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Measurement of *Ascaris lumbricoides* IgG antibody, associated risk factors and identification of serum biochemical parameters as biomarkers of pathogenicity: among patients with gastrointestinal complains in Pakistan

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Abstract. Soil transmitted helminths are causing significant morbidity worldwide and the most common infection is *Ascaris lumbricoides* in developing countries. The present study aimed to determine the immuno-epidemiological status of ascariasis among patients with gastrointestinal complaints and to identify the associated risk factors and eventual changes in serum biochemical parameters to reflect its pathogenicity. This study was conducted on 700 respondents aged between 5-45 years. A total of 356 patients participated in an enzyme-linked immunosorbent assay (ELISA) study to determine anti-*Ascaris* IgG levels and biochemical parameters. The overall seroprevalence was 58.4%, with 100% sensitivity and 84.4% specificity of the assay. The infection was highest among the 21-28 year age group (14.0%), and ascariasis was found to be not significantly ($P>0.05$) different between the age groups. The results showed that the risk of ascariasis was significantly ($P<0.05$) increased in individuals who had no contact with soil (OR=4.6, 95% CI: 1.9-10.8), eating unwashed vegetables one month prior to the study (OR=2.7, 95% CI: 1.4-5.2), eating mixed food (OR=2.4, 95% CI: 1.2-4.7), drinking pressure pump water (OR=3.4, 95% CI: 1.9-6.1), and those who had no complain of vomiting (OR=3.1, 95% CI: 1.6-5.8) and nausea (OR=1.9, 95% CI: 1.1-3.2). The results showed significantly ($P<0.05$) elevated level of serum alanine aminotransferase, alkaline phosphatase, serum cholesterol, total protein and globulin in anti-*Ascaris* IgG positive cases than the control group. The study concluded that patients who visited health care centres with gastrointestinal complain were at higher risk of ascariasis as compared to other diseases. In conclusion epidemiological studies are needed to establish baseline data for public health authorities in order to plan and implement health education programs to reduce the impact of the disease.

INTRODUCTION

Soil transmitted helminthiasis (STH) is a global health problem affecting more than one billion people, particularly the rural communities in the developing world (Hotez *et al.*, 2007). Soil-transmitted helminths are a group of parasitic nematode worms that includes *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms. The World Health Organization placed them under the neglected tropical diseases (Dunn *et al.*, 2016). Adult *A. lumbricoides* reside in the gastrointestinal tract of humans and often causes no clinical signs or symptoms. Asymptomatic ascariasis can be detected by observing the presence of eggs in the stool (Pal, 2014). However, infection with a large number of worms is associated with clinical disease comprised of pulmonary, intestinal, appendicular, hepatobiliary and pancreatic ascariasis (Sundriyal *et al.*, 2015). In endemic countries,

Ascaris infection is a common cause of protein-energy and micronutrient deficiencies which may lead to stunted growth, impaired learning, defective immune regulation and increased risk of other parasitic infections (Papier *et al.*, 2014). Changes in serum biochemical parameters have been associated with nematode infections (Singh *et al.*, 2004).

In Pakistan *Ascaris lumbricoides* infection is widely prevalent, with variable distribution in all parts of the country. Therefore, accurate diagnosis of soil-transmitted helminthiasis is important for individual patient management, drug efficacy evaluation, identification of infected individual, monitoring control programs and elimination (McCarthy *et al.*, 2012; Speich *et al.*, 2015). The detection of antibodies or antigens could provide a simpler, more rapid diagnosis of *Ascaris* infection than conventional stool microscopy (WHO, 2015). In control programmes, the potential to reduce STHs may be associated with antibodies to provide a good marker of infection in areas where people are frequently exposed to intestinal pathogens (Zakzuk *et al.*, 2013; Moss *et al.*, 2014). However, studies on application of immunodiagnostic tests to provide epidemiological data on *A. lumbricoides* are limited in Pakistan. The present study was designed to provide sero-epidemiological data of ascariasis among patients who had visited health care centers with gastrointestinal complaints and to measure the serum biochemical changes which reflects early development and could predict eventual disease.

MATERIALS AND METHODS

Sample Selection and Surveys

This study was approved by the institutional review board of Quaid-i-Azam University, Islamabad and Pakistan Institute of Medical Sciences (approval no. F.1-1/2015/ERB/SZABU/). Written consent was obtained from all participants in this study; parents consented for children below 18 years of age. Blood sampling was conducted from August 2015 to March 2017 on patients who that visited clinics for gastroenteritis. The participants in this study were inhabitant from different localities of Pakistan including: Islamabad, Rawalpindi, Peshawar, Abbottabad, Muzaffarabad, Tank and Mianwali (Figure 1). The sample size was determined by using the formula: $n= Z^2 P (1-P)/d^2$ (Daniel, 1999), where n is the sample size, Z is the statistic corresponding to level of confidence, P is expected prevalence, and d is precision. The final study consisted of 356 participants aged between 5-45 years (Figure 2). The sensitivity and specificity of the assay was determined by using the control sera (n=47). The *A. lumbricoides* positive control sera (n=15) were confirmed by stool examination. The negative control sera (n=13) taken from healthy individuals were confirmed by serology (ELISA, immunofluorescent antibody tests, polymerase chain reaction) and faecal examination techniques (i.e. direct wet mount in saline/iodine/haematoxylin stain, sedimentation and Kato-Katz methods). The sera positive for other parasitic infections (n=19) includes, fascioliasis, enterobiasis, malaria, amebiasis and giardiasis.

Questionnaire Administration

After completion of the consent process, questionnaires were administered to participants that were translated to their local language. The questionnaire included information on age, gender, educational background, socio economic status, family size, history of anthelmintic treatment, access to safe water source, sanitation facility, medical symptoms, footwear, and finger nails status.

