Iranian J.Publ. Health
1987, Vol.16, No.1-4

THE PUZZLE OF THE FALSE POSITVE REACTION IN CR.NEOFORMANS'ES CAPSULAR ANTIGEN SLIDE LATEX AGGLUTINATION TEST BY SERA OF PATIENTS WITH RHEUMATOID ARTHRITIS.

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Key words: Cr.neoformans, Latex agglutination test (LAT), False Positive reaction (FPR)

ABSTRACT

Different examination have shown that Rheumatoid Factor is not responsible for false positive reaction (F.P.R.) in Cr. neoformans'es free capsular antigen latex agglutination test, whereas heigh levels of iron in Sera of patients with Rheumatoid Arthritis as well as other sera was responsible not only for this F.P.R., but also had an important role in the production of F.P.R. in many slide latex agglutination tests. This is because of Iron's Ion reaction with reagent's preservative: sodium azid and/or negative charge of the antigens fixed to the latex particles.

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INTRODUCTION

The latex agglutination test (LAT) for detection of Cr. neoformans antigen in serum and cerebrospinal fluid (2,4) is a specific and rapid reliable method in diagnosis of meningeal cryptococcosis, used commonly in Medical Mycology laboratories. Since false positive results were recorded by authors in LAT with sera from patients with Rheumatoid Arthritis (1,3,5,6), the present study was undertaken to investigate the reasons which are responsible for production of FRR in the LAT.

MATERIALS AND METHODS

One hundred samples of sera were obtained from blood which had been aseptically collected by venipuncture from patients suffereing from Rheumatoid Arthritis.

Test 1:

All the 100 samples of sera were tested for R.F. as mentioned by Cambridge Biochemical R.A latex test kit. (Lanwades Business Park, Kennett, New Market, CB&7PW,U.K.)

Test 2:

The 100 R.F. Positive sera were examined for LAT as recommanded by crypto-LA-test (International Biological Labs, INC. Dist) after inactivating of the sera at $56^{o}c$ for 30 minutes.

Test 3:

C.Reactive protein (C.R.P) was investigated in 5

randomly selected sera which were R.F.and L.A.T positive according to the method suggested by Human, R.F. quick test kit (6204 taunusstein-Neuhof).

Test 4:

Amount of serum-γ-globulins were detected in five sera which were L.A.T. and R.F. positive by the Acetate cellulose method as mentioned by Millipore (Millipor House, Abbey Rd. London, NW 10 7SP).

Test 5:

Sera which were R.F. and L.A.T. positive were tested with other several commercially produced slide latex agglutination reagents for wright and widal (Combridge Biomedical), L.E.test (Hyland Diagnostics, Division of Cooper Diagnostics INC, Malvern, Pennsylvania 19355,U.S. A), Mono test (Cooper Biomedical, Diagnostic Division, Freehold, NJ, 07728, U.S.A), Beta H.C.G test (Merck, D-6100 Darmstadt, F.R. Germany), slide meningite recommended by Biomerieux (Marcy l'Etoile, 69260, charbonnieres les Bains, France).

The above tests were repeated for the iron standard solutions (wako and Hyland kits) and also for sera of patients with Thallasemia Major with iron levels higher than 200 $\mu g dl^{-1}$.

Test 6:

Five R.F. and L.A.T. positive sera and twenty R.F. positive but L.A.T. negative sera were examined for their serum iron and TIBC according to the method described by

Wako (Wako pure chemicals industries Ltd., 10 Doshomachi 3 chome, Higashi Ku,Osaka, 541, Japan).

Test 7:

Two different iron standard solutions were examined with naked latex particals prior and after adding the sodium azide to latex particals.

RESULTS AND DISCUSSION

In examinations done on sera of 100 patients with Rheumatoid Arthritis (R.A.) whose sera were positive for Rheumatoid Factor (R.F.), only 14 showed F.P.R. in slide latex agglutination test for Cr.neoformans'es free capsular antigen.

This result seems to be contrary to the hypothesis described by Bennet and Bailey (1) in which R.F is responsible for F.P.R.

