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The Surveillance of Avian Influenza Virus in Pulmonary Tuberculosis Patients Admitted in Gulab Devi Hospital Lahore, Pakistan

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ABSTRACT

Influenza infection is an important risk factor for tuberculosis. Sero-prevalence of avian influenza virus was studied in clinically declared *Mycobacterium tuberculosis* (Mtb) patients hospitalized in Gulab Devi Hospital, Lahore Pakistan. The patients were categorized into different groups on the basis of age and location. Blood samples were collected and tested for presence of anti-avian influenza antibodies by means of Haemagglutination Inhibition test (HI). In all parameters of clinical and laboratory confirmed TB patients, anti-avian influenza geometric mean HI-antibody titer of sub-serotype H9N2, H7N3 and H5N1 were 8.52±0.83, 2.90±2.44 and 8.08±1.43, respectively. Surveillance of sero-prevalence of avian influenza subtypes in 176 *Mycobacterium* infected patients revealed that high prevalence of avian influenza virus subtype H9 followed by H7 and H5 in all parameters of study. It was observed that co-infection of three subtypes (H5, H7 and H9) of avian influenza viruses in laboratory confirmed *Mycobacterium tuberculosis* patients displayed different clinical presentations at Gulab Devi Hospital Lahore, Pakistan. Whereas, tuberculosis and influenza co-infection has increased the disease severity and risk to death as compared to tuberculosis infection alone.

Key words: Influenza A virus, pulmonary tuberculosis, *Mycobacterium tuberculosis,* Haemagglutination inhibition, sero-prevalence

INTRODUCTION

The Lahore city is a capital of the Punjab province, Pakistan. It is located between 31°15 -31°45 N and 74°01 -74°39 E. Lahore weather conditions comprise extremely hot and long summers (months of May, June and July) and temperature fluctuate between 40-48°C (104-118°F). The monsoon season starts from late July to August, with heavy rainfall[1]. Malnutrition, diabetes, host genetic factors, infected with Human Immunodeficiency Virus (HIV), pollution by solid fuel burning and active smoking are reported to be tuberculosis (TB) risks globally and in view of increasing pollution, TB cases are increasing day by day[2].

Wild birds play an important role in the spread of influenza virus. They carry influenza virus without being harm for longer period of time and serve as the source of disease

transmission in animal and human population[3,4,5]. Influenza is a common respiratory infection that affects both birds and mammals. It is highly contagious, occurs in annual outbreaks and causes substantial morbidity and mortality[6,7]. It is caused by RNA viruses which is enveloped, negative sense single stranded. Influenza virus can be transmitted through air by cough or sneezes of the affected patient. It is also a common zoonotic infection transmitted by direct contact with bird droppings or through contact with contaminated surfaces of their farms[8,9].

The viruses have the potential to cause extremely severe respiratory illness in humans and of the 169 cases reported to the World Health Organization as of February 13, 2006, 91 (54%) have been fatal[10]. Many of the viruses isolated from humans have been found to be genotypically resistant to the adamantine and resistance to oseltamivir has also been described[11]. Although human to human transmission appears at present to be rare the development of an effective vaccine against influenza A virus is a matter of considerable urgency. Despite the use of efficacious treatment, tuberculosis remains the second most infectious disease related death worldwide after Acquired Immune Deficiency Syndrome (AIDS) and human immune deficiency syndrome (HIV)[12]. HIV infection is an important risk factor for tuberculosis and HIV-infected individuals have a 20 times greater risk of developing tuberculosis as compared to HIV-uninfected individual [13]. The mechanism of re-assortment in influenza viruses subsequently compels the organism towards genetic shift results in failure of vaccination. Substantial morbidity and mortality was observed due to emerging subtypes of influenza virus despite of excessive vaccination[14].

