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The value of the blood group in ascariasis and toxocariasis

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Abstract
The predisposition of blood group type to some diseases has been established. It has been shown that some parasites have an affinity for A and B blood group antigens. The purpose of this study was to reveal the prevalence of ascariasis and toxocariasis depending on the blood group type in patients with bone and joint pathology, and to establish the features of hematological and biochemical parameters depending on the presence of a parasitic infection. Patients with bone and joint pathology underwent determination of hematological and biochemical parameters, detection of the blood group type, immune group-specific antibodies and IgG antibodies to *Ascaris lumbricoides* (*A. lumbricoides*), and *Toxocara canis* (*T. canis*). A high frequency of seropositivity to *A. lumbricoides* was revealed in individuals with blood group A and a weak B antigen, whereas seropositivity to *T. canis* was mostly found in persons with blood group B. The persons with seropositivity to *A. lumbricoides* showed by eosinophilia, leukopenia and thrombocytopenia, increased serum urea and immune group-specific antibodies. The persons with seropositivity to *T. canis* demonstrated eosinophilia, monocytopenia and hogh level of immune group-specific antibodies. The immune antibodies showed a direct correlation with IgG antibodies to *A. lumbricoides* and *T. canis*. A high level of immune antibodies was determined in individuals with weak A and B blood group antigens and was associated with anemia and eosinophilia. Monitoring the level of immune group-specific antibodies in individuals with seropositivity to *A. lumbricoides* and *T. canis* may allow preventing anemia in this category of patients.

Introduction

In recent years, the blood group type of the patients has begun to attract the attention of the researchers. Various distributions of the alleles of the erythrocyte A and B antigens are associated with different survival rates from diseases (1). ABO blood groups and helminth infections have been reported to be associated (2). The certain blood

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group expression may increase or decrease host susceptibility to parasitic infection. *Blood* group antigens facilitate uptake or adhesion through providing receptors or coreceptors for parasites and organization of membrane microdomains. Thus, in giardiasis, ya high incidence of persons with blood group A has been registered (3). A correlation was found between human blood types with giardiasis, as most blood type A persons were symptomatic patients (4). The association between ABO human blood group and *Ascaris lumbricoides (A. lumbricoides), Ancylostoma duodenale, Trichuris trichura, Enterobius vermicularis, Taenia solium* and *Hymenolepis nana* was studied (5). The dominant parasitic infections of O and A blood groups were *A. lumbricoides* and *Ancylostoma duodenale* in male and *A. lumbricoides* and hookworm in female. In B blood group male and female were infected with roundworm and hookworm.

The researchers have noted a high incidence of parasitic infection in patients with bone and joint pathology. The echinococcal disease is considered to be the most frequent form of osteoarticular parasitic infections. Bone form is the worst form of the disease. The histopathological lesions of bone are not well characterized. Bone echinococcal disease is an invasive disease due to the absence of a pericyst, limiting the extension of the lesions (6). The studies on the prevalence of *toxocariasis* and *ascariasis* in persons with bone and joint pathology remain few. *Toxocara canis* (*T. canis*) and *A. lumbricoides* were reported to share similar glycoproteins to human blood group antigens (7, 8). The type substances, especially A substance, of *A. lumbricoides*, have been shown to have a certain correlation with the blood group types (9). The experiments suggest that *A. lumbricoides* might absorb A and B antigens from the host, and modify the cuticular carbohydrates expression as a kind of antigenic mimicry.

Larval parasites have been shown to export glycosylated macromolecules with methylated oligosaccharide structures, similar to the mammalian blood group antigens. *Toxocariasis*, caused by the parasite *Toxocara*, affects millions of pediatric and adolescent populations globally (10, 11). Four forms of human *toxocariasis* have been reported: common *toxocariasis*, visceral larva migrans, ocular larva migrans, *neuro toxocariasis*, and asthma (12). *Toxocara* antigens have been reported to be similar to human blood group antigens and blood group substances have been used for the diagnosis of *toxocariasis* (13). The antibodies, generated to *T. canis* infection were reported to recognise the GalNAc residue. Antibodies generated to helminth infections other than *T. canis* were unreactive with the glycans. The human dendritic cell lectin, was found to bind both *Toxocara* products and mammalian blood group antigen H. A sensitive enzyme-linked immunosorbent assay (ELISA) that detects IgG-specific antibodies for the study of humoral immune response in the AB0 system after intravenous injection of helminthic A and B blood group antigens (*T. canis*) has been applied. After transfusion, high levels of anti-A-IgG were revealed. The studies showed that unusual presentation of A antigen to host leads to IgG reaction (14).

