Comparison of RT 23 and Local Tuberculin (PPD) Produced by Razi Institute

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Summary

To Comparison between the local tuberculin produced by Razi institute and RT23, 86 people were participated. They were confirmed tuberculosis patients and apparent healthy students that randomly divided in two groups. For the first group the local tuberculin and RT23 respectively injected to right and left hand simultaneously and for the second group the reverse was done. To compare the agreement of two PPD in different tuberculosis prevalence, which has practical importance, results simulated in populations with different tuberculosis infection rates. The agreement between tuberculins was assessed by weighted kappa coefficient. The weighted kappa coefficient, with 37% tuberculosis prevalence, was 63% (95% CI: 55-71). However, in two simulations in suspected cases of tuberculosis in a referral clinic and apparent healthy people were 48% and 43%, respectively. Based on estimated kappa coefficients the agreement between local PPD and RT23 for clinical and epidemiological proposes was moderate. In addition, with the reduction of the tuberculosis infection in these groups, the agreement between two tuberculins would decrease. The main recommendation is to allocate a tuberculin product in Iran for human usage.

Key words: Tuberculin test, local PPD, RT23, Iran

Introduction

The tuberculin skin test is used for diagnosis of individual's tuberculosis infection. At the time being, it is recommended for making decisions upon the chemoprophilaxis

and diagnosis of children tuberculosis. Moreover, this test is used as a tool in tuberculosis epidemiological investigations (Crofton *et al* 1992).

A purified protein derivative (PPD) provides by chemical fractionation of local tuberculin in Razi institute, the biological assay of product is performed on guinea pig. Since the process of standardization and characterization of the worldwide used tuberculin require researches on human (Wijsmuller & Bardine 1972); the purpose of this study is to make a comparison between the local tuberculin and the RT23. The latter was selected because it has been confirmed as a standard PPD for field works by different health authorities such as WHO, UNICEF and IUATLD (Arnadottir *et al* 1996).

Materials and Methods

Subject. 86 persons were participated, among which some were patients admitted by Dr.Masih Daneshvary hospital and others were students studying at Dr. Ahari Child Care Center, Tehran University of Medical Sciences. The reason for this was that, since all the subjects from the first hospital were regarded as tuberculosis patients and remaining weren't obviously, a broad range of tuberculin responses could be evaluated. The necessary criteria for individuals to be included in the study were their voluntarily agreement to participate in the study (they had over 15 years of age) and the opportunity of getting in touch with them till 48h after the injection (for follow up the result of the study). The exclusion criteria were measles, rubella, mumps, chicken pox, whooping cough and or immune depressant conditions and also self-report of measles, rubella, mumps and poliomyelitis vaccination during one month before start of the study. In addition treatment using immune depressants within previous two weeks was another exclusion criterion.

Skin test. The study subjects who met the criteria mentioned above were randomly divided in two groups by using random table. For first group the local tuberculin injected to the right hand and RT23 was simultaneously injected to the left by mantoux technique, and for the second group the reverse was done. The results were measured through Sokal procedure (Arnadottir *et al* 1996) and a physician in all cases did it.

Data analysis. First of all the subjects under the study were characterized. Then the correlation between the results of the tuberculins was determined and the difference between them was examined based on the different categories of the response to RT23. Consequently, since the reliability of the two tests was different due to the prevalence of the characteristic under the study (here tuberculosis infection), the data were gained of the subjects were simulated in two different situations. First, for a population for whom the prevalence of tuberculosis is rare, e.g. condition would happen under epidemiological investigation in whole population, and secondly using the sample data on a tuberculosis and lung disease clinic prevalence. This was done to compare the agreement of two PPD in different tuberculosis prevalence, which has practical importance. For this, a population was selected using the data obtained from investigations of the tuberculin results on 11'116 primary school students in Bam, southern Iran. Moreover, for later group tuberculin response of suspected cases in a tuberculosis clinic, "Tuberculosis and Lung Diseases Clinic" in Park-Shahr Tehran, obtained. Consequently, the expected results in two populations were studies by simulating observed results of the RT23 and local tuberculin.

For conducting these comparisons, the results of the tuberculins were classified to "4mm or below", "5-9 ", "10-14" and "15mm or above" (Ravinglion & O'Brien 1998). The agreement between two groups was assessed by weighted kappa coefficient (Altman 1995). The data entered in Epi-info and analysis was done by SPSS for Windows.