Concurrent bacterial infections and influenza virus interaction result in more severe disease. Mortality data from the 1918 influenza demonstrates significant overlap between the populations most affected by influenza and tuberculosis. Influenza virus might have impact on patients having preexisting TB or latent TB in terms of severity in clinical presentation or even may cause mortality[15]. Whereas, in Thailand chronic respiratory disease is referred as predisposing factor for severe disease with seasonal influenza, as demonstrated and data from 2009 influenza A (H1N1) pandemic suggests that TB predisposed H1N1infected patients to a severe clinical course[16,17]. For most serious human illness and high rate of mortality, Asian Highly pathogenic avian influenza H5N1 and H7N9 viruses have been responsible[18]. The current study was therefore designed to investigate the prevalence of different serotypes of avian influenza viruses in T.B. infected patients admitted in Gulab Devi Hospital, Lahore.

MATERIAL AND METHOD

Data collection: A standardized questionnaire was used to collect clinical data and medical history of the patient. Collection of blood samples, hospitalization record, clinical presentation and data regarding tuberculosis was obtained according to attending-physician discretion. Specific Mtb and AI virus clinical signs such as chest pain, productive cough, coughing up blood, shortness of breath, difficult breathing, pneumonia, acute respiratory distress and weight loss were keenly observed of each patient and record was maintained for reconciliation with anti AI-HI antibody titers to compare the influence of AI sero-prevalence.

TB diagnosis was confirmed based on indoor hospital documentation for each patient. The documentation includes presence of clinical symptoms, a chest X-ray examination (CXR), microscopic detection of acid-fast bacilli in sputum and a positive culture of Mtb. Since, clinical picture of suspected avian influenza virus infection in humans is highly confusing with other respiratory tract pathogens. Therefore, direct detection of specific antigen and significant raised antibodies in response of avian influenza virus during laboratory testing is indication of the virus. The control subjects underwent the same examinations as the patients.

Collection of samples: The 2.5 mL fresh venous blood was collected randomly from laboratory confirmed Mtb adult patients admitted in wards marked by the Deputy Medical Superintendent. For negative control samples, same quantity of blood was collected from each of the ten volunteers selected on the basis of healthy clinical presentation. Whereas, 2.5 mL of blood samples were also obtained from serologically confirmed influenza positive and non-Mtb persons those have been working in commercial poultry farm for last two years. Blood samples were collected in 5 mL sterile disposable plastic syringes under complete sterile and aseptic measures from upper limb veins of the patients. The blood samples were transported to Research and Development Laboratory of Ottoman Pharma (Immune Division) located at 10 km-Raiwind road, Lahore and maintained in slanting position under room temperature.

Storage of serum samples: When the sufficient sera had separated from blood in the syringes, it was collected into 2 mL micro centrifuge eppendorf's tube under sterile conditions. The tubes containing serum were centrifuged at 1800 g for 10 min. Clear supernatant was transferred to sterile eppendorf's tubes marked with the same serial numbers as of the syringes and questionnaires of the respective days. The

grand total of serum samples of all the three days was 176. The samples were stored at -80°C till further use.

Avian influenza virus antigens: Avian influenza virus antigens used in this study were collected from Ottoman Pharma (Immuno Division), alicensed poultry vaccine manufacturing unit. The details of the virus antigens are A/KHI-OP/03/01 (H5N1), A/KHI-OP/09/03 (H7N3) and A/GP-OP/126/16 (H9N2)[19].

Haemagglutination inhibition assay (HI): All the serum samples were subjected for H5, H7 and H9-specific anti-HI antibodies as described by using four HA units of virus and 1% horse red blood cells as described by Stephenson *et al.*[20]. Declaration of influenza virus in Mtb patients was based on the following parameter.

Positive ≥ 1:5 HI antibody titer
Negative ≤ 1:4 HI antibody titer

Personal Protection Equipment (PPE): Experimentation was performed in Class B facility in R and D section of the Ottoman Pharma, Lahore and standard protocols for handling of TB infected samples were adopted during execution.

Statistical analysis: The data was analyzed by mean standard deviation and subsequently through one way analyses of variance using SPSS in which significance of probability was p>0.05.