The standard test for diagnosing toxocariasis and ascariasis is ELISA that detects IgG-specific antibodies against *T. canis* and *A. lumbricoides* and is recommended by the Centers for Disease Control and Prevention (15). The term "glycan mimickry" has been proposed as an active strategy of parasites to use their glycans to target cells within the host to promote their survival (16). Researchers point to antigenic mimicry of group erythrocyte antigens and parasitic antigens (17). Antigenic mimicry complicates an effective immune response and may lead to autoimmunization and chronicity of parasitic infection. In this regard, it is important to study not only the intensity of the serological response (the level of IgG antibodies) to a parasitic infection, but also to investigate group-specific immune antibodies, since immune antibodies may be produced under the influence of bacterial, viral and parasitic antigens. The aim of the study was to identify the incidence and pathological effects of *A.lumbricoides* and *T.canis* infection in persons with bone and joint pathology depending on blood group type by changes in hematological and biochemical blood parameters, native agglutinating and immune antibodies.

Materials and Methods

96 individuals aged 64.2±7.1 years old with bone and joint pathology (coxarthrosis, gonarthrosis, ligamentum ruptures and bone fractures) were examined. All patients underwent clinical blood analysis, biochemical blood analysis, determination of blood group type, IgG antibodies to *A. lumbricoides* and *T. canis*, and immune group-specific antibodies.

Blood group B with a weak A antigen was determined in 11 individuals, and blood group A with a weak B antigen was identified in 10 individuals. A and B blood groups were determined in 58 and 17 persons, respectively. The control group included 35 persons without weak A and B antigens with seronegativity to *A. lumbricoides and T. canis.*

Blood collection

The plasma samples measured biochemical parameters and IgG antibodies against *T. canis* and *A. lumbricoides*.

Blood group typing has been performed according to the protocols (18).

The native agglutinating and immune group-specific antibodies were evaluated at 4°C and 37°C. Immune antibodies were detected by haemagglutination method (19). The plasma samples were diluted with 0.9% saline (1:4) and heated at 56°C for 30 minutes. The heated plasma (100 µl) was added to 50 µl of 2% washed in 0.9% saline suspension of erythrocytes and incubated at 37°C for one hour. The agglutination was estimated under microscope MicroMed XS-3330 from strongly positive (4+) to negative (-).

Analysis of hematological parameters

The level of erythrocytes, hemoglobin, leukocytes, neutrophils, lymphocytes, eosinophils, monocytes, erythrocyte sedimentation rate (ESR) and platelets were analyzed by the autohematological analyzer MYTHIC 3CRP - 3 DIFF (Cormay Diagnostics, Poland).

Urea

The content of urea was measured by the reaction between diacetyl monoxime and urea in the presence of sulfuric acid, phosphoric acid, thiosemicarbazide and ferric chloride (20). The absorbance was measured at a wavelength 520 nm.

Creatinine

The level of creatinine was detected by color Yaffe reaction using a commercial kit (Felicit diagnostics) (21). The optical density of the analyzed and standard samples was measured after 30 seconds (E1 and E2) and 90 seconds (E3 and E4) at a wavelength of 505 nm. Creatinine concentration was calculated using the formula: Concentration of calibrator sample × (E3-E1)/(E4-E2).

Aspartate aminotransferase activity

Aspartate aminotransferase (AST) was detected by dinitrophenylhydrazine method using Multiparametric Photocolorimetric biochemical analyzer ALIZE (Lisabio, France) (22). Substrate-buffer solution (0.25 ml) was added to the blood serum (0.05 ml) and incubated for 60 minutes at 37°C. 0.25 ml of 2,4 dinitrophenylhydrazine was added to the mixture and incubated for 20 minutes at room temperature. 0.4 M sodium hydroxide solution (2.5 ml) was added and incubated for 10 minutes at room temperature. The optical density of the test sample for AST was measured against a control sample on a photometer at a wavelength of 500-560 nm.

Alanine aminotransferase activity (ALT)

For this purpose, 1 ml of working solution (substrate and coenzyme-enzyme reagent) was mixed with 0.1 ml of serum. The optical density was measured at a wavelength of 340 nm after 1 (E1) and 3 (E2) minutes. The difference in extinction (E2-E1)/3 was calculated.