Sample Collection and Processing

Blood (1 ml) was taken from each participant in non EDTA vacutainers. The blood samples were separated by centrifugation and sera stored at –20°C until used for immunological assays.

Measurement of Serum IgG Against *Ascaris* Antigen

Commercial ELISA kits were used to detect IgG antibodies against *Ascaris lumbricoides*

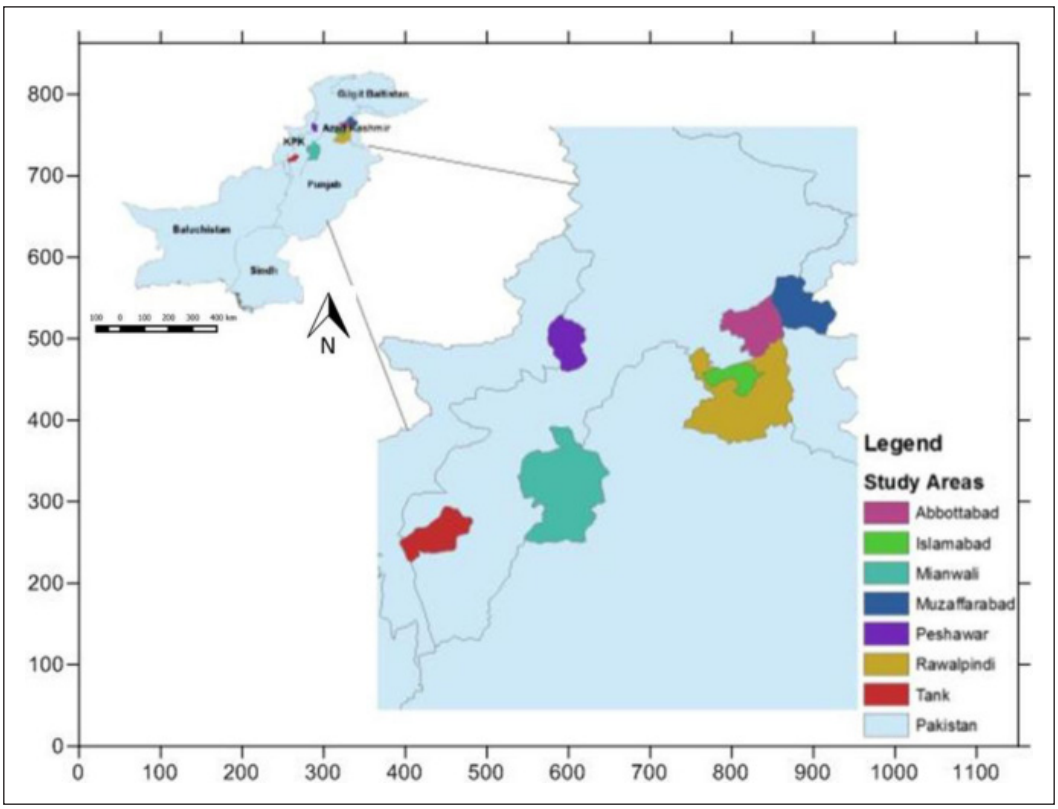

Figure 1. Map of Pakistan showing studied area.

(AccuDiag™). The assay was performed according to manufactures instructions. Briefly, 100 µl of control and unknown sample (diluted 1:100) were added to adult worms extract coated wells and incubated at room temperature for 10 minutes. After washing 3 times, wells were incubated with 2 drops of enzyme-conjugate. After another washing, 2 drops of chromogen (tetramethylbenzidine) and a substrate (hydrogen peroxide) were added to wells and incubated for 5 minutes at room temperature. Addition of 2 drops of stop solution ends the reaction and absorbance was recorded at 450 nm using a microplate reader (Bio-Red). The cut-off was set by the mean optical density (OD) of the negative reference serum, plus three times standard deviations (0.16+3*0.05=0.31). Serum samples with OD≥0.3 (cut-off) were considered as positive. The sensitivity and specificity of the assay was determined with following formulae:

$$\text{Sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

$$\text{Sensitivity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

Biochemical Assays

The serum total protein, globulin, albumin, glucose, cholesterol-liquizyme and liver enzymes i.e. aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), alkaline phosphatase (ALP) were measured according to manufactures instructions (Spectrum and Futura System Group) by using a biochemistry analyzer and spectrophotometer.

Data Management and Statistical Analysis

All data generated from this study were maintained in Microsoft Excel (2010), and statistical analyses were carried out in SPSS

