SEROPOSITIVITY OF TOXOPLASMA GONDII LATENT INFECTION IN PATIENTS WITH NEUROPSYCHIATRIC DISORDERS

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ABSTRACT

OBJECTIVE: To detect Toxoplasma latent infection seropositivity as anti-toxoplasma antibodies and its serointensity as anti-toxoplasma gondii IgG (ATG-IgG) levels in target groups with neuropsychiatry diseases.

METHODOLOGY: This prospective cross sectional study was conducted from September 2013 to August 2014. Target subjects were taken from Kohat and Karak districts. Cases were in grouped in A & B per selection criteria with healthy control. Group A included children with mental retardation & group B included adult patients using neuropsychiatric drugs. Latex agglutination and ELISA tests were run on blood samples for ATG-IgG.

RESULTS: Group A included 69 patients and 36 controls with mean age of 8.61±2.09 & 8.42±1.71 years respectively. ATG-IgG were positive in 17/69(24.6%) cases and 5/36(13.9%) controls (p>0.05). Mean value of ELISA (IU/ml) for ATG-IgG was 43.56±12.87 for positive cases and 11.84±8.45 for negative. There was gradual increase in mean value of ELISA for mild, moderate, severe as 17.11, 21.08 and 32.94 respectively.

Group B included 73 patients and 40 controls with mean age of 31.88±11.35 & 31.82± 9.95 years respectively. ATG-IgG were positive in 41/73(56.2%) cases and 7/40 (17.5%) controls (p<0.01). Among cases, 41 while, among controls 7 were positives for ATG-IgG. The mean value of ELISA (IU/ml) was 102.80±89.52 and for negative cases 11.362±7.11. The mean value of ELISA for degree of disease presentation as mild moderate and severe was 37.72, 64.65, and 88.31 respectively.

CONCLUSION: Significant number of patients with psychiatric disorders expressed seropositivity for anti T. plasma antibodies. Antibodies levels increase with severity of psychosis.

KEY WORDS: Latent infection, Toxoplasma gondii, Neuropsychiatric disorders.

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INTRODUCTION

Toxoplasmosis is a protozoal disease caused by Toxoplasma gondii (T. gondii) and prevalent globally including Pakistan.[1] T. gondii, an obligate intracellular protozoan, is primarily hosted not only in cats but also in rates, rabbits, dogs, farmyards and wild animals. Humans may become infected with T. gondii by ingestion of food or drinking water contaminated with oocysts shed by cats or undercooked or raw meat containing tissue cysts from cattle or other infected animals. The parasite prefers to form cyst in brain and muscles.[2]

On infection, seropositivity remains throughout life. Toxoplasmosis can be detected by measuring immunoglobulin G (IgG) antibodies in the blood.[3] Most immune-competent adults and children are asymptomatic usually,[4,5] while some may resolve spontaneously having mild symptoms leading to latent infections. Slow but cumulative effects of latent toxoplasmosis are responsible for the decrease of psychomotor performance of infected subjects.[6] Effects of acute infection are established facts in immunocompromised but latent chronic infection was thought to be benign in immunocompetent persons.[7] T. gondii primary infection during pregnancy is transmitted to the fetus who may develop blindness mental retardation and other complications later in life.[8]

Psychosis is the term used for brain structural and functional abnormalities which may have infectious causation for disease like Schizophrenia and mental retardation.[9] T. gondii may change behavioral parameters that play a role in the etiology of brain diseases like schizophrenia, depressive disorders, epilepsy, headache, mental retardation, suicide attempts and work accidents.[10] Even impaired hearing and frequent headache was found associated with sero-positivity to T. gondii.[11]

As T. gondii has a high affinity for brain tissue where tachyzoites may form tissue cysts and persist for a life long time, this study was designed to detect the seropositivity and serointensity of T. gondii latent infection in patients of psychosis.

METHODOLOGY

This prospective cross sectional study was designed at the Microbiology Department Kohat University of Science and Technology Kohat and conducted during September to 2013 to August 2014.

Target subjects included from community of different rural localities in districts Kohat and Karak. Cases were divided into two groups (A and B) with healthy Control. Group A consisting of children with mental retardation and learning disability, group B consisting of adults patients using neuropsychiatric drugs for the disease. Those with history of genetic disorder, birth asphyxia, kernicterus, birth trauma, accidental trauma to brain and meningitis were excluded. Informed consent was taken from the patients/ their relatives. Proforma was filled about the demography of patients. Participants were provided written informed consent about purpose and procedure of study participation. They were given time then to come on specific time, date and venue.

This study was approved by Institutional Review Board for Bioethics, Khyber Medical University Institute of Medical Sciences Kohat.