RESULTS

Avian Influenza Haemagglutination Inhibition assay of 176 serum samples was performed against characterized avian influenza viruses subtypes H5N1, H7N3 and H9N2. The target patients were admitted in the hospital and on anti-tuberculosis treatment. No patient was on any immunosuppressive medicine. All the patients belonged to Lahore division and its surrounding districts. The patient age ranged from 14 to 87 years with mean of 44.20±17.12 years. Reconciliation of clinical signs and serological results on Master sheet data revealed that patients in either group showing high anti-AIH5-HI antibody titer had severe difficulty in breathing along non-productive cough. Whereas, patients show high anti H9 did not show any severity in respiratory distress. 96 well HI plate shown in Table 1.

On physical examination, ten volunteers in each negative control group were found free from Mtb and avian influenza and did not show any specific Tb/avian influenza signs and symptoms. Laboratory diagnosis revealed that such personals had normal chest X-ray examination (CXR), absence of acid-fast bacilli in sputum sample during microscopy. Whereas, immunological analysis of serum showed none of the person in the group had anti-influenza H5-HI, anti-influenza H7-HI and anti-influenza H9-HIantibody titer. Furthermore, serum obtained from ten influenza exposed volunteers in influenza positive (Mtb negative) group showed mean 8.08±1.43 anti-influenza H5-HI antibody titer, mean 7.47±0.86 anti-influenza H7-HI antibody titer and mean 9.23±1.72 anti-influenza H9-HI antibody titer. The incidence of anti AIV5 antibody titer in TB infected patients based on different age group and locality association was evaluated, using one way ANOVA and subsequently Duncan Multiple Range test (DMR).

The age group based mean antibody response of 1 and 2 is significantly lower than that of 4 ($p<0.05$) (Fig. 1) as compare to the mean antibody titer in 3 which is significantly higher ($p>0.05$). Whereas in case of AIVH7 mean antibody response of 1 and 2 is significantly lower than that of 4 ($p<0.05$) (Fig. 1) as compare to the mean antibody titer in 3 which is significantly higher ($p>0.05$). Moreover, AIVH7 mean antibody response of 1 and 2 is significantly lower than that of 4 ($p<0.05$) (Fig. 1) as compare to the mean antibody titer in 3 which is significantly higher to that of 3 and 4.

In locality based seroprevalence of AIV5 in TB infected exposed patients showed significantly higher mean anti body titers in patients associated with 1, 2 and 3 as compare to 4,5 and 6 ($p>0.05$). Whereas, mean anti H7 antibody titer of A and B is significantly lower than that of 4 ($p<0.05$) (Fig. 1, 2). Whereas, in case of AIVH9 mean antibody response of 1 and 2 is significantly lower than that of 4 ($p<0.05$) (Fig. 2) to that of 3 which is significantly higher ($p>0.05$).

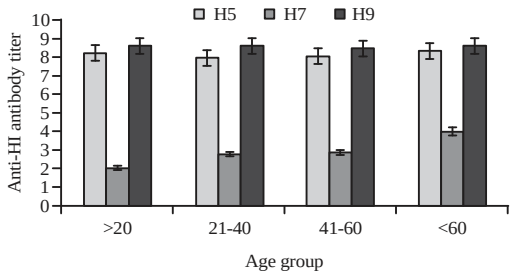

Fig. 1: Anti AIV-HI antibody titer in different age groups

Table 1: 96 well HI plate row from 1-12th well to heamagglutination inhibition units