Detection of anti-T. canis and anti-A. lumbricoides IgG antibodies

Commercial ELISA kits (Abcam, UK) were used to measure serum *anti-T. canis* and *anti-A. lumbricoides* IgG antibodies. The serum and control samples (IgG-positive, IgG-negative, IgG cutoff, and substrate blank) were prepared. 100 µl of the control or serum samples were added to the pre-coated 96-well plates and incubated for 1 hour at 37 °C. The plates were washed three times with washing buffer, incubated with 100 µl of protein A horseradish peroxidase conjugate for 30 minutes at room temperature, washed three times, and incubated with 100 µl of 3,3′,5,5′-Tetramethylbenzidine substrate solution for 15 minutes at room temperature. 100 µl of the stop solution was added. The absorbance was measured at 450 nm. IgG index: >1.0: positive; 0.91-0.99: equivocal result; <0.9: negative.

Statistical analysis

All the data were analyzed using Statistica 10.0 software (StatSoft, Kraków, Poland). The mean and standard deviation (SD) were used to describe the data. Differences were considered statistically significant at $p < 0.05$. The reliability of the results was evaluated by the Mann–Whitney U test. Correlation analysis was performed using Spearman's correlation test.

Results

59.3% of the persons with bone and joint tissue pathology (n=57) demonstrated seropositivity to *T. canis*, and 40.6% of the studied patients (n=39) showed seropositivity to *A. lumbricoides*. The distribution of blood group types in individuals with seropositivity to *A. lumbricoides* was as follows: Group A: n=10, Group A with weak B antigen: n=20, Group B: n=3, Group B with weak A antigen: n=5. The pattern of blood group types in persons with seropositivity to *T. canis* was the following: Group A: n=6, Group A with weak B antigen: n=22, Group B: n=23, Group B with weak A antigen: n=6. The seropositivity to *A. lumbricoides* was more often detected in persons with A blood group with weak B antigen and seropositivity to *T. canis* was more frequent in persons with B blood group.

The hematological and biochemical parameters for the persons with weak blood group antigens are displayed in Tables 1 and 2. Persons with blood group B and weak antigen A showed increased levels of eosinophils, warm group-specific natural and immune antibodies and seronegativity to *T. canis*. Persons with group A and weak antigen B demonstrated decreased levels of leukocytes, monocytes and increased levels of eosinophils, warm immune antibodies and IgG antibodies to *A. lumbricoides*.

The immune antibodies were inversely associated with the levels of erythrocytes and hemoglobin and directly correlated with ESR, eosinophils, thymol probe, creatinine, C-reactive protein, IgG antibodies to *T. canis* and *A. lumbricoides* (Table 3). Natural agglutinating antibodies were directly correlated with ESR, eosinophils, and antibodies to *A. lumbricoides*.The antibodies to *T. canis* were inversely correlated with the level of ESR, hemoglobin and were directly associated with the levels of eosinophils, immune antibodies and glucose (p<0.05). The antibodies to *A. lumbricoides* were directly associated with the levels of eosinophils, urea, creatinine, and thymol probe (p<0.05). Importantly, the antibodies to *A. lumbricoides* in the group with weak blood group B antigen showed association with natural agglutinating and warm immune antibodies (r=0.4; r=0.4) (p<0.05), whereas the antibodies to *A. lumbricoides* in the group without weak blood group B antigen showed association only with natural agglutinating antibodies (r=0.2) (p<0.05).

Table 1. Clinical blood analyses of persons with bone and joint tissue pathology (mean±SD)

Variables	Group I BA+ (n=11)	Group II B (n=17)	Group III AB+ (n=10)	Group IV A (n=58)
Erythrocytes (10^{12}/l)	4.95±0.27	5.01±0.34	5.02±0.36	4.77±0.3
Hemoglobin (g/l)	144.42±8.3	140.6±8.1	146.7±8.7	142.25±8.3
Platelets (10^9/l)	239.54±16.4	263.6±17.63	270.4±17.8	262.22±17.1
Leukocytes (10^9/l)	6.2±0.35	6.47±0.38	6.35±0.37**	7.4±0.43
ESR (mm/h)	9.2±3.2	10.9±1.8	6.25±2.1	8.96±1.7
Eosinophils (%)	2.81±0.6*	1.76±0.43	3.33±0.43**	2.0±0.41
Neutrophils (%)	55.5±7.2	55.94±7.56	57.6±6.54	56.6±6.1
Lymphocytes (%)	31.72±3.74	34.2±3.85	32.8±4.2	31.33±4.0
Monocytes (%)	7.3±0.78	5.7±0.74	5.0±0.32**	6.67±0.6
T. canis abs	0.91±0.02*	1.43±0.04	1.12±0.03	1.15±0.03
A. lumbricoides abs	0.99±0.03	0.97±0.03	0.94±0.02**	0.80±0.02
Natural abs at 4°C	3.31±0.2	2.9±0.2	2.9±0.2	2.41±0.35
Natural abs at 37°C	2.62±0.2*	2.04±0.15	2.5±0.2	2.44±0.2
Immune abs at 4°C	1.57±0.15*	2.15±0.17	2.5±0.2**	1.66±0.17
Immune abs at 37°C	2.43±0.2*	1.72±0.14	3.0±0.34**	1.91±0.2