Blood samples were taken and transported to the laboratory of KMU-IMS. Blood was centrifuged to obtain serum. Total and differential leukocytic count was done before centrifugation. Serum anti toxoplasma IgG was detected by ELISA BioChek BC-1085 kit. Cut-off value for the kit used was 32 IU/ml. SPSS version 19 was used for statistical analysis.

RESULTS

Subjects of group A consisting of children between age six to twelve years. There were 69 cases (33 were males and 36 females) with mental retardation and learning disabilities and age matched 36 controls (21 males and 15 females). Mean age of cases was 8.61±2.09 years and mean age of controls was 8.42±1.71 years.

In the group B, there were 73 adult cases with mean age of 31.88±11.35 years and 40 controls for the neuropsychiatric drug users enrolled with age matching from same locality. The mean age for control was 31.82±9.95 years (Table I).

There were 17 positive (24.63%), 52 negative individuals in cases and five positive (13.88%) and 31 negative individuals in controls of group A. While in Group B, out of 73, there were 41 positive (56.16%) and 32 negative individuals in cases and only seven out of 40 controls were positive (17.5%) for anti T. gondii antibodies. Gender distribution in group A and B and Mean of ELISA test value was 43.56 for the 22 positives cases of

TABLE 1: DEMOGRAPHIC DETAILS OF GROUP A AND B

Base line characteristics		Group A# (n=105)			Group B$ (n=113)		
		Cases	Controls	Total	Cases	Controls	Total
Gender	Male	33 (47.82%)	21(58.33%)	54	29(39.72%)	15(37.50%)	44
	Female	36(52.18%)	15(41.67%)	51	44(60.28%)	25(62.5%)	69
	Total	**69**	**36**	**105**	**73**	**40**	**113**
Mean age in years +SD		8.61± 2.095	8.42 ±1.713	8.54±1.966	31.88±11.352	31.82±9.956	31.86±10.833

#: Group A consisting of children with mental retardation and learning disability
$: group B consisting of adults patients using neuropsychiatric drugs for the disease

TABLE II: MEAN VALUE FOR POSITIVE AND NEGATIVE RESULTS OF ELISA FOR CASES AND CONTROL IN GROUP A & B

		Group A# (n=105)			Group B$ (n=113)		
		Positive (n=22)	Negative (n=83)	Total (n=105)	Positive (n=48)	Negative (n=65)	Total (n=113)
Cases	Male	10	23	33	13	16	29
	Female	07	29	36	28	16	44
	Total	**17**	**52**	**69**	**41**	**32**	**73**
Controls	Male	01	20	21	0	15	15
	Female	04	11	15	7	18	25
	Total	**05**	**31**	**36**	**7**	**33**	**40**
Mean of ELISA		43.56	11.84	18.48	102.80	11.36	50.20

#: Group A consisting of children with mental retardation and learning disability
$: group B consisting of adults patients using neuropsychiatric drugs for the disease

TABLE III: DEGREE OF DISEASE SEVERITY AND MEAN VALUE OF ELISA FOR ANTI TOXOPLASMA IgG

Degree of Disease	Group A#		Group B$	
	Mean of ELISA ± SD	No. of subjects	Mean of ELISA ± SD	No. of subjects
Mild	17.11±12.95	41	37.72±48.75	44
Moderate	21.08±18.90	18	64.65±47.77	21
Severe	32.94±24.12	10	88.31±87.62	08
Normal	14.74±13.11	36	48.73±99.51	40

#: Group A consisting of children with mental retardation and learning disability
$: group B consisting of adults patients using neuropsychiatric drugs for the disease

TABLE IV: SEROPOSITIVITY FOR ANTI T. GONDII ANTIBODIES IN CASES AND CONTROLS OF GROUP A & B

Groups		Anti T. Gondii Antibodies		Total	P Value (Chi Square Test)
		Positive	Negative		
Group A#	Case	17 (24.6%)	52 (75.4%)	69	>0.05
	Control	5 (13.9%)	31 (86.1%)	36	
	Total	22 (21%)	83(79%)	105	
Group B$	Case	41(56.2%)	32(43.8%)	73	<0.01
	Control	7 (17.5%)	33 (82.5%)	40	
	Total	48(42.5%)	65(57.5%)	113	

#: Group A consisting of children with mental retardation and learning disability
$: group B consisting of adults patients using neuropsychiatric drugs for the disease

anti T. gondii and 11.84 for 83 negative cases (Table II).

When degree of severity of disease was related with seropositivity and serointensity in terms of mean values of ELISA, there was a linear co-relation. The disease severity increased with antibodies intensity in plasma against T. gondii. Mental retardation and learning disability are related to Ig G serointensity but may be caused by seropositivity. Results are shown in Table III.