1	2	3	4	5	6	7	8	9	10	11	12
2	4	8	16	32	64	128	256	512	1024	PC	NC

Table 2: Anti avian Influenza H5 antibody titer in different age groups

Age group	Sample No.	Titer	Mean±SD
≤20	106	5	8.23±1.11
	4, 41, 121, 124	7	
	55, 49, 67	8	
	48, 133, 33, 113, 156, 168, 138, 170, 58, 46, 77, 84, 171	9	
21-40	162, 128, 149	5	7.98±1.23
	21, 22, 11, 118	6	
	108, 141, 2, 20, 8, 109, 122, 111, 125	7	
	14, 16, 30, 37, 116, 163, 18, 27, 54, 99, 26	8	
	59, 66, 86, 93, 147, 173, 36, 61, 68, 95, 96, 131, 135, 143, 146, 160, 35, 74, 155, 97, 151, 40, 64, 98, 152	9	
41-60	107, 126	5	8.03±1.73
	15, 23, 24, 112, 119, 145	6	
	1, 7, 17, 100, 53, 103, 114, 102, 104, 117, 123	7	
	3, 10, 51, 176, 29, 31, 164, 19, 32	8	
	136, 34, 73, 79, 154, 167, 43, 47, 57, 65, 69, 78, 81, 82, 88, 89, 137, 150, 172, 175, 42, 60, 87, 94, 101, 115, 129, 142, 174, 45, 52, 63, 70, 83, 90, 105, 144, 72, 85, 132, 153, 166, 39, 91, 139, 159, 165	9	
>60	13, 25	6	8.32±0.99
	5, 127, 148	7	
	6, 28, 56, 62, 161	8	
	9, 44, 50, 75, 92, 130, 157, 169, 80, 134, 38, 76, 158, 140, 71	9	

Table 3: Anti avian Influenza H5 antibody titer in district Lahore and its surrounding districts

Location	Sample No.	Titer	Frequency	Mean±SD
Gujranwala	103	7	1	7±2
	136	9	1	
	162	5	1	
Kasur	128	5	1	8.04±1.17
	21	6	1	
	7, 41, 100, 108, 114, 121, 141	7	6	
	14, 28, 55	8	3	
	34, 48, 59, 66, 73, 79, 86, 93, 133, 147, 154, 167, 173,	9	13	
Okara	107	5	1	7.64±2.53
	13	6	1	
	27, 54, 90	8	3	
	40, 58, 72, 85, 132, 140, 153, 166	9	8	
Lahore	1	5	1	8.28±0.99
	2, 3	6	2	
	6, 9, 10, 16, 17, 20, 23, 24, 30, 33, 36	7	11	
	37, 43, 44, 47, 50, 51, 57, 61, 62, 65, 68, 69	8	12	
	75, 78, 81, 82, 88, 89, 92, 95, 96, 102, 104, 110, 113, 116, 117, 123, 124, 127, 130, 131, 135, 137, 143, 146, 149, 150, 156, 157, 160, 161, 163, 169, 172, 175, 176	9	35	
Mureedkay	15, 22	6	2	8.28±1.02
	8, 109, 122, 148	7	4	
	29, 49, 56, 67	8	4	
	35, 42, 60, 74, 80, 87, 94, 101, 115, 129, 134, 142, 155, 168, 174	9	15	
Nankanasahib	11, 25, 118	6	3	7.5±1.29
	4, 111, 125	7	3	
	18, 31, 164	8	3	
	38, 45, 52, 63, 70, 76, 83, 90, 97, 105, 138, 144, 151, 158, 170	9	15	
Sheikhpura	106, 126	5	2	7±1.58
	112, 119, 145	6	3	
	5, 53	7	2	
	19, 26, 32	8	3	
	39, 46, 64, 71, 77, 84, 91, 98, 139, 152, 159, 165, 171	9	13	

Sero-prevalence of anti avian influenza virus H5N1 antibody titer: Mean antibody titer against H5N1 was considerably raised in all serum samples irrespective of area and age group. As a whole the mean titer was 8.08±1.43 and it ranged from 7±2 for Gujranwala region to 8.28±1.02 for Mureedkay and Lahore. Twenty-eight (14.77%) and one hundred (56.81%) patients had high titer values of 8 and 9, respectively. Only two (1.13%) patients had equal and less than 4 HI units (Table 2, 3 and Fig. 1, 2).

Sero-prevalence of anti avian influenza virus H7N3 antibody titer by area and age: Anti H5 and H9-HI antibody titers in the current study were high in different areas and all age groups whereas H7 remained quite low. As a whole the mean titer was 2.90±2.44 and it ranged from 2.52±2.41 for Mureedkay to 3.14±2.18 for Okara. Sixty-three (35.80%) patients had zero titer but without regard to area and age group predilection. Only one serum sample showed anti-influenza H7-HI antibody titer value of 9 (Table 4, 5 and Fig. 1, 2).

