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Multilevel modeling of household contextual determinants of tuberculin skin test positivity among contacts of infectious tuberculosis patients, Umerkot, Pakistan

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Abstract

This cross-sectional study sought to identify household contextual determinants that might be associated with Mycobacterium tuberculosis infection as assessed by tuberculin skin test (TST) positivity among familial contacts of index patients of infectious pulmonary tuberculosis (TB) while controlling for the effects of individual-level factors. We analyzed data on TST results on 359 household contacts of 77 index cases of acid-fast bacilli (AFB) sputum smear-positive pulmonary TB using multilevel logistic regression analysis with characteristics of household contacts at the first level and that of households at the second level. The prevalence of M. tuberculosis infection as assessed by TST positivity among household contacts of index TB patients was 49.9% (179/359). After taking into account the individual-level risk factors, household-level contextual determinants significantly associated with contact's TST positivity were gender of index TB patient (adjusted odds ratio [OR] = 2.2; 95% confidence interval [CI]: 1.3-3.9%) and density of AFB sputum smear (adjusted OR = 3.2; 95% CI: 1.9-5.5%). Household variances in multilevel models indicated significant inter-household heterogeneity in TST positivity among contacts. This study provided the evidence for substantive effects of householdlevel contextual variables on the TST positivity among contacts of index TB patients. Thus, both individual-level and household-level characteristics need to be taken into account while prioritizing contacts for investigations to improve TB control and prevention in resource-constrained countries such as Pakistan.

Introduction

Tuberculosis (TB) is the leading cause of death from a curable infectious disease in low-income and middle-income countries.[1,2] The most recent estimates of the worldwide TB epidemic are for 2004, when there were 8.9 million new cases and 1.7 million deaths.[3] About 3.9 million cases were sputum smear positive for Mycobacterium tuberculosis - the most infectious form of the disease. 3-5 About 80% of the newly diagnosed TB cases live in the 22 most populous countries. Varying levels of endemicity of infection with M. tuberculosis have been reported worldwide, and Southeast Asia seems to be the most afflicted: 44% of its population is reported to be M. tuberculosis infected.[4] Pakistan has been ranked fifth among the 22 highest TB incidence countries.[4,6]

Transmission of M. tuberculosis from an infectious source case to people with whom they share air is governed by several factors. These factors, which determine whether M. tuberculosis transmission will occur and establish a new infection, are related to the source case, the organism, the environment, and the people who are exposed to the source case. Contacts of patients with infectious TB constitute a high-risk group for acquiring M. tuberculosis infection,[7,8] as approximately 30% of close contacts demonstrate evidence of infection, and at least half of infected contacts exhibit progression to disease in the first 2 years.[7,8] The risk of acquiring infection with M. tuberculosis among contacts may increase with increasing closeness of the contact 8 and with increasing infectivity of the index case,[9] resulting from environmental factors such as overcrowding and social factors including poverty.[10-12] Investigations of close contacts of infectious TB patients constitute a key target in TB control because they can detect new recent infections with M. tuberculosis . Because newly infected contacts are at high risk for progression to active TB, contact investigations should be prioritized over other TB screening efforts to prevent substantial future TB cases.[13]

Household contacts of an infectious TB patient are a particularly high-risk population for latent infection with M. tuberculosis and for development of active TB.[6,7] Furthermore, M. tuberculosis transmission risk greatly varies between households within communities and between individuals in the same household.[12] Risk factors underlying this variation seem to operate at the individual-level and household-level and need to be

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taken into account while planning rationale preventive measures. Hitherto, only a limited number of studies have evaluated the risk factors associated with acquiring infection with M. tuberculosis among household contacts of index cases of pulmonary TB.[8,14-16] However, to our knowledge, thus far none has quantified simultaneously in a multilevel setting, the relative contributions of host (individual-level) and householdlevel factors in the M. tuberculosis transmission among the household contacts of infectious index TB cases. Therefore, the primary goal of this study was to identify householdlevel factors that might be associated with tuberculin skin test (TST) positivity while controlling for the effects of individuallevel factors so that we could explain the inter-household heterogeneity in TST positivity among familial contacts of index patients of infectious pulmonary TB in a resource-constrained setting of Umerkot, Pakistan.