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

Table 2. Biochemical parameters of persons with bone and joint pathology (mean±SD)

Variables	Group I BA+ (n=39)	Group II BA- (n=17)	Group III AB+ (n=10)	Group IV AB- (n=34)
Glucose (mmol/l)	5.53±0.24	5.21±0.2	5.61±0.25	5.74±0.27
Total protein (g/l)	72.9±6.13	78.83±7.7	78.57±7.63	78.2±7.4
Alt (un/l)	16.6±1.31	19.6±1.4	28.4±2.0	23.32±1.56
Ast (un/l)	24.6±1.77	24.4±1.64	33.6±2.41	31.82±2.11
Urea (mmol/l)	3.56±0.48	4.44±0.6	4.4±0.6	4.36±0.56
Creatinine (mcmol/l)	73.33±6.41	77.6±7.2	81.5±8.4	79.0±7.5
Thymol probe (units)	2.34±0.2*	3.1±0.34	2.51±0.15	2.76±0.27

Table 3. Correlation analysis of the studied parameters in persons with bone and joint pathology

Parameters	AB+ and BA+		
	Immune abs	Abs to *Ascaris lumbricoides*	Abs to *Toxocara canis*
Er	-0.6	-0.2	-0.3
Hb	-0.3	-0.38	-0.3
ESR	.0.4	.0.3	0.62
Eos	0.27	0.15	
Urea		0.46	
Glu			0.56
Crea	0.6	0.42	
Thymol probe	0.4	0.47	
Abs to *Toxocara canis*	0.3		
Abs to *A. lumbricoides*	0.4		
Natural abs		0.23	

AB+: group A with weak B antigen, BA+: group B with weak A antigen, abs: antibodies, Er: erythrocytes, Hb: hemoglobin, ESR: erythrocyte sedimentation rate, Eos: eosinophils, Crea: creatinine.

Table 4. The studied parameters according to the seropositivity to *T. canis* and *A. lumbricoides* (mean±SD)

	Seropositivity to *T. canis* (mean±SD) N=57	Seronegativity to *T. canis* (mean±SD) N=39	Seropositivity to *A. lumbricoides* (mean±SD) N=39	Seronegativity to *A. lumbricoides* (mean±SD) N=59
Monocytes (%)	5.4±0.3*	6.8±0.43		
Eosinophils (%)	2.46±0.15*	2.1±0.11		
Thymol probe (units)	3.2±0.2*	2.2±0.13	2.83±0.2**	2.3±0.19
CRP (units)	8.25±0.67*	2.3±0.17		
Platelets (10^9)			237.64±16.4**	272.0±18.1
Leukocytes (10^9)			6.53±0.41**	7.27±0.5
Urea (mmol/l)			5.67±0.31**	4.2±0.27
Natural abs			3.5±0.24**	2.7±0.2

Abs: antibodies, CRP: C-reactive protein, *: $p<0.05$ compared to the group with seronegativity to *T.canis*, **: $p<0.05$ compared to the group with seronegativity to *A. lumbricoides*.

In the correlation analysis of the entire group of persons with bone and joint pathology, the antibodies of *T. canis* showed a direct association with the titer of group-specific anti-A antibody (r=0.54) ($p<0.05$).

Hematological and biochemical parameters of the persons with bone and joint pathology have been analyzed according to the seropositivity or seronegativity to *T. canis* and *A. lumbricoides*. Thus, the persons with seropositivity to *T. canis* showed decreased levels of monocytes and increased levels of eosinophils, thymol probe, and C-reactive protein (Table 4). The persons with seropositivity to *A. lumbricoides* showed decreased levels of platelets and leukocytes and increased levels of thymol probe, urea, and natural agglutinating antibodies ($p<0.05$).