Results

The participants and censored subjects characteristics including sex, nationality, age and tuberculosis involvement are demonstrated in tables 1 and 2, respectively. Because of four subjects were absent the results of 82 were obtained. Since the comparison between two tuberculins was performed simultaneously for both arms, there was no threat of the confounding results with another variables. The only possible difference could be the side of the arm, which was injected. Likewise, this proved to have no difference due to the random assignment of the injections to both arms. Although four subjects were loss to follow up, it didn't make any significant

Table 1. *Description of study subject*

Variable	Levels	Count	Percent
Sex	Female	45	54.9
	Male	37	45.1
Nationality	Iranian	57	69.5
	Afghani	25	30.5
Age group (year)	15-24	17	20.7
	25-34	25	30.5
	35-44	16	19.5
	45-54	8	9.8
	55-64	9	11
	65 and/or above	7	8.5
Health status	Patient	30	36.6
	Healthy	52	63.4
Study group	Local-RT23	38	46.3
Right-left (hands)	RT23-local	44	53.7
Total		82	100

difference regarding to arm allocated, e.g. 46.3% in contrast to 53.7%. Thus for doing comparisons, there would be no need to use multivariate analysis for the adjustment of confounding variables.

Table 2. *Characteristics of loss to follow up persons*

Variable	Levels	Count
Sex	Female	2
	Male	2
Nationality	Iranian	4
	Afghani	0
Age group (year)	15-24	2
	25-34	2
	35and/or above	0
Health status	Patient	2
	Healthy	2
Study group	Local-RT23	3
Right-left (hands)	RT23-local	1
Total		4

Figure 1 demonstrates scatter diagram of two tuberculins response in study participants, which correlation and determinant coefficients are (r =) 0.77 and (r^2=) 0.54 respectively.

Table 3 shows the mean difference between two PPD according to categories of the based tuberculin, which is RT23. The difference of two tuberculins was significant in "4 mm and below" category ($P < 0.001$). Consequently, despite of such result global

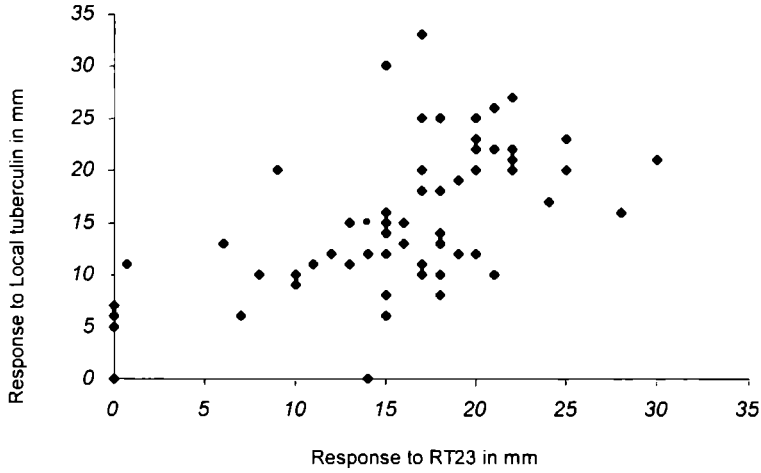

Figure 1.*Simultanous reaction to tuberculins*

comparison of two tuberculins was ignored. In other three categories, however, the difference was not significant. For comparing the results of the diverse groups, table 4 is presented which pointed out the distribution of the simultaneous results with two PPD. The weighted kappa coefficient for this table is 63% with 8% standard deviation (95% CI: 55–71 percent).

As the prevalence of tuberculosis in this population, according to table, was 37% the obtained result could occur in neither epidemiological investigation nor clinical practice. Therefore, the potential distribution of the results of both tuberculins and their agreement in two populations, i.e. suspected to tuberculosis and apparently healthy primary school children, are demonstrated in tables 5 and 6.

Table 3. *Mean and standard deviation of responses difference between two tuberculins*

Tuberculin response group	Count	Difference	
		Mean	Standard deviation
0-4mm	20	-2.95	3.56
5-9mm	4	-4.75	5.31
10-14mm	10	1.80	4.49
15mm and or above	48	1.00	5.99
All cases	82	-0.15	5.60

Table4. *Frequency of simultaneous responses to each category of tuberculins**

Responses in mm		Local Tuberculin				Total
		0-4	5-9	10-14	15 & or above	
RT23 Tuberculin	0-4	11	8	1	0	20
	5-9	0	1	2	1	4
	10-14	1	1	6	2	10
	15 & or above	0	3	13	31	48
Total		12	13	22	35	82

* Estimated weighted kappa coefficient = 63%

Table5. *Expected frequency of simultaneous responses to each category of tuberculins, simulated based on tuberculosis infection situation in a tuberculosis and lung disease clinic *, ***