Study Population and Methods

Study design, Setting, and Study Population:

The study procedures, including a description of study design, setting, and study population, have been described elsewhere,[15] and are briefly outlined here. A cross-sectional study was conducted during August and September 1999 in Umerkot, a remote district of Sindh Province covering an area of 5,608 km 2 with a population around 0.7 million, 83% of whom live in rural areas. Topographically the Umerkot district has two distinct parts, i.e., the irrigated area in the north-west and rain-fed desert in the south-east. Agriculture is the main occupation in the area. About 57% of the housing units are single room houses with an average household size of 5.4 persons. The Muslims constitute 51% of the total population of the district and about 90% of the people speak Sindhi.[17]

Our study site, the Umerkot Taluka an administrative subunit of district Umerkot, has a population of about 0.3 million. The local health care system includes a government health center, a non-governmental organization clinic, namely Umerkot Anti-tuberculosis Association (UATA) clinic, and about 20 private clinics. Tuberculosis control is provided for most patients of this study area by UATA clinic. The majority of patients are diagnosed and treated as outpatients. Our study population comprised household contacts of index TB patients in Umerkot Taluka. The index TB case was defined as the first member of the household, aged 15 years or more, presented and registered for TB treatment at the UATA clinic, and had at least one sputum smear positive for acid-fast bacilli (AFB). The initial TB diagnosis in the index case at UATA clinic is based on the combination of two or more methods, including TST, chest radiography, AFB sputum-smear status, and bacterial culture. 18,19 The household contact was defined as any person who was over 3 months of age, had lived in the same house as the index case for at least 3 months, and had slept in the same house for at least 4 nights per week on an average, throughout the 3-months period.[19]

Selection of Index Cases and Household Contacts:

For the estimation of prevalence of M. tuberculosis infection among household contacts of index TB cases, we intended to include 385 contacts of 77 index cases based on the following assumptions; estimated prevalence of M. tuberculosis infection (assessed by TST positivity) among household contacts of infectious TB cases as 0.5 with a bound on error of 0.05 at a significance level of 0.05. 20 This sample size was also sufficient for identification of factors associated with TST positivity with an odds ratio (OR)^3 2 and study power of at least 80%.[21]

A list frame of 680 TB cases maintained at UATA clinic was used to select a simple random sample of 77 TB index cases. Using the Ziehl-Neelson staining method,[22] each selected TB case was screened for AFB in three sputum samples taken one on spot and two on two consecutive days in early morning. If a TB case turned out to be AFB smear positive on at least one sputum smear, that case was enrolled as an index TB case and interviewed. If a registered TB case turned out to be AFB sputum smear negative or selected patient declined to participate in the study, then the next TB case in the list frame was contacted and the screening procedure conducted. During the first visit of the household for enrollment of contacts of an infectious TB index case, verbal informed consent was obtained from all the persons 12 years of age or more and from the parents of household contacts less than 12 years of age who fulfilled our contact definition. The study protocol was reviewed and approved by the departmental ethical review committee.

Tuberculin Skin Test and Data Collection:

In addition to one of the authors (SKR), three (two male and one female) medical graduates were recruited for interview purposes. They had at least two years of experience in the field since their graduation. All were quite fluent in Sindhi. SKR briefed the interviewer team

on the interviewing process during the training session, which included seeking the consent of the subject, being polite and considerate, standardize delivery of questions, recording the responses, and performing the TST. A predesigned and pre-tested questionnaire was used to collect data on potential risk factors from the index case and from household contacts. The information on household-level variables was collected from the head of household or his/her representative on the day of performing TST. For the household contact of age < 12 years, mother/guardian answered the questions on behalf of the contact. The bacillus Calmette-Guérin (BCG) vaccination records were not available for most of the contacts. Therefore, BCG vaccination status was assessed through physical examination for the presence or absence of BCG scar. The reported sensitivity of BCG scar, as an indicator of BCG vaccination status, was 98.5% when evaluated using the vaccination card as a gold standard.[23]