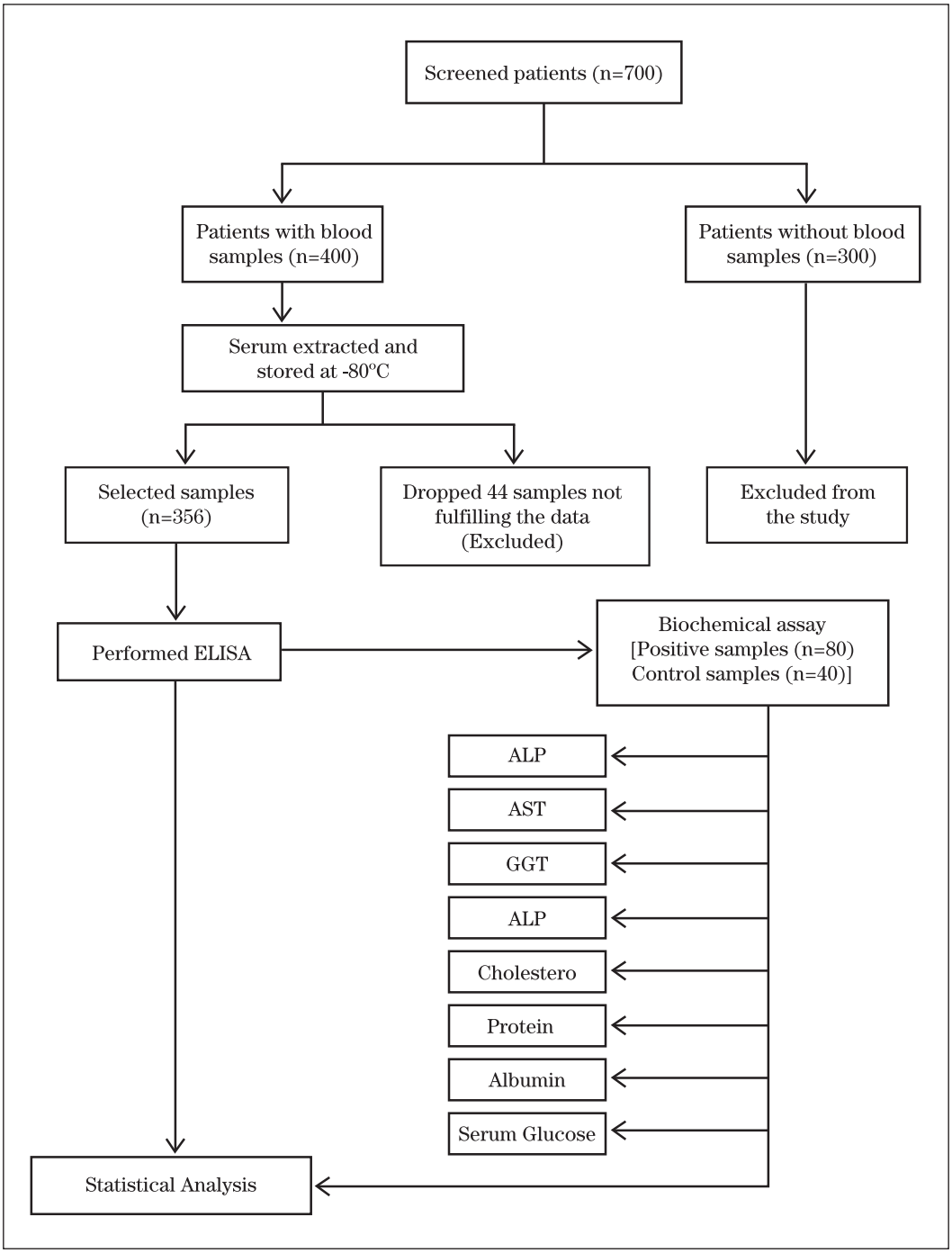

Figure 2. Participation selection, diagnostic procedures and data collection.

version 20.0. Statistical methods included were percentages for categorical variables, while mean (±SD) for numerical variables. Chi-square test and the odds ratio were computed to measure the strength of association. Logistic regression model was subsequently conducted for multivariate analysis and adjusted odds ratio with 95% confidence interval (CI) was calculated for risk factors identification. The level of significance was set at $P \leq 0.05$. Independent sample t-test was applied to analyze bio-

chemical parameters by comparing the means of cases and controls. The software Graph Pad Prism V. 5 was used for graphical representation of OD values of each sample.

RESULTS

Study Subject Characteristics

A total of 356 participants were included in the study and a 100% response rate was obtained in filling out the questionnaires. The mean age of the studied participants was 22.3 ± 10.9 years. Of the total studied participants, 166 (47.0%) were females and 190 (53.0%) males. The literacy rate among participants was 29.0%, 71.0% were illiterate (below primary <5 years). The participants residing in rural communities were 152 (43.0%), whereas 204 (57.0%) were living in urban areas. Family size that comprised of 3-7 family members were 118 (33.0%), and 238 (67.0%) were those with 8 or more family members.

Overall Sero-prevalence and Anti-*Ascaris* IgG level

The sensitivity (100%; 95% CI: 78.2-100) and specificity (84.3%; 95% CI: 67.2-94.7) of the assay was determined to test the diagnostic performance of *Ascaris* IgG human ELISA test (Figure 3A). Of the 356 participants, 58.4% were found seropositive, 148 (41.6) were negative.

Sero-prevalence of *Ascaris* IgG across Socio-demographic Characteristics

Table 1 shows sero-prevalence of anti-*Ascaris* IgG across socio-demographic characteristics of studied participants. The prevalence of anti-*Ascaris* IgG in male and female participants was equal (29.0%). The