Sero-positivity for anti T. gondii antibodies in cases and controls of group A & B has been compared in Table IV. In group A, ATG-IgG were positive in 17/69(24.6%) cases and 5/36(13.9%) controls showing no statistically significant difference between cases and controls ($p>0.05$). In group B, ATG-IgG were positive in 41/73(56.2%) cases and 7/40 (17.5%) controls, showing highly significant difference for the controls and cases of neuropsychiatric drugs users in evaluating positive or negative for anti T. plasma antibodies ($p<0.01$).

DISCUSSION

Our study was community oriented in rural areas of the two districts Kohat and Karak having nearly the same geographic position of populations with hilly areas and good drainage slopes for water. The areas were partially cultivated and dry. Mostly peoples used open-well water for drinking. There were no custom to take raw meat in routine. Vegetables in salads are used un-boiled. Cats wonder by their own, no special keen for them to be domestic animals.

The two groups of cases children up to age 12 were included in interest to detect seropositivity of anti T. gondii Ig G if congenitally or even acquired infections has any association with mental retardation while excluding the possible other factors by excluding criteria. Controls from the same localities for this age group were taken irrespective of genders.[2.12.13]. The other group of cases was with the same excluding criteria but all those adult patients who were advised neuropsychiatric drugs by doctors. Again the controls for the adult patients were from same localities of patients and willing to participate in study. The division of patients in terms of mild, moderate and severe was in broad categorization of public language and entered accordingly. Patients in the areas are not only for the family only rather for the locality. Public enlarge share for the situations in religious and local customs.

Quantitative detection of anti T. gondii IgG was done by ELISA in preference to other methods to screen for latent infections.[13-15] Children were taken as a group in our study to see mental retardation due to T. gondii congenital infection as such latent infection remain without symptoms up to late childhood even later as reported by Delair et al; 2011.[16] In our study, children cases with seroprevalence 24.63% and control was 13.88% too low to the 62.6% in school children study by Fan et al; 20126 from West Africa and 46.4% in elementary school children of age 6-10 years by Lopes et al;2008.17 Although statistically p valve is > 0.5 for comparison of cases and control in

our results yet there is increase in the serointensity of anti T. gondii Ig G with degree of severity in mental retardation. Two such studies of Dunn et al; 1999[18] and Gilbert et al; 2001[19] were done but in relation fetus and infants severity T. gondii to symptoms degrees in mothers.

As T. gondii prefer brain tissue to encyst[20], the Bradzoites although not proliferating, yet act as non-self. Hence there will be constant response of inflammation. Inflammatory mediators in integration to complements affect the release of neurotransmitters.[20] Different neuronal centers produce different neurotransmitters. When affected, modified or increased levels of the neurotransmitters may release which produce variably behavioral changes.[21,22] Our study results with the mean age of 31.88±11.35 years of cases and 31.82±9.95 years of controls with seroprevalence of T. gondii 56.16% and 17.50% respectively in group B (Neuropsychiatric disorder) are reflected in results for cases with Schizophrenia and controls by Alvarado-Esquivel et al 2006 study[23] in Mexican city but with different age and percentages of seroprevalence. This may be probably due to the dry and slope climate of our study area as reported by in another study by Alvarado-Esquivel et al 2012[24] that seroprevalence in mountains and dry area is low. Other study by Delgado García; 1979[25] reported 60% and Mahmoud and Hasan; 2009 reported 53% prevalence of T. gondii in psychiatric patients with significantly higher than healthy subjects. The prevalence of our study and others[23,25] are different in % but similar in pattern that psychiatric patient had significant higher prevalence than controls.

The seroprevalence of T. gondii in controls with higher values but without neuropsychitatric presentation is probably on the basis that muscles is also priority tissue for T. gondii[2] which have no distant affect on brain.

CONCLUSION

T. gondii as a latent infection persists in tissues, detected by antitoxoplasma antibodies. Seropsoitivity in form of IgG anti T. gondii were significantly present in adults neuropsychiatric patients as compared to controls. There was no significant difference in seropsoitivity of IgG anti T. gondii in diseased children compared to controls. Mean value of seropositivity as ELISA IgG anti T. gondii goes on increasing with degree of disease severity in adults and children.

We recommend the research for kit development with neuromarkers to differentiate between brains and muscles involvement of T. gondii. At present all patients with seropositivity for T. gondii should be given prophylactic chemotherapy in early age as indicated in our cases of children to avoid latent infection in more severe form later in life as in group B above. Further cohort studies with large sample size for T. gondii infection in brain tissue and serointensity with advancement in age are required.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

AMK: Concept, acquisition and analysis of data, drafting the manuscript, final approval of the version to be published

JK: Study design, drafting the manuscript, critical revision, final approval of the version to be published

HR: Drafting the manuscript, final approval of the version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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