Household contacts of an index TB case were subjected to TST using mantoux method, a recommended technique for mass screening of M. tuberculosis infection in epidemiologic studies.[24,25] It was performed on the volar aspect of the forearm with 0.1 mL of Tubersol (purified protein derivative [PPD-RT 23],[5] tuberculin units; Connaught Laboratories, North York, Ontario, Canada). Contacts were visited again 72 hours later to read the TST. During that visit any household contact not present on the first visit was also interviewed and subjected to TST and revisited 72 hours later to read the result. At the most, we made three visits to read the TST results. The BCG unvaccinated household contact of index case was considered M. tuberculosis infected, if his/her skin induration was 10 mm or more at 72 hours post-TST. For BCG vaccinated household contacts, a cut-off value of 15 mm or more at 72 hours post- TST was used as an indication of M. tuberculosis infection.[25,26]

Statistical Analysis:

Descriptive statistics and univariable analysis. We previously published descriptive statistics on the individual- level variables and their univariable associations with TST positivity of household contacts of index TB patients.[15] We computed the descriptive statistics and evaluated univariable associations with TST positivity for household contextual variables, which included the epidemiologic and clinical characteristics of the index TB case as well as social (total monthly household income) and environmental factors (i.e., crowding).

Multilevel Modeling:

Because of the hierarchical structure of the data, household contacts of AFB smear positive index TB patients nested within households and the possibility of intrahousehold correlation regarding the likelihood of TST positivity, we used multilevel logistic regression analysis.[27] Multilevel models are generally used to simultaneously examine the effects of group-level and individual-level variables on individual- level outcomes. A series of multilevel models were fitted with characteristics of familial contacts at the first level and household contextual effects at the second level. Parameters were estimated by constructing generalized linear models (GLM) using second-order penalized quasi-likelihood (PQL) and second-order linearization with a binomial distribution assumption and a logit link. In model A (null model), the variation in TST positivity was modeled by random intercept. In model B, we fitted the main effects of the same individual-level variables (i.e., age in completed years, sleeping site of contact relative to index case, and presence or absence of BCG scar) that were significantly associated with TST positivity in the previous analysis.[15] Finally in model C, we examined household contextual effects and significant individual-level characteristics identified at the second stage to assess whether householdlevel effects were explained by individual-level characteristics. With this strategy, we were able to isolate the effects at familial contact-level and household-level to gain an understanding of the distribution and determinants of TST positivity.

To estimate the fixed effects, we quantified the associations of the variables in the models and TST positivity of contact by computing adjusted OR with 95% confidence intervals (CI) obtained from regression coefficients and their standard errors (SE). We used the variance partition coefficient (VPC) and median odds ratio (MOR) to quantify the inter-household variance in TST positivity. We also applied 80% interval OR (IOR-80), which integrates random effects (inter-household variation) in the measurement of fixed effects (i.e., householdlevel variables).[28,29] The IOR-80 takes into account the magnitude of inter-household variation in TST positivity when interpreting the influence of household variables.

Sensitivity Analyses:

To test the influence of missing data on our results, we carried out sensitivity analyses. We analyzed the TST positivity by assuming that TST readings of the contacts with missing data on this outcome variable would have followed the same distribution as of those who

remained under observations (group mean substitution),[30] Subsequently, the results from sensitivity analyses were compared with that of main analyses (complete-case analysis).[31]

Results

Descriptive and Univariable Analyses:

The participation rate was 99.5% (383/385) when the contacts were explained the benefit of TST and if needed, followed by free prophylactic or curative TB therapy as appropriate. Of the 385 TB contacts included, only two female contacts of female index TB patients refused to undergo TST and to report either age or to be examined for BCG scar. Of the remaining 383 participants, 24 (6.3%) could not be traced on repeated visits for TST reading. Of these 24 contacts, most (16) were a second degree relative of the index TB patient, male (16), never married (18), and all 24 were contacts of female index patients. Thus, the effective sample size for all further analyses was 359 contacts.

