons. The fact that the extract did not inhibit the agglutination against anti-A, anti-B and anti-AB antibodies demonstrated the absence of A and B epitopes in *A. lumbricoides* obtained from blood group O persons (Ponce de León and Valverde, 2003; Ponce de León et al., 2005, 2006).

The antigens of *A. lumbricoides* are divided serologically into two groups, A+ and A-, according to the presence of A antigen. The majority of the parasites in persons with blood types A, AB or B are referred to A+ group, meanwhile worms from O blood type persons belong to A- group. Immune anti-A antibodies showed a strong association with the level of antibodies to *A. lumbricoides*. Importantly, persons with Blood group A demonstrated the lowest percentage of seropositivity to *A. lumbricoides* (13.7%). Thus, anti-B antibodies seem to protect against *A. lumbricoides*. The study revealed high level of immune antibodies in persons with weak blood group A and B antigens, associated with IgG antibodies to *T. canis* and A. *lumbricoides*. Both immune and IgG antibodies to *T. canis and* A*. lumbricoides* showed positive association with anemia.

The seropositivity to *T. canis* was accompanied by monocytopenia and eosinophilia, increased thymol probe and CRP; whereas, seropositivity to A. *lumbricoides* was associated with leukopenia, thrombocytopenia and increased serum urea and natural antibodies. The heterogeneity in the ABO substances expression of *T. canis a*nd *A. lumbricoides* might be involved in the escape of the host's immune response. Blood group-specific antigens are associated with many diseases. Human beings are confronted with microbial and parasite ABO blood group-like substances and produce antibodies without affecting their own blood group antigens (Beinart et al., 2017; Ella et al.,2011). It has been shown that IgG and IgM antibodies reactive with autologous A and B antigens are present in normal serum and are controlled by the specific antibodies complementary to these autoantibodies. Since IgM antibodies have been reported to be eliminated by heat inactivation, the immune antibodies in the present study were detected in the heated serum (Al-Muzairai et al., 2008; Özen et al., 2018; Dielievska et al., 2020).

The study revealed the presence of an association between anemia, group-specific immune antibodies and positive serology for *T. canis* and *A. lumbricoides.* Clinicians should target and screen for parasitic infection in persons with coxarthrosis, gonarthrosis and bone fractures.

The study provides group-specific data concerning the prevalence of *T. canis* infection among persons, carrying B antigen and A*. lumbricoides* infection among persons with weak blood group A and B antigens. The control of the level of immune antibodies in persons with seropositivity to *T. canis* and A*. lumbricoides* would prevent anemia and improve biochemical status in patients with coxarthrosis, gonarthrosis and bone fractures. The data indicate, that *T. canis* and *A. lumbricoides* share similar to human blood group antigens glycoproteins and may initiate the production of immune group-specific antibodies.

Conclusion

A high incidence of seropositivity to *A. lumbricoides* was found in people with Blood group B and weak A antigen and Blood group A with weak B antigen. The persons with blood group B showed increased seropositivity to *T. canis.* Seropositivity to *T. canis* and *A. lumbricoides* initiates the production of immune group-specific antibodies, associated with anemia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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