Table 4: Anti avian Influenza H7 antibody titer in different age groups

Age group	Sample No.	Titer	Mean±SD
≤20	48, 84, 133	3	2.05±2.46
	55, 67	4	
	124, 156, 58	5	
	168	6	
	77	7	
21-40	66, 96, 131	2	2.77±2.40
	64, 131, 135, 143	3	
	162, 11, 141, 2, 16, 163, 99, 26, 173,74,97,40,98	4	
	128, 149, 20, 125, 27, 160, 151	5	
	14, 147, 146, 155, 152	6	
	68	9	
41-60	145, 1, 65, 94	2	2.88±2.42
	24, 102, 31, 43, 81, 172, 101, 166,	3	
	119, 103, 7, 100, 123, 53, 3, 51, 176, 164, 32, 78, 88, 175, 52, 139	4	
	10, 154, 167, 47, 57, 60,72,132,153,39,159,165	5	
	73, 69, 89, 174, 83	6	
	79, 87, 63, 144	7	
	150	8	
>60	62, 134, 124	3	4±2.33
	25, 44, 127	4	
	5, 13, 148, 28, 161, 56, 157, 38	5	
	9, 75, 76	6	
	50, 80, 158	7	

Sero-prevalence of anti avian influenza virus H9N2 antibody titer: In this study, avian influenza virus H9N2 had the highest antibody titer and as a whole the mean antibody titer was 8.52±0.83. Individually in samples, anti-influenza H9-HI antibody titer values ranged from 4 to 9. In all the areas, the majority of cases had anti-influenza H9-HI antibody titer

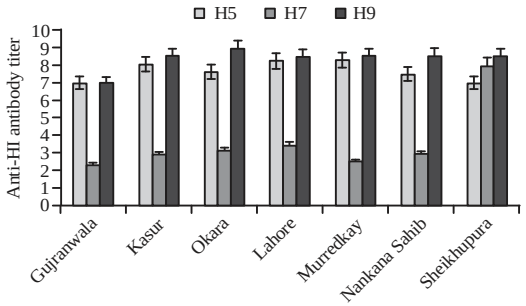

Fig. 2: Anti AIV-HI antibody titer in district Lahore and its surroundings

Table 5: Anti avian influenza H7 antibody titer in district Lahore and its surrounding districts

Location	Sample No.	Titer	Frequency	Mean±SD
Gujranwala	136, 162	4	2	2.33±1.53
Kasur	66	2	1	2.92±2.46
	48, 133	3	2	
	7, 55, 100, 141, 173	4	5	
	28, 128, 154, 167	5	4	
	14, 73, 147	6	3	
	79	7	1	
Okara	140, 166	3	2	3.14±2.18
	40, 99	4	2	
	13, 27, 58, 72, 132, 153	5	6	
Lahore	124	4	1	3.46±0.96
	2, 20	6	2	
	43, 51, 135, 89	7	4	
	78, 81, 150, 3, 61, 82, 9, 37, 47, 57, 88, 44, 163, 110	8	14	
	10, 23, 24, 50, 96, 123, 130, 131, 137, 143, 172, 6, 62, 102, 17, 117, 146, 149, 156, 175, 30, 36, 68, 69, 116, 176, 1, 16, 33, 65, 95, 161, 169, 75, 113, 127, 157, 92, 160, 104	9	40	
Mureedkay	8	6	1	2.52
	101	7	1	
	15, 22, 35, 42, 56	8	5	
	109, 122, 29, 49, 115, 129, 142, 94, 134, 67, 74, 148, 60, 155, 168, 174, 80, 87	9	18	
Nankanasahib	45, 70, 11, 38	7	4	2.96±1
	4, 105, 52, 63	8	4	
	118, 111, 18, 90, 138, 170, 31, 25, 164, 97, 125, 151, 76, 83, 144, 158	9	16	
Sheikhpura	46, 71, 26, 39	7	4	8±1
	126, 64, 32, 5	8	4	
	12, 106, 112, 19, 91, 171, 145, 84, 119, 53, 98, 139, 159, 165, 152, 77	9	16	