The prevalence of TST positivity among household contacts of AFB sputum-smear positive index TB patients was 49.9% (179/359). Over 50% of the contacts were 15 years of age or older, 53% were male, 61% were never married, BCG scar was invisible in 85% of the contacts, and about 48% of the familial contacts were the children of index pulmonary TB patients (Table-1). The C_2 analysis of individual-level variables showed that contact's age (years), education, marital status, relationship with index case, visibility of BCG scar in contact, sleeping site of contact relative to index case, sharing utensils with index case were significantly ($P \leq 0.05$) associated with TST positivity (Table-2).

Table-1: Frequency distribution of individual-level characteristics of household contacts of the smear-positive tuberculosis cases recorded during a cross-sectional study of risk factors associated with tuberculin skin test positivity among household contacts of index cases, Umerkot, Pakistan, August-September 1999 (N = 385).

Variables	Number	%
Age of household contact (years)		
0.25–4	68	17.7
5–14	83	21.6
5–24	103	26.8
≥25.0	130	33.9
Gender		
Male	205	53.2
Female	180	46.8
Education (years of schooling)		
Nil	277	71.9
1–5 years	36	9.4
6–10 years	45	11.7
> 10 years	27	7.1
Marital status		
Ever married	149	38.7
Never married	236	61.3
BCG scar		
Present	57	14.8
Absent	327	85.2
Relationship of contact with index case		
Husband	28	7.3
Wife	34	8.8
Child	184	47.8
Sibling	23	6
Father	8	2.1
Mother	8	2.1
Grandparent	1	0.3
Uncle/Aunt	2	0.5
Others	97	25.2

Table-2: X^2 analysis of individual-level factors from a cross-sectional study of tuberculin skin test (TST) positivity among household contacts of smear-positive tuberculosis cases, Umerkot, Pakistan; August-September 1999 (N = 359).

Variables	TST positive contacts* n (%)	Total	X^2	P
Age of contact (years)				
0.25–4	14 (23)	60	27.5	< 0.001
5–14	39 (52)	75		
15–24	46 (47)	99		
≥25	80 (64)	125		
Gender				
Male	94 (50)	189	0.03	0.96
Female	85 (50)	170		
Education				
Illiterate†	142 (55)	259	9.2	0.002
Literate	37 (37)	100		
Marital status				
Ever married	87 (61)	143	11.5	0.001
Never married	92 (43)	216		
Relationship of contact with index case‡				
Spousal	41 (66)	62	8.6	0.014
First degree	103 (48)	215		
Second degree	35 (43)	82		
BCG scar in contact				
Present	34 (62)	55	3.7	0.054
Absent	145 (48)	304		
Sleeping site relative to index case				
Same bedroom	118 (56)	211	7.5	0.006
Different bedroom	61 (41)	148		
Sharing of utensil with index case				
Yes	132 (56)	238	8.9	0.003
No	47 (39)	121		

* Contact was considered MTB (Mycobacterium tuberculosis) infected, if his/her skin induration ≥10 mm without bacillus Calmette-Guérin (BCG) vaccination and ≥15 mm with BCG vaccination at 72 hours post-tuberculin test, otherwise he/she was regarded as Mycobacterium tuberculosis non-infected.

† Illiterate = had no formal school education; literate = had one or more years of schooling.

‡ Relationship of contact with index case: Spousal: (husband or wife); First degree: (child, sibling, and parents; second degree: grandparents, uncle, aunt, brother-in-law, and sister-in-law, others).

Household contextual variables significantly (P £ 0.05) related with contact's TST positivity were gender of index case, education of index case, duration of TB in index TB case, intensity of AFB smear status in index case, and haemoptasis status of index case (Table-3).