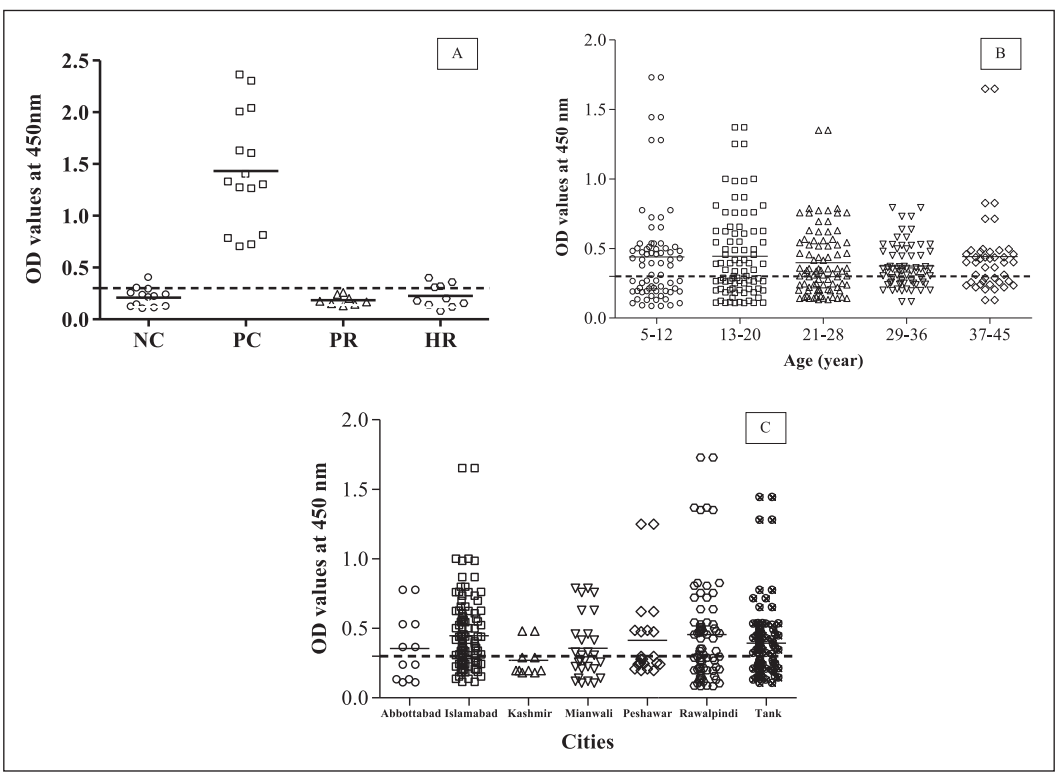

Figure 3. Scatter plots depicting the ranges of OD values obtained with *Ascaris* IgG ELISA test (A) Diagnostic performance of *Ascaris* IgG human ELISA test with controls: positive control (PC), negative control (NC), protozoan reference (PR) and helminth reference (HR); (B) with respect to age groups and (C) cities. A serum is considered positive when its absorbance value is above cut-off value OD>0.3. Dotted line represents cut-off point.

Table 1. Results of univariate analysis showing association between sero-prevalence of *Ascaris* IgG and socio-demographic characteristics among studied participants

Characteristics	Total N (%)	Positive N (%)	χ^2	Odds ratio (OR)	95% CI Lower-upper	P-value
Gender						
Female	166(46.6)	104(29.2)	2.28	1.39	0.91-2.12	0.131^{NS}
Male	190(53.4)	104(29.2)		Reference		
Locality						
Rural	152(42.7)	122(34.3)	52.07	5.58	3.43-9.08	0.000**
Urban	204(57.3)	86(24.2)		Reference		
Age in years						
5-12	72(20.2)	40(11.2)	2.71	Reference		
13-20	86(24.2)	46(12.9)		0.92	0.49-1.73	0.795^{NS}
21-28	86(24.2)	50(14)		1.11	0.59-2.09	0.744^{NS}
29-36	68(19.1)	44(12.4)		1.47	0.74-2.89	0.27^{NS}
37-45	44(12.4)	28(7.9)		1.40	0.65-3.03	0.392^{NS}
Weight (kg)						
13-27	92(25.8)	46(12.9)	3.77	0.66	0.39-1.12	0.125^{NS}
28-42	58(16.3)	36(10.1)		1.08	0.57-2.03	0.817^{NS}
43-57	136(38.2)	82(23)		Reference		
>57	70(19.7)	44(12.4)		1.11	0.62-2.02	0.721^{NS}
Education						
Illiterate	252(70.8)	122(34.3)	35.62	0.19	0.11-0.35	0.000**
Secondary school	104(29.2)	86(24.2)		Reference		
Months						
Jan-Feb	210(59)	118(33.1)	7.33	1.41	0.73-2.74	0.310^{NS}
July-Aug	34(9.6)	20(5.6)		1.57	0.63-3.92	0.332^{NS}
Sept-Oct	42(11.8)	20(5.6)		Reference		
Nov-Dec	70(19.7)	50(14)		2.75	1.24-6.10	2.750^{NS}
Climate						
Cold and Dry	60(16.9)	40(11.2)	5.06	3.00	1.06-8.52	0.039*
Cold and Humid	220(61.6)	130(36.5)		2.17	0.85-5.51	0.105^{NS}
Hot and Dry	56(15.7)	30(8.4)		1.73	0.61-4.88	0.300^{NS}
Hot and Humid	20(5.6)	8(2.2)		Reference		
Size of family						
3-7	238(66.9)	56(15.7)	8.74	Reference		
>7	118(33.1)	152(42.7)		0.51	0.32-0.8	0.003*
District						
Abbottabad	12(3.4)	6(1.7)	0.26	0.67	0.19-2.23	0.51^{NS}
Islamabad	118(33.1)	82(23)		1.52	0.85-2.70	0.155^{NS}
Muzaffarabad	10(2.8)	2(0.6)		0.17	0.03-0.83	0.029*
Mianwali	28(7.9)	14(3.9)		0.67	0.28-1.56	0.351^{NS}
Peshawar	22(6.2)	8(2.2)		0.38	0.15-1.00	0.05*
Rawalpindi	76(21.3	42(11.8)		0.82	0.44-1.53	0.538^{NS}
Tank	90(25.3)	54(15.4)		Reference		
Socioeconomic status						
Poor	160(44.9)	156(43.8)	182.66	108.0	38.1-306.1	<0.0001**
Good	196(55.1)	52(14.6)		Reference		
Occupation						
Government job	237(66.6)	171(48)	54.99	0.17	0.11-0.282	0.000**
Other job	160(44.9)	156(43.8)		Reference		
Animals at home						
Large ruminants	48(13.5)	20(5.6)	47.49	1.35	0.66-2.73	0.413^{NS}
Small ruminants	98(27.5)	34(9.6)		Reference		
Pet animals	210(59)	154(43.3)		5.18	3.09-8.68	0.000**
Concrete floor at home						
Yes	142(39.9)	110(30.9)	35.25	4.07	2.53-6.55	0.000**
No	214(60.1)	98(27.5)		Reference		
Total	**356**	**208(54.8)**				