Table 6: Anti avian Influenza H9 antibody titer in different age groups

Age group	Sample No.	Titer	Mean±SD
≤20	48	6	8.59±.796
	46	7	
	4, 33, 41, 55	8	
	106, 121, 110, 49, 113, 138, 170, 171, 133, 84, 67, 124, 156, 58, 168, 77	9	
21-40	8	6	8.56±0.73
	11, 26, 37, 96	7	
	2, 16, 14, 21, 22, 54, 59, 36, 35, 66, 64, 128	8	
	118, 108, 109, 122, 111, 30, 18, 86. 93, 61, 95, 116, 131, 135, 143, 162, 141, 163, 99, 173, 74, 97, 40, 98, 149, 20, 125, 27, 160, 151, 147, 146, 155, 152, 68	9	
41-60	88	4	8.45±0.94
	23, 103, 104	6	
	39, 45, 47, 70, 101, 136	7	
	3, 7, 10, 15, 24, 32, 34, 42, 43, 51, 52, 63, 69, 105, 126, 137	8	
	1, 12, 17, 19, 29, 31, 53, 57, 60, 65, 72, 73, 78, 79, 81, 82, 83, 85, 87, 89, 90, 91, 94, 100, 102, 107, 112, 114, 115, 117, 119, 120, 123, 129, 132, 139, 142, 144, 145, 150, 153, 154, 159, 164, 165, 166, 167, 172, 174, 175, 176,	9	
>60	6, 38, 71,	7	8.56±0.71
	5, 44, 56, 62, 157	8	
	9, 13, 25, 28, 50, 75, 76, 80, 92, 127, 130, 134, 140, 148, 158, 161, 169	9	

Table 7: Anti avian Influenza H9 antibody titer in district Lahore and its surrounding districts

Location	Sample No.	Titer	Frequency	Mean±SD
Kasur	48	6	1	8.52±0.714
	21, 41, 34, 59, 66, 7, 55, 128, 14	8	9	
	108, 114, 121, 86, 133, 93, 100, 141, 173, 28, 154, 167, 73, 147, 79	9	15	
Okara	54	8	1	8.93±0.267
	13, 27, 40, 58, 72, 85, 99, 107, 120, 140, 166, 132, 153	9	13	
Lahore	2, 10, 20, 23, 24, 43, 50, 51, 78, 81, 96, 123, 130, 131, 137, 143, 150, 172	0	18	8.46±2.38
	6, 62, 102, 135	2	4	
	3, 17, 61, 82, 117, 146, 149, 156, 175	3	9	
	9, 30, 36, 37, 47, 57, 68, 69, 88, 116, 124, 176	4	12	
	1, 16, 33, 44, 65, 89, 95, 161, 163, 169	5	10	
	75, 110, 113, 127, 157	6	5	
	92	7	1	
	160	8	1	
	104	9	1	
Mureedkay	94	2	1	8.5±1.87
	101, 134	3	2	
	67, 74	4	2	
	148, 56, 60	5	3	
	155, 168, 174	6	3	
	80, 87	7	2	
Nankanasahib	31	3	1	8.50±1.58
	11, 25, 164, 52, 97	4	5	
	125, 38, 151	5	3	
	76, 83	6	2	
	63, 144, 158	7	3	
Sheikhpura	145	2	1	8.50±1.87
	64, 84	3	2	
	119, 53, 26, 32, 98, 139	4	6	
	5, 39, 159, 165	5	4	
	152	6	1	
	77	7	1	

values of 8 and 9 with 37 (21.02%) and 119 (67.61%) cases respectively. Only one patient had the minimum value of less than 4. Similarly, titer values of 5, 6 and 7 were also detected in few patients (Table 6, 7 and Fig. 1, 2).