Multilevel Models

Multilevel logistic regression analysis of the fixed effects showed that compared with contacts 4 years of age or less, contacts were significantly more likely to be TST positive if their age was 5-14 years (adjusted OR = 2.60; 95% CI: 1.32-5.01) or 15-24 years (adjusted OR = 4.45; 95% CI: 1.98-9.97) or 25 years or more (adjusted OR = 7.16; 95% CI: 3.39-15.10). Furthermore, familial contacts were significantly more likely to be TST positive if they had slept in the same room as that of the index TB patient (adjusted OR = 2.64; 95% CI: 1.48-4.71) (Table-4 ; model C). Over and above the individual-level variables, household contacts were significantly more likely to be TST positive if the index TB patient was a female (adjusted OR = 2.20; 95% CI: 1.25-3.87) or if the intensity of AFB sputum-smear positivity in index TB patient was graded more than 2 (adjusted OR = 3.19; 95% CI: 1.86-5.46). However, IOR-80s for household-level variables in the model were very wide and contained 1 both for gender (IOR-80: 0.25-19.77) and intensity of AFB smear positivity of index patients (IOR-80: 0.36-28.65) (Table-4 ; model C). Therefore, the contextual effects of these two variables on the contact's TST positivity were not strong enough when compared with the residual household-level heterogeneity.

Table-3: X^2 analysis of household-level variables from a cross-sectional study of tuberculin skin test positivity among the contacts of 77 index patients with infectious pulmonary tuberculosis, Umerkot, Pakistan, (N = 359).

Variables	TST positive contacts n (%)	Total	X^2	P
Age (years) of the index case*			4.6	0.204
≤25	42 (60)	70		
26–34	25 (49)	51		
35–49	56 (44)	127		
≥50	56 (51)	111		
Gender of index case			7.3	0.007
Male	84 (43)	194		
Female	95 (58)	165		
Education of index case (years of formal schooling)			7.7	0.005
≥5	174 (52)	336		
< 5	5 (22)	23		
Home ownership			2.9	0.089
Yes	124 (47)	263		
No	55 (57)	96		
Crowding index			0.1	0.757
≤ 2	20 (48)	42		
> 2	159 (50)	317		
Duration (year) of TB in index case†			7.8	0.005
< 1	75 (42)	177		
≥1	104 (57)	182		
Intensity of AFB smear positivity in index case††			17.2	< 0.0001
≤ + +	111 (43)	258		
> + +	68 (67)	101		
Index case ever had haemoptasis			5.9	0.015
No	60 (42)	143		
Yes	119 (55)	216		
Index case had chest cavity§			0.4	0.554
No	114 (49)	234		
Yes	65 (52)	125		

* Index case: The first member of household presented to NGO clinic at Umerkot.
† Number of months since the diagnosis of TB in assumed index case.
†† Intensity of sputum smear-positivity: + = 1–10 acid-fast bacteria in the whole slide.
++ = 1–10 acid-fast bacteria per microscopic field (×1,000); +++ = more than 10 acid-fast bacteria per microscopic field .
§ Cavitary lesion present on chest x-ray of index case.

Multilevel logistic regression analysis of random effects showed that household intercept variance (±SE) in model A, B, and C was 2.00±0.54, 3.31±0.83, and 1.47±0.70, respectively, and was substantially large in each case. Consequently, MORs and VPCs in all three models were also large (Table-4 ; models A-C). In addition, as noted earlier, IOR-80s for both the contextual variables in the model included 1, thus confirming the low importance of these contextual effects in the present study.

Sensitivity Analyses:

We assigned TST positive status to 14 randomly selected contacts from the total of 24 contacts with missing TST readings to reflect the proportion of TST positivity among the contacts of female index TB patients. With this approach, the overall prevalence of TST positivity (50.3%) was nearly the same as that of main analysis (49.9%). Furthermore, we found no meaningful differences in the effects obtained from the sensitivity analyses compared with those of the main analyses.

Discussion

Individuals with exposure to an infectious TB patient in congregate settings (e.g., household, close social, work place contacts) are considered as high-priority contacts for rapid evaluation. Resultant identification and chemoprophylactic therapy of contacts infected by a TB patient helps to prevent future TB cases.[32] The TST continues to be the standard test for detecting latent infection with M. tuberculosis using purified protein derivatives of M. tuberculosis . However, problems

Table-4: Estimates of individual-level and household-level fixed and random effects obtained from multilevel logistic regression analysis of tuberculin skin test positivity among contacts of index tuberculosis patients, Umerkot, Pakistan, 2002.