SINCE

1- The degree of agglutination (F.P.R.) were not correlated with the degree of R.F in sera with positive results.

2- The sera which had F.P.R in slide latex agglutination test for Cr. neoformans'es capsular antigen, showed positive reaction in many other silde latex agglutination tests as Wright, Widal, L.E test, Mono test, Beta H.C.G test, Slide Meningite, especially when the time of exposure was more than 5 mi-

nutes (10 minutes), whereas the sera which were negative in slide latex agglutination test for yeast's capsular antigen were also negative for all of the above tests.
3- F.P.R was not seen when positive control reagents of R.F kits was tested for Cr. neoformans'es capsular antigen.
4- There is some statistical correlation between serum's R.F and F.P.R, but it is low (Pt = 0.274).
So there was something in those 14 sera responsible for the F.P.R in all of the above mentioned slide latex agglutination tests for detection of different antigens and antibodies which was excluded in the rest of R.F positive sera.
C.Reactive Protein, Protein electrophoresis
were done for five positive randomly selected sera, but the results showed no similarity between them.
Another five positive and twenty negative sera were selected by chance and examined for serum iron and total iron binding capacity.
In these experiments when the mean amount of more than 200 $\mu g\ dl^{-1}$ of serum iron in the first five sera (Table 1) were compared with those of less than 150 $\mu g\ dl^{-1}$ (Table 2) of next twenty sera, an evaluation with student t test showed a significant difference, with t=15.246 and $p < 0.01$.

Table 1. The amount of serum iron in five sera with positive latex agglutination test:

No.	Serum Iron/ $\mu g dl^{-1}$
1	275
2	253
3	228
4	235
5	250

Mean=248.2 SD=18.213

Table 2- The amount of serum iron in twenty sera with negative latex agglutination tests:

No.	Serum Iron/$\mu g dl^{-1}$	f
1	72	1
2	77	1
3	78	1
4	84	1
5	85	1
6	87	1
7	94	3
8	95	1
9	97	1
10	103	1
11	107	1
12	112	1
13	115	1
14	123	1
15	126	1
16	127	1
17	130	1
18	137	1

Mean=101.85 SD = 19.3996

So it is suggested that serum iron plays an important rule in F.P.R not only in latex agglutination test for yeast's free capsular antigen, but for F.P.R in all of the above latex agglutination tests.

Other examinations have shown that the minimal amount of iron in serum for F.P.R is about 150 $\mu g dl^{-1}$.

To determine whether or not the iron affects all slide latex agglutination tests, 200 $\mu g l^{-1}$ iron standard solutions were tested with different above slide latex agglutination tests as well as for Cr. neoformans'es capsular free antigen. Iron standard solutions gave F.P.R in all of the above tests especially when the time of exposure was 10 minutes. Overall the above tests were repeated with serum of patients with Tallasemia Major who had iron levels higher than 200 $\mu g dl^{-1}$, and the same results were obtained as above. Since the chemical structure of antigens and antibodies were not similar in those reagents, F.P.R might not be caused by them, and on the other hand when the iron standard solutions (200 $\mu g dl^{-1}$) was examined with naked latex particles, no reactions were shown. But when the experiments were repeated with addition of 1 g L^{-1} sodium azide to the naked latex particles the result was strogly positive. It may, therefore, be concluded that observation of F.P.R was due to combination of iron with sodium azide. It seem's that sodium azide which act's as preservative in reagents will dissociate in solutions to produce azide ions (Anion: N^{3-}) and sodium ion (Cation: Na^{+})(7). These ions will absorb

to the surface of latex particles and will make incomplete valence bonds with them. On the other hand when a serum has high iron level, some of them will not bind to transferrin and will be as free ions(Fe^{2+}, Fe^{3+}).
The cations of iron will react with azides anions which are absorbed to latex particles. Or it might react non-specifically with the antigens which are absorbed to the latex particles, because of their negative charges and iron ions will act as bridges between latex particles and will cause F.P.R.
Mercaptoetanol can inhibit F.P.R if added to serum prior, to use, because it reduces iron cations, but its effect on R.F has no rule in inhibition of F.P.R.

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