DISCUSSION

Anti-avian influenza Geometric Mean Titer (GMT) of Heamagglutination Inhibition (HI) against three serotypes of AIV was measured in blood obtained from laboratory

confirmed Mtb infected patients of various age groups from Lahore division and its surrounding districts. It showed that the overall GMT of H9N2 subtype was higher as compared to the other two serotypes but there was mild variance between H5N1and H9N2 GMT values. The 88.63 and 72.72% patients had GMT levels greater than or equal to 8 HI units for H9N2 and H5N1 respectively. During last 5 years, there was no official confirmation of H7N3 outbreaks in poultry farms of Punjab, Pakistan[21] that might be the reason of low titer of H7N3 subtype in TB patients. It is also evaluated the sera of same pulmonary TB infected patients for H5N1 and it was revealed that like H9N2, they are found highly seropositive against H5N1. The highest GMT 8.32±0.99 was observed in the older age group and the lowest GMT 7.98±1.23 was observed in the 21-40 years old people. However, GMT values of HI in most of the patients in current study were in higher range. But the relevant authorities have not reported the outbreaks of H7N3 in this region. However, the presence of antibodies against this antigen is the evidence for the presence of field antigen in the environment.

This sero survey to H7 subtype of AIV revealed that the highest GMT 4.00±2.334 was observed in old age group while the lowest GMT 2.05± 2.46 was observed in the younger group. It might be due to immunosuppressive health status of the patients or recurrent exposure of the field antigen. District wise distribution showed that the highest GMT 3.14±2.18 was observed in Okara and the lowest GMT 2.52±2.41 was observed in Mureedkay. The reason of high anti AI GMT antibodies in the Mtb infected hospitalized patients belonged to the Okara is the presence of many fold high broiler population in the form of environmental controlled sheds as compare to Mureedkay.

In the present study collected samples from the patients in random order. It reveals that the seropositive GMT above 8.5 against H9N2 was recorded in patients of all age groups which might be due to more prevalence of H9N2 virus in the environment because of frequent outbreaks in the poultry farms. High titer values might be due to frequent exposure to the antigen.

Total of 176 test samples when compared with 10 control samples in each positive and negative control group were found according to the optimized values. Positive control group in which volunteers were declared as Mtb free but had exposure to avian influenza showed 8.08±1.43, 7.47±0.86 and 9.23±1.72 GMT for H5, H7, H9, respectively. However, Negative control group did not show anti influenza HI antibody titer for either of the subtype. Influenza sero-positive volunteers were commercial poultry farms workers those have been engaged in general farm management for last two years. The direct contact with the influenza infected commercial birds for long duration might have been the cause of such raised anti influenza HI antibody titer[22].

Since, Interferon-γ mediated immune responses play a major role in the control of *Mycobacterium tuberculosis* (Mtb) infection, are inhibited by type I interferons[23,24]. Although, type I interferons are profusely induced during replication of influenza virus, it was observed that influenza viruses infection play a role in the development of active TB directly by the exposure to Mtb or indirectly through reinfection of latent Mtb[25]. Both influenza and *Mycobacterium tuberculosis* have been known to impair immune system such as host T-cells responses. *Mycobacterium tuberculosis* patient is more prone towards influenza infections whereas, influenza viruses may auscultate *Mycobacterium tuberculosis* infection in latent TB infected host[26]. Wild aquatic birds are the natural host for a large variety of influenza A virus. Occasionally these viruses are transmitted to other species and cause devastating outbreaks as in domestic poultry or give rise to human influenza pandemics[27]. Preexisting TB might have predisposed to death from influenza or influenza mortality might have been highest among TB patients[16]. Chronic respiratory disease is a known risk factor for severe disease with seasonal influenza, as demonstrated in Thailand and data from 2009 influenza A pandemic suggests that TB predisposed H5N1infected patients to a severe clinical course[17,18].

CONCLUSION

Based on data obtained in current sero-prevalence study it is not evident that severity in clinical presentation of Mtb patient was due to the avian influenza virus rather severity in clinical presentation might be due to recurrent exposure of influenza virus. Furthermore, current study focused the presence of anti-influenza HI antibody titer and its co-relation with clinical presentation. It is necessary to examine the biochemical and immunological pathway induced during the replication cycle of Mtb and avian influenza virus. However, it might be hypothesized that immunosuppression nature of Mtb may support influenza virus to create co-lateral damage which might get confused in diagnosis of the either disease. This study has been done to evaluate the presence of influenza virus in environment, its impact on immunosupressive disease host and to differentiate the avian influenza exposure in TB infected patients in relevance to the influenza positive control group.

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