Discussion

This study presents the distribution of blood group types and main changes in the biochemical and hematological blood parameters of the persons with seropositivity to *T. canis* and *A. lumbricoides* suffering from bone and joint pathology. This is the first serological report of *T. canis* and *A. lumbricoides* seropositivity among persons with bone and joint pathology, carrying weak blood group antigens. The high frequency of seropositivity to *T. canis* and *A. lumbricoides* revealed in persons with bone and joint pathology might be explained by their unique capacity of directly altering hematopoiesis in the bone marrow. Many parasites have been reported to invade bone marrow. Once inside the hosts, the parasites reside in monocyte-derived macrophages, in the liver and spleen. However, it has also been widely reported that they can be found in the bone marrow of infected individuals (23). Although parasitic infections generally do not lead to mortality; chronic infections may lead to considerable morbidity, since parasites suppress immune responses to non-parasite antigens and other infections. The high level of seropositivity to *T. canis* and *A. lumbricoides* in persons with bone and joint pathology reflects its importance as a public health concern and supports the need to increase public awareness of this issue.

High level of IgG antibodies to *T. canis* in persons with B blood group type agree with the data of the researchers, suggesting evidence for blood-group-like oligosaccharides in *T. canis* (24). Thus, a blood group A substance was revealed on the surface coat of *T. canis* infective larvae using fluorescent anti-A1 Helix Pomatia lectin by immuno-electron microscopy. Blood group antigen A of *Toxocara* was reported as a marker for successful diagnosis of *toxocariasis*. Mass spectrometry defined two major O-linked glycans, containing N-acetylgalactosamine (A blood group antigen), galactose (B blood group antigen), and fucose (H blood group antigen). The persons with A blood group type and weak B antigen demonstrated increased levels of IgG antibodies to *A. lumbricoides*. *A. lumbricoides* has been associated with the ABO system (25). Human anti-A and anti-B agglutinins were neutralized by *A. lumbricoides* antigens (26). Egg agglutinins produced in response to antigens of *A. lumbricoides* are responsible for fighting parasites, carrying carbohydrate antigens (27). *A. lumbricoides* isolated from O blood group persons demonstrated the presence of H antigen. The extract did not inhibit the agglutination against anti-A, anti-B and anti-AB antibodies demonstrating the absence of A and B epitopes in *A. lumbricoides* extracts from O blood group persons (28). The antigens of *A. lumbricoides* were reported to be divided serologically by the presence or absence of A substance into A+ and A- groups. The majority of the parasites in persons with A, AB or B blood types belong to A+ group, whereas those worms from O blood type persons belong to A- group.

The heterogeneity in the ABO epitope expression of *T. canis* and *A. lumbricoides* might be involved in the escape of the host's immune response. The present study revealed the direct association of seropositivity to *T. canis* and *A. lumbricoides* with the level of eosinophils and immune antibodies. Interestingly, the level of immune antibodies was significantly higher in persons with weak blood groups A and B antigens. Immune antibodies showed a negative association with erythrocytes and hemoglobin. Similarly, the negative association with erythrocytes and hemoglobin was revealed for IgG antibodies to *T. canis* and *A. lumbricoides*. Human beings are confronted with microbial and parasite ABO blood group-like antigens and develop an immune response against pathogens without affecting their blood group antigens. It has been shown that anti-A and anti-B specific IgG may develop as natural antibodies and may be found in all persons lacking A or B antigens. It has been revealed that IgG and IgM antibodies reactive with autologous A and B antigens are present in normal serum. This autoantibody activity to A and B antigens has been reported to be controlled by antibodies complementary to the V regions of autoantibodies. Since IgM antibodies are reported to be eliminated by heat inactivation, the immune antibodies in the present study were detected using the heated serum (29-31).

This study complements existing data on the association of blood group antigens with parasite antigens (32). Our findings 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. Since immune antibodies tend to disappear as the antigenic stimulus decreases, the quantitative determination of these antibodies would allow the evaluation of the treatment efficacy.

Monitoring the level of immune antibodies in persons with seropositivity to *T. canis* and *A. lumbricoides* might reflect the dynamics of hematological and biochemical parameters in persons with bone and joint pathology. The blood group antigens will continue to be used as a major tool in the investigation of the causative agents for diseases, whether they are viral, bacterial, or parasitic in nature.

Conclusion

Individuals with blood group B are predisposed to infection with *T. canis*, whereas persons with blood group A and weak expression of B antigen are susceptible to infection with *A. lumbricoides.* The production of immune group-specific antibodies caused by parasitic infection negatively affects hematopoiesis. Therefore, the determination of group-specific immune antibodies can be used as a method for assessing the impact of parasitic infection on hematological and biochemical parameters in individuals with seropositivity for *T. canis* and *A. lumbricoides.*

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Conflict of interest

There are no conflicts of interest.

Ethics approval

The study was approved by the Ethical Committee of Kharkiv National Medical University (Institutional approval N 5, 26. 01. 24). Informed consent was obtained from all the participants. The study adhered to the ethical guidelines of the Declaration of Helsinki.

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