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