Effects	Model A	Model B adj. OR (95% CI)	Model C adj. OR (95% CI)
Fixed effects			
Individual-level variables			
Age of the contact (years)			
5–14 (vs. 0.5–4)		6.44 (2.32–17.84)	2.60 (1.32–5.01)
15–24		12.48 (4.04–38.06)	4.45 (1.98–9.97)
> 25		24.58 (8.91–67.85)	7.16 (3.39–15.10)
Sleeping site relative to index case (same vs. separate room)		3.92 (1.88–8.16)	2.64 (1.48–4.71)
Household-level variables			
Gender of index patient (female vs. male)			2.20 (1.25–3.87)
IOR-80			(0.25–19.77)
Intensity of AFB sputum-smear positivity of index case (> + + vs. £ + +)			3.19 (1.86–5.46)
IOR-80			(0.36–28.65)
Random effects			
Household intercept variance (SE)*	2.00 (0.54)*	3.31 (0.83)*	1.47 (0.70)*
VPC (%)	37.83	50.14	30.91
MOR	3.84	5.63	3.17

* (SE), standard error of the variance.
Adj. OR = adjusted odds ratio; CI = confidence interval; IOR-80 = interval odds ratio-80; VPC = variance partition coefficient; MOR = median odds ratio.

resulting from prior immunization with BCG vaccine or environmental mycobacteria and low TST sensitivity in immunosuppressed individuals, such as those infected with human immunodeficiency virus (HIV), underline the need for more specific and more sensitive tests. Substantial progress has been made for the development of new diagnostic tests to detect latent infection with M. tuberculosis , and in several low endemic countries such tests have been included in the panel of procedures for investigating contacts of patients with infectious TB. However, the potential use of these high cost tests in TB endemic countries is still debated.[33] Therefore, despite some of these shortcomings TST continues to be the standard test for use in field epidemiologic studies in less developed countries.

Prevalence of TST Positivity:

In this cross-sectional study, the prevalence of TST positivity among the household contacts of AFB smear-positive patients was 49.9%. To our knowledge similar data from the study area or other areas of Pakistan are not available for comparison. However, comparable data on the prevalence of M. tuberculosis infection among the contacts of TB patients as high as 41% in Spain,[34] 63.9% in Brazil,[35] and 66% in Uganda, 36 have been reported. In a high TB incidence area, 34% of children under the age of 5 years and living in the same household as an index TB case, were reportedly diseased and 14% were infected with M. tuberculosis.[37] Recently, during an investigation in the United States, 39% of high priority contacts (work place) of index infectious TB patients were TST positive.[38] The difference in the prevalence of TST positivity among high priority contacts in various settings may be the result of different contact types (household versus workplace) with variable exposure intensity and its duration and difference in background rates of exposures to M. tuberculosis. Nonetheless, the results of the present study corroborate the earlier findings that the close contact with an AFB sputumsmear positive index case poses a substantially higher risk of acquiring M. tuberculosis infection in less developed countries. We could not assess the sputum smear status of the TST positive contacts, but one could speculate that a considerable number of these contacts could have active TB and contribute to the continued spread of M. tuberculosis infection in their households and in the community at large. It is known that once M. tuberculosis infection has occurred, the risk for development of TB is influenced by the duration of infection[39] and intrinsic characteristics, such age,[40] body build, BCG vaccination,[41] HIV infection,[42] and host genetic susceptibility.[42,44] The role of such factors in influencing the progression from M. tuberculosis infection to disease in this population merits further investigations.

In this study, we have identified several risk factors associated with TST positivity among household contacts of index TB patients. The role of these factors in potential intra-household transmission of M. tuberculosis from index patients to contacts has been previously described.[15,45-47] However, we are unaware of any published data that have been analyzed in a multilevel setting to evaluate the household contextual determinants of TST positivity among the contacts after taking into account individual-level characteristics. Our main motivation for this analysis was to obtain the estimates of the fixed effects of household contextual parameters. Therefore, the multilevel approach was an appropriate analytical technique as it gave less biased estimates along with appropriate standard errors.