** P<0.01, ^{NS} P>0.05, Reference: Null value OR=1 Exposure does not affect odds of outcome.

anti-*Ascaris* IgG level varied among age groups (Figure 3B), lowest in the 37-45 year group (7.9%). The higher prevalence was found in the 21–28 year group (14.0%) and the association between age groups and infection was not significant (P=0.6). The prevalence was 34.3% for rural participants and 24.2% among those residing in urban areas which was significantly different (χ^2=52.0; P<0.0001; OR=5.6). Significantly higher prevalence was found among illiterate subjects (34.3%: χ^2=35.6; P<0.0001; OR=0.2) and among those with family size >7 (42.7%: χ^2=8.7; P=0.003; OR=0.5). District wise, significantly (χ^2=0.3; P=0.006) different prevalence of anti-*Ascaris* IgG (Figure 3C) was observed with the highest in Islamabad (23.0%) followed by Tank (15.4%), Rawalpindi (11.8%), Mianwali (3.9%), Peshawar (2.2%), Abbottabad (1.7%) and Muzaffarabad (0.6%). The infection was significantly (χ^2=182.6; P<0.0001; OR=108) higher in subjects with poor socio-economic status (43.8%), then those who belonged to middle or high status (14.6%). Participants who had concrete floor houses (30.9%) showed significant (χ^2=35.2; P<0.0001; OR= 4.1) difference from those that did not live in concrete floor houses (27.5%). The infection was significantly (P<0.001) associated with participants occupation (OR=0.2) and their pet animals (OR=5.2).

Sero-prevalence of *Ascaris* IgG across Hygiene and Environmental Conditions

The sero-prevalence of anti-*Ascaris* IgG in relation to hygiene and environmental conditions of the participants is shown in Table 2. The prevalence of ascariasis in subjects who had contact with soil was 26.4% and 32.0% in those with no soil contact. The risk of infection was lower in participants with soil contact (χ^2=50.2; P<0.0001; OR=0.2). The habit of eating soil and hand washing practices after using the toilet with water only (44.4%) or with soup (14.0%) did not show significant difference (P>0.05). Regarding sanitary facility, 48.3% of participants who had poor sanitation facility were anti-*Ascaris* IgG positive, in comparison to 10.1% who had satisfactory sanitation. The type of sanitation facilities was significantly (χ^2=10.9; P=0.001; OR=2.3) different with infection. Significant difference (χ^2=10.2; P=0.001; OR= 0.1) was observed associated with drinking water, 52.8% of participants who drank untreated water were infected, compared to 5.6% of subjects who drank treated water. Source of drinking water showed significant (χ^2=4.7; P=0.031; OR=0.6) difference, 32% were infection positive who used public pipeline and 26.4% with pressure pump water. Significantly (P<0.05) lower risk association was observed in those who had sand pits at home/school (OR= 0.2), habit of wearing no shoes (OR= 0.4), and in those with finger nails not trimmed (OR=0.6). The subjects who had habit of eating vegetables and fruits without washing also showed significantly (OR= 0.2; P=0.001) lower risk of infection than those who ate washed. The study also showed significantly (P<0.05) lower risk of infection among subjects with history of eating unwashed vegetables over the previous month (OR=0.3), eating improperly cooked food (OR=0.4), those who consumed more vegetables (OR=0.2) and used a common knife for cutting all type of foods (OR=0.3).

Sero-prevalence of *Ascaris* IgG in Relation to Clinical Symptoms

Clinical symptoms observed among anti-*Ascaris* IgG positive participants are shown in Table 3. Among anaemic studied subjects, 12.4% were *Ascaris* IgG seropositive, compared to 46.1% who did not show anaemia. The difference was significant (χ^2=4.9; P=0.027; OR=1.9). A significantly lower risk of infection (χ^2=4.2; P=0.04; OR= 0.6) was observed among participants with diarrhoea (16.9%) compared to positive cases who did show diarrheic symptoms (41.6%). The participants who showed symptoms of vomiting (7.3%; OR=0.3), nausea (18.3%; OR=0.5) and fever (17.7%; OR=0.6) had significantly (P<0.05) lower risk for anti-*Ascaris* IgG, compared to those who did not show these symptoms. A significant (χ^2=14.0; P<0.0001) difference with appetite was observed, 27.2% was positive with poor appetite (OR=2.4) compared to 31.2% that had good eating habits. A significantly (p<0.05) lower risk of infection was observed in

Table 2. Results of univariate analysis showing association between sero-prevalence of *Ascaris* IgG and environmental risk factors among studied participants