Responses in mm		Local Tuberculin				Total
		0-4	5-9	10-14	15 & or above	
RT23 Tuberculin	0-4	11	33.24	0.64	0	44.80
	5-9	0	4.15	1.27	0.57	5.99
	10-14	1	4.15	3.82	1.14	10.11
	15 & or above	0	12.46	8.27	18.29	39.02
Total		12	54	14	20	100

* Estimated weighted kappa= 48%

** Cells filled by simulation, so the data were expected value and might be decimals.

Table 6. *Expected frequency of simultaneous responses to each category of tuberculins, simulated based on tuberculosis infection situation in a primary school children study*

Responses in mm		Local Tuberculin				Total
		0-4	5-9	10-14	15 & or above	
RT23 Tuberculin	0-4	54.1	17.8	0.5	0	72.40
	5-9	0	2.2	1	0.03	3.23
	10-14	4.9	2.2	3	0.06	10.16
	15 & or above	0	6.8	6.5	0.91	14.21
Total		59	29	11	1	100

As presented in table 7, which is a summary for two previous tables, weighted kappa coefficient for two populations were respectively 48 and 43 percent. This result emphasized on the direct relationship of agreement with the rate of tuberculosis infection.

Table 7. *Comparison of Local and R23 tuberculins agreements in different populations based on various tuberculosis expectations*

Study population	Tuberculosis probability	Greater 10 mm response(%)	Weighted kappa(%)
Present study	37%	57	63
Cases of a lung clinic	5-10 %	34	48*
Appearance healthy school children	Less than 5 per 100,000	12	43*

*Calculations of these two cells were based on tables 5 and 6; expected frequencies of present study.

Discussion

Based on kappa coefficients, which indicated in table 7 the agreement between local PPD and RT23 for clinical and epidemiological proposes was moderate. In addition, with the reduction of the tuberculosis infection in these groups, the agreement between two tuberculins would decrease. Although absolute value of differences in small reactions expected to be smaller than groups with greater responses and it must be considered with caution in using absolute values; but in interpretation of table 3 it `is obvious that the absolute value of difference in "0-9 mm" group is more that it in "10and or above" reactions. Therefore, the result of this table, too, was indicative of the main conclusions of the study on the agreement of two tuberculins in higher rates of tuberculosis infection. Based on the conclusions of the study, the questions raised by the previous researches on the local tuberculin could be removed. At least the studies in which the authors of this paper had been involved (Golkari *et al* 1995, Sharifi *et al* unpublished) the interpretation of results have always been performed with doubts on the accuracy and precision of local PPD.

It could certainly much more satisfactory if the study would be performed in two distinct researches. One of which studying the patients who suffered from symptoms

compatible to tuberculosis and the other one the whole population, i.e. respectively for clinical setting and epidemiological investigations, each of them takes adequate samples. What made the investigators to select the subjects under the study between two populations, however, was their desire to study a diverse range of responses. On the other hand, as indicated in previous studies, in performing tuberculin skin test (especially in the general population) the mode of responses would be "no reaction" or zero (Reider 1995, Edwards *et al* 1969). Due to the limitations in supplying RT23, there would be a threat of wasting the sample. Thus, it was decided to recruit these groups of subjects. In data analysis, however, the results were simulated to two groups, which are to the point of interest in applied practice.

In addition to the prevalence of the characteristics under study, two factors influenced the estimated kappa. The manner of classification, regarding both the numbers of groups and selection the cut-of points, is crucial. Consequently, the selection of classifications in a different way could vary the estimated kappa. The classifications considered in this study, nevertheless, were based on the recommendations used in common clinical and epidemiological practices (Ravinglion & O'Brien 1998). One of the applications of such study might be determination of the groups within whose limits, the local tuberculin enjoy higher validity and reliability. For instance, based on table 4, 32 individuals out of 35 (91%) whose results on the local tuberculin were "15mm or above" performed the same results with RT23, too.

Finally, the results of the study raised quest for a broad investigation upon local tuberculin. It's necessary to mention that the present RT23 which is confirmed by WHO is a product which was firstly produced in 1950s and through rigorous experiments, it's standardization on guinea pigs and humans have performed and the accuracy and precision on the final product have been verified in large scale experiments (Guld *et al* 1958). The products made in Iran are produced within a short interval of one year or so. Their standardization and characterization, therefore, aren't examined on human beings. The investigation of the relative potency of such tuberculin is performed on guinea pigs based on Central Veterinary Laboratory instructions in Weybridge, UK. To sum, the main recommendation is to allocate a tuberculin product of Razi institute for human usage that could last for years. In this

case, the standardization and characterization of such a product would include all the essential research process in humans as well as guinea pigs.

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