Fixed Effects:

Individual-level factors. Consistent with previous findings, a trend of increasing odds of TST positivity with increasing age was observed in this study,[47] perhaps as a result of an extended duration and a higher frequency of close intimacy with index TB cases. Additionally, household contacts with increasing age might have been exposed to other AFB smearpositive TB cases in the neighborhood, because in an area of high TB incidence most of the transmission of M. tuberculosis infection takes place outside the household.[12] In a similar setting in rural India, with a culture of extended families and the contact network spanning beyond the nuclear family, 80% of M. tuberculosis infected persons in any age group occurred in households without TB cases.[16] We focused on the well-defined social network of a household and did not delineate contact network outside the household. Therefore, how much exposure to M. tuberculosis infection has occurred outside the household is unknown in this study. Furthermore, the proportion of exposure to M. tuberculosis outside the household in such settings may differ from region to region depending on the prevalence of TB and mixing patterns of infectious cases. 16 Any future investigations in this and similar settings may consider taking into account the amount and pattern of exposure within and outside the households of index TB patients; preferably using molecular epidemiologic techniques.

To assess the level of proximity of contact made with the index case, the sleeping site of household contact relative to index TB patient was considered. In this study TST positive contacts were significantly more likely than TST negative contacts to have had slept in the same bedroom with index case. This type of intimacy of contacts with index case is quite common in less developed countries, wherein household contacts often share either the same bedroom or same bed with index case. This type of prolong contact has been shown to be independently associated with TB status of household contacts. 16 This behavior seemed to have resulted due to over-crowding, because most of the housing units in the study area are one bedroom with an average household size of 5.4.

In our analysis, we could not demonstrate significant association between the presence of BCG scar and a positive TST among contacts of index infectious TB patients. There are conflicting reports regarding the influence of BCG vaccination on TST. Our previous analysis 15 and a recent study[47] showed a marginally significant association between BCG scar status and positive TST. A meta-analysis showed that BCG vaccination increased the TST positivity,[48] which may have several alternate explanations. For example, several studies have demonstrated that after BCG vaccination, skin test reaction wanes with time and the vaccine-induced protection is likely to last for 15 to 20 years. Furthermore, BCG vaccine has a documented protective effect against TB meningitis and disseminated TB among infants and young children. However, it does not prevent primary infection with M. tuberculosis and, more importantly does not prevent reactivation of endogenous latent M. tuberculosis infection, which is the principal source of spread of M. tuberculosis infection in the community. 49,50 Nevertheless, the role of BCG vaccination on the duration of protection against infection with M. tuberculosis needs to be reassessed so as to devise an effective revaccination scheduled in this and similar setting in less developed countries with a greater load of mycobacterial exposure.

Household-level Factors:

The AFB sputum-smear positive grade of index case was associated with increased odds of TST positivity among household contacts of index TB cases in this study. These findings are consistent with previous data showing that index cases with high-grade sputum smears are highly infectious to others,[51-53] wherein positive TB rates among contacts of index TB patients with a smear grade of 2 or more were 7 times higher than among contacts of index TB patients with a grade of 1.[54]

The household contacts of female index patients compared with male index patients were twice as likely to be TST positive. Previous studies have looked at the

gender of index TB patient in relation to the risk of M. tuberculosis infection among household contacts of such patients.[16,45-47] However, to our knowledge none has implicated the role of index TB patient's gender in disproportionate increase in the risk of acquiring M. tuberculosis infection among household contacts. This is an interesting observation; the possible explanation is that female index TB patients in this and similar settings in the region tend to spend more time at home and thus a relatively extended duration of exposure to their contacts compared with male TB index patients. However, this finding needs to be verified in future studies. Education of index case, duration of TB in index case, and index case ever had haemopytasis had no apparent independent associations with TST status as assessed in multilevel models in this study.