Characteristics	Total N (%)	Positive N (%)	χ^2	Odds ratio (OR)	95% CI Lower-upper	P-value
Contact with soil						
Yes	216(60.7)	94(26.4)	50.26	0.18	0.11-0.29	0.000**
No	140(39.3)	114(32)		Reference		
Geophagia						
Yes	58(16.3)	30(8.4)	1.28	0.72	0.41-1.27	0.259^{NS}
No	298(83.7)	178(50)		Reference		
Sand pit at home/school						
Yes	174(48.9)	72(20.2)	40.72	0.24	0.15-0.38	0.000**
No	182(51.1)	136(38.3)		Reference		
Hand washing after toilet						
With water only	280(78.7)	158(44.4)	2.16	0.67	0.39-1.14	0.143^{NS}
With water and soap	76(21.3)	50(14)		Reference		
Habit of wearing shoes						
Yes	320(89.9)	194(54.5)	6.29	Reference	0.21-0.84	0.014*
No	36(10.1)	14(3.9)		0.41		
Sanitation facility						
Poor	272(76.4)	172(48.3)	10.97	2.29	1.39-3.77	0.001**
Satisfactory	84(23.6)	36(10.1)		Reference		
Finger nail status						
Trimmed	238(66.9)	148(41.6)	4.18	Reference	0.40-0.98	0.042*
Not trimmed	118(33.1)	60(16.9)		0.63		
Hand washing before food handling						
With water only	350(98.3)	202(56.7)	nc	0.10	0.01-1.88	0.125^{NS}
With water and soap	6(1.7)	6(1.7)		Reference		
Habit of washing vegetables and fruits before eating						
Yes	336(94.4)	204(57.3)	12.88	Reference	0.05-0.49	0.001**
No	20(5.6)	4(1.1)		0.16		
Eaten raw unwashed vegetables last month						
Yes	112(31.5)	44(12.4)	24.65	0.32	0.19-0.50	0.000**
No	224(68.5)	164(46.1)		Reference		
Cooking method						
Raw cooked	40(11.2)	16(4.5)	6.30	0.431	0.22-0.84	0.014*
Properly cooked	316(88.8)	192(53.9)		Reference		
Using common knife for cutting food						
Yes	332(93.3)	188(52.8)	6.57	0.26	0.09-0.78	0.016*
No	24(6.7)	20(5.6)		Reference		
Food type						
Mostly vegetables	70(19.7)	22(6.2)	26.15	0.25	0.14-0.43	<0.0001**
Mixed	286(80.3)	186(52.2)		Reference		
Sources of drinking water						
Pressure pump	144(40.4)	94(26.4)	4.67	Reference	0.40-0.96	0.031*
Public pipeline	212(59.6)	114(32)		0.61		
Drinking water						
Treated	22(6.2)	20(5.6)	10.19	Reference		
Untreated	334(93.8)	188(52.8)		0.129	0.03-0.56	0.006**
Total	**356**	**208(58)**				

** P<0.01, ^{NS} P>0.05, Reference: Null value OR=1 Exposure does not affect odds of outcome.

Table 3. Results of univariate analysis showing association between sero-prevalence of *Ascaris* IgG and its clinical presentation

Characteristics	Total N (%)	Positive N (%)	χ^2	Odds ratio (OR)	95% CI Lower-upper	P-value
HB level						
Low	60(16.9)	42(11.8)	5.04	1.78	0.98-3.24	0.06^{NS}
Normal	282(79.2)	160(44.9)		Reference		
High	14(3.9)	6(1.7)		0.572	0.19-1.69	0.312^{NS}
Anaemia						
Yes	62(17.4)	44(12.4)	4.86	1.94	1.07-3.51	0.029*
No	294(82.6)	164(46.1)		Reference		
Jaundice						
Yes	38(10.7)	20(5.6)	0.59	0.77	0.39-1.51	0.444^{NS}
No	318(89.3)	188(52.8)		Reference		
Abdominal pain						
Yes	206(57.9)	126(35.4)	1.51	1.31	0.85-2.00	0.22^{NS}
No	150(42.1)	82(23)		Reference		
Diarrhoea						
Yes	118(33.1)	60(16.9)	4.18	0.63	0.40-0.98	0.042*
No	238(66.9)	148(41.6)		Reference		
Vomiting						
Yes	72(20.2)	26(7.3)	18.50	0.32	0.19- 0.54	0.0001**
No	284(79.8)	182(51.1)		Reference		
Nausea						
Yes	133(37.4)	65(18.3)	7.98	0.54	0.35-0.83	0.005**
No	223(62.6)	143(40.2)		Reference		
Appetite						
Good	219(61.5)	111(31.2)	14.04	Reference		
Poor	137(38.5)	97(27.2)		2.36	1.49-3.7	0.000**
Lassitude						
Yes	218(61.2)	124(34.8)	0.55	0.85	0.55-1.31	0.457^{NS}
No	138(38.8)	84(23.6)		Reference		
Complain of indigestion						
Yes	164(46.1)	92(25.8)	0.68	0.84	0.55-1.28	0.41^{NS}
No	116(32.6)	116(32.6)		Reference		
Fever						
Yes	123(34.6)	63(17.7)	3.02	0.64	0.41-0.99	0.046*
No	233(65.4)	145(40.7)		Reference		
Other infections						
Asthma	18(5.1)	10(2.8)	13.50	0.81	0.31-2.11	0.659^{NS}
Eczema	18(5.1)	4(1.1)		0.18	0.06-0.58	0.004**
Epigastric pain	21(5.9)	16(4.5)		2.06	0.73-5.81	0.172^{NS}
Others	59(16.6)	32(9)		0.76	0.43-1.36	0.356^{NS}
No	240(67.4)	146(41)		Reference		
Diabetes						
Yes	12(3.4)	10(2.8)	3.17	3.69	0.79-17.08	0.095^{NS}
No	334(96.6)	198(55.6)		Reference		
Learning Skills						
High	12(3.4)	10(2.8)	4.65	10.00	1.03-97.50	0.048*
Normal	338(94.9)	196(55.1)		2.76	0.49-15.28	0.245^{NS}
Slow	6(1.7)	2(0.6)		Reference		
Worms in stools						
Yes	48(13.5)	32(9)	1.55	1.50	0.79-2.85	0.215^{NS}
No	308(86.5)	176(49.4)		Reference		
Trauma						
Yes	350(98.3)	4(1.1)	0.17	1.43	0.26-7.92	0.681^{NS}
No	6(1.7)	204(57.3)		Reference		
Anthelmintic drugs						
No	356(100)	208(58.4)	nc	1.41	nc	nc

** P<0.01, ^{NS} P>0.05, Reference: Null value OR=1 Exposure does not affect odds of outcome.

eczema participants than those who had no other infection (χ^2=13.5; OR=0.18). Factors like, Hb level, jaundice, lassitude, complain of indigestion, learning skills, worms in stool and trauma were not significant (P>0.05).