Random Effects:

We examined whether household had a general contextual effect on TST positivity of a familial contact of an index TB patient. As noted earlier, this contextual phenomenon was measured by VPC (%) and MOR. We also computed IOR-80s, which integrated the inter-household variance in the evaluation of household-level fixed effects. In model C, IOR-80s both for gender of index TB patient and intensity of AFB-sputum smear positivity of index TB patient included 1, thus indicating low importance of these two household- level fixed effects for TST positivity among familial contacts of index TB patients. The MORs was substantially large and ranged from 3.17 to 5.63 in three models, suggestive of considerable household differences among familial contacts' probabilities of being TST positive. The household intercept variances in three models were large ranging from 1.47 to 3.30, reflecting substantial differences in the probabilities of being TST positive among the familial contacts across the households. Therefore, there are still unknown household-level factors conditioning the probability of M. tuberculosis transmission in this and perhaps in other settings with similar characteristics. Earlier studies also suggested the household as a complex system of interacting risks for M. tuberculosis infection and subsequently its progression to disease.[16] Further research needs to clarify these modes of M. tuberculosis transmission using better definitions and assessment of household attributes.

Limitations of the Study:

Several potential limitations should be considered in interpreting the results of this study. First, the study is limited by cross-sectional design so temporality (cause-and-effect relationships) cannot be established. Second, from a given household, the first subject registered for treatment at the UATA clinic was assumed to be the index case. We are however, uncertain whether he/she is indeed the first person who was infected or is a secondary contact of another infected member in the household, but exhibited disease earlier than the other M. tuberculosis infected household member. Third, we could not distinguish between contacts that developed TST positivity resulting from exposure to an identified index TB patient in the household or from exposure to another TB patient in the neighborhood. It has been shown that in a high-incidence area M. tuberculosis transmission takes place mainly outside the household.[12] Fourth, after the exposure to M. tuberculosis the ability of TST to detect a delayed type of hypersensitivity reaction on an average is 4-12 weeks.[33] Therefore, some of the non-infected household contacts might have been incubating the M. tuberculosis but could not be detected by TST directing the results towards the null. Fifth, the duration of contact of each household member with the index case could not be ascertained, because the exact dates of diagnosis of M. tuberculosis infection in most of the index TB cases were not available. Sixth, because all of the index cases were AFB smear-positive, we cannot draw any inference regarding the risk of M. tuberculosis transmission in the household of AFB sputum-smear negative index TB cases. Seventh, in this study we could not assess the intra-observer and inter-observer variability of reading the TST results. However, we draw contention from the fact that such variation possibly was minimal because all the interviewers were medical graduates and their prior training, before the start of the actual study, may have given them sufficient experience to conduct TST and objective measurement of the TST results. Finally, we had missing data on TST results for 6.3% of contacts of female index TB patients. Therefore, it is possible that this might have slightly biased the results and/or reduced the study power.[31] However, we found no meaningful differences between the results of the main analysis and those from sensitivity analysis. Also, reassuring were the comparable findings of a study in Brazilian families, wherein TST positivity was 54% and 40% among the household contacts of female and male index TB patients, respectively,[55] the figures nearly similar to those in our study.

In this study, we could not determine whether index TB patients were suffering from multidrug-resistant TB. The evaluation of such TB patients and their required treatment should be the priority to help shrink the pool of infectious individuals. Investigation of household

and other close contacts of an infectious TB patient is a standard practice in developed countries to identify subjects having M. tuberculosis infection and TB disease. 13,46 However, contacts' evaluation is not an integral component of a TB control program in many less developed countries. Therefore, the results of this study reinforce the need for active case-finding among household contacts of infectious cases in TB high burden countries such as Pakistan. This procedure may identify substantially more subjects who could qualify for chemoprophylaxis or supervised therapy under DOTS (directly observed treatment short course) program and help achieve the World Health Organization (WHO) set targets of 70% case detection and 85% cure rate.[56] The individual-level and household-level characteristics identified in this study should be considered while prioritizing contacts for investigations to use the available resources effectively. Enhanced motivation of household contacts of infectious TB patients through educational intervention, based on the findings of this study, may further improve TB prevention and control in the near future in resource-constrained settings in Pakistan and perhaps other countries in the region.

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