Risk factors Associated with *Ascaris* IgG Seropositivity

In a multivariate analysis, stepwise logistic regression was used to avoid an excessive number of variables and unstable estimates in the subsequent models. The relative effect of the independent variable on the outcome variable is shown in Table 4. Ascariasis risk was found significantly (P<0.05) lower for literacy rate (OR=0.3, 95% CI: 0.1-0.8), weight group of 28-42 (OR=0.2, 95% CI: 0.1-1.0), month of Nov-Dec (OR=0.1, 95% CI: 0.0-1.1), cities (OR=0.0, 95% CI: 0.0-1.0; Rawalpindi: OR=0.03, 95% CI: 0.0-0.8), socioeconomic status (OR=0.00), geophagia (OR=0.4, 95% CI: 0.2-0.8) and habit of washing vegetables and fruits before eating (OR=0.2, 95% CI: 0.1-0.7). The results showed significantly (P<0.05) increasing infection risk in individuals who had no contact with soil (OR=4.6, 95% CI: 1.9-10.8), eating unwashed vegetables last month (OR=2.7, 95% CI: 1.4-5.2), eating mixed food (OR=2.4, 95% CI: 1.25-4.7), drinking pressure pump water (OR=3.4, 95% CI: 1.9-6.1), and those who had no complain for vomiting (OR=3.1, 95% CI: 1.6-5.8) and nausea (OR=1.9, 95% CI: 1.1-3.2).

Biochemical Analysis

Results showed that serum alanine aminotransferase (P=0.003) and alkaline phosphatase (P=0.012) were significantly elevated in *Ascaris* positive patients as compared to control group. The serum aspartate aminotransferase and gamma glutamyltransferase were not significantly (P>0.05) different. Total protein (P=0.01), globulin (P=0.018) and cholesterol (P=0.000) were significantly elevated in *Ascaris* infected patients as compared to controls. There was no significant difference in albumin and glucose levels (P>0.05) between infected and control groups (Table 5).

DISCUSSION

The applications of serological assays are established methods for diagnosis of intestinal parasitic infections when sensitivity and specificity is satisfactory (McCarthy *et al*., 2012). The disadvantage of stool examination is it requires adult worms to produce eggs (Corcoran *et al*., 2016). Often stool examination is difficult for diagnosis in prepatent period and for ectopic worm infections. Serological evidence of *A. lumbricoides* infection has varied among study sites and these differences could be explained by geographic condition and living standard of study participants (Begna *et al.*, 2016). The prevalence rates of *A. lumbricoides* reported in Pakistan were 48.0% in Abbottabad (Ahmed *et al*., 2003), 51.7% district Bagh (Khan *et al*., 2004), 16.5% Karachi (Mehraj *et al.*, 2008), 39.8% Swat (Khan *et al.*, 2012), 54.5% Dir (Ullah *et al.*, 2014), 56.9% Attock (Ali *et al.*, 2014), 18.0% Bannu (Ahmed *et al*., 2015) and 38.3% in Peshawar (Attaullah *et al*., 2016).

Among gender, no difference in prevalence of ascariasis was recorded due to equal exposure of both sexes to acquire *Ascaris* eggs (Okyay *et al*., 2004). Lower infection has been reported among older age groups most likely due to development of immunity in advanced age (Yadav & Parkash, 2017). This study found that the majority of people who suffered from ascariasis were illiterate with poor socio-economic conditions, which indicates that education directly or indirectly plays a key role by ensuring family and other society members to understand the significance of hygiene and cleanliness (Ross *et al*., 2017; Bhardwaj *et al*., 2017). Due to lack of education, people did not know about the problems regarding hygiene and open defecation (Bhardwaj *et al*., 2017).

This study showed higher prevalence among rural participants who did not receive any medication and drank untreated water. The participants had muddy floors in their houses, which is favourable environment

Table 4. Multinomial logistic regression models of risk factors (by odds ratio and 95%CI) associated with anti-*Ascaris* IgG seropositivity

	*Coefficient	Sig.	Odd Ratio	95% CI for OR	
				Lower	Upper
Education					
Illiterate	-1.28	0.016	0.28	0.10	0.78
Secondary school	0[b]				
Weight(kg)					
28-42	-1.50	0.05	0.22	0.05	1.02
43-57	0[b]				
Months					
July-Aug	7.69	0.002	nc	nc	nc
Sept-Oct	0[b]				
Nov-Dec	-2.70	0.05	0.07	0.00	1.09
Cities					
Peshawar	-4.06	0.049	0.02	0.00	0.98
Rawalpindi	-3.54	0.035	0.03	0.00	0.79
Tank	0[b]				
Socioeconomic status					
Good	-5.99	0.000	0.00	0.00	0.01
Poor	0[b]				
Animals at home					
Pet animals	2.31	0.001	10.08	2.60	39.01
Small ruminants	0[b]				
Contact with soil					
No	1.54	0.000	4.66	1.99	10.88
Yes	0[b]				
Geophagia					
No	-1.00	0.013	0.37	0.17	0.81
Yes	0[b]				
Habit of washing vegetables and fruits before eating					
No	-1.65	0.015	0.19	0.05	0.73
Yes	0[b]				
Eaten raw unwashed vegetables last month					
Yes	1.00	0.003	2.73	1.42	5.24
No	0[b]				
Food type					
Mixed	0.89	0.009	2.42	1.25	4.72
Mostly vegetables	0[b]				
Sources of drinking water					
Pressure pump	1.23	0.000	3.43	1.90	6.18
Public pipeline	0[b]				
Vomiting					
No	1.13	0.000	3.10	1.64	5.84
Yes	0[b]				
Nausea					
No	0.66	0.013	1.94	1.15	3.29
Yes	0[b]				

*Only significant regression coefficients () are shown.
[b]This parameter is set to zero because it is redundant.

Table 5. Biochemical changes between *Ascaris* IgG positive cases and controls

Variables	Infected group (N=80) Mean± SD	Control group (N=40) Mean ± SD	t-value	P-value
AST (U/l)	29.75±14.78	19.61±15.61	1.85	0.08^{NS}
ALT (U/l)	28.34 ± 14.36	19.20 ± 5.75	3.14	0.003**
ALP (U/l)	89.854±44.101	57.539±29.912	2.750	0.012*
GGT (U/l)	18.86±15.64	21.65±25.57	0.33	0.74^{NS}
Total Protein (g/dL)	7.53±1.32	6.44±1.04	2.77	0.01*
Albumin (g/dL)	3.98±0.190	4.18±0.83	0.76	0.46^{NS}
Globulin(g/dL)	3.548±1.257	2.264±1.364	2.702	0.018*
Cholesterol (mg/dl)	155.07±52.68	84.76±17.41	7.04	0.000**
Glucose (mg/dl)	46.58±33.99	59.06±65.25	0.58	0.57^{NS}

** P<0.01, ^{NS} P>0.05

for soil-transmitted helminths. Muddy floors allow the persistence of *A. lumbricoides* eggs for up to 15 years after excreted from infected individuals (Sungkar *et al.*, 2015). Animals at home were found significantly associated with ascariasis and this could be due to eggs of *A. lumbricoides* attached to the fur of animals, and contact with these animals may transfer eggs to the human (Rajoo *et al.*, 2017). Results are in general agreement with findings that large number of family members is linked with ascariasis (Okeke *et al.*, 2015).

Identified environmental risk factors associated with infection in this study are lack of hand washing practices after defecation, before eating, food handling and eating raw vegetables/lettuce/fruits without washing. Results are consistent with previous studies (Sungkar *et al.*, 2015; Mama and Alemu, 2016). It has been reported that washing of vegetables before eating was protective against soil transmitted helminths (Mukhtar *et al.*, 2016). The positive association of *A. lumbricoides* infection and geophagy is likely due to the parasite being mainly transmitted by orally ingesting materials contaminated with infective eggs. Similar observations have been reported in other studies (Mughini-Gras *et al.*, 2016; Ivoke *et al.*, 2017).

Depending upon the parasitic load, *A. lumbricoides* infection has variable clinical signs and symptoms. Nausea, vomiting, fever, lassitude, diarrhea and abdominal pain were significantly associated with ascariasis in this study and are in agreement with others (Gupta *et al.*, 2017; Agrawal *et al.*, 2016; Kiani *et al.*, 2016; WHO, 2008; Azhar *et al.*, 2015). The poor appetite associated with infection may be due to parasite consumption of nutrients in the intestine that may lead to reduced absorption of those nutrients eventually leading to decreased appetite, gastrointestinal disturbances and damaged intestinal mucosa (Ridwan *et al.*, 2015). The positive association of anti-*Ascaris* IgG with anemia is in agreement with Lone *et al.* (2012); however, some studies reported no association between anaemia and soil transmitted helminthiasis due to low prevalence of helminths infection (Foo *et al.*, 2004; Sagin *et al.*, 2002). Although ascariasis influences nutritional status, its effect on anaemia is less clear (Silva *et al.*, 2003). In this study, very few cases were found of *Ascaris* infected patients who had history of food allergy, skin allergy, eczema, or asthma. Helminths can regulate host immune responses, in a way that enhances their survival in the host and limits host tissue damage (Smits *et al.*, 2010; Cooper *et al.*, 2009).

Results showed that elevated levels of some liver enzymes i.e. ALP, AST and ALT were associated with ascariasis. This elevation is indicative of hepatic injury which may be due to higher nematode infection (Sorathiya *et al.*, 2017; Hussein *et al.*, 2016). Chronic ascariasis may cause

liver abscesses, in which patients show intermittent pyrexia and tender hepatomegaly (Andrade *et al.*, 2016). The total protein elevation observed in this study is not in agreement with other studies that reported low level of serum proteins during parasitic infection (Solanki *et al.*, 2017). Hypoproteinaemia may be due to severe infection of the liver, which may result in destruction of liver parenchyma and drastic alteration in protein values (Matanović *et al.*, 2007). There was no association between albumin and *Ascaris* infection which is in agreement with Ridwan *et al.* (2015). This could be explained by that heavy intensity infection that may lead to nutritional disturbance and hypoalbuminemia (Chaichisemsari *et al.*, 2011). The high level of globulin may be due to infection stimulating globulin synthesis as a result of immunogenic response (Lone *et al.*, 2012). The higher cholesterol level may be attributed to the extensive synthesis of bile acid from cholesterol in the liver. The increased output of epinephrine and corticosteroid could be responsible for elevation of serum cholesterol (Atasoy *et al.*, 2015). Reduced blood glucose was associated with *Ascaris* infection in this study, which is consistent with Okoye *et al.* (2013). This may be due to depression in voluntary feed intake and hepaticglycogenic pathways. Hypoglycaemia may be due to disturbance of gluconeogenesis, which can result from hepatic disorder. In addition, elevation of ketone bodies from gastroenteritis could result in depression in blood glucose (Phiri *et al.*, 2007).

CONCLUSION

This study concluded that ascariasis is of public health concern among the Pakistani community and ingestion of *Ascaris* eggs occurs due to contaminated food, unwashed and raw cooked food, contact with soil, habit of geophagia, poor sanitation, using untreated water and contact with animals. Pathophysiological effect of *Ascaris* infection alters some biochemical parameters. Furthermore, there is a need to promote health education and awareness programs to reduce the transmission risk of *A. lumbricoides* in Pakistan and other endemic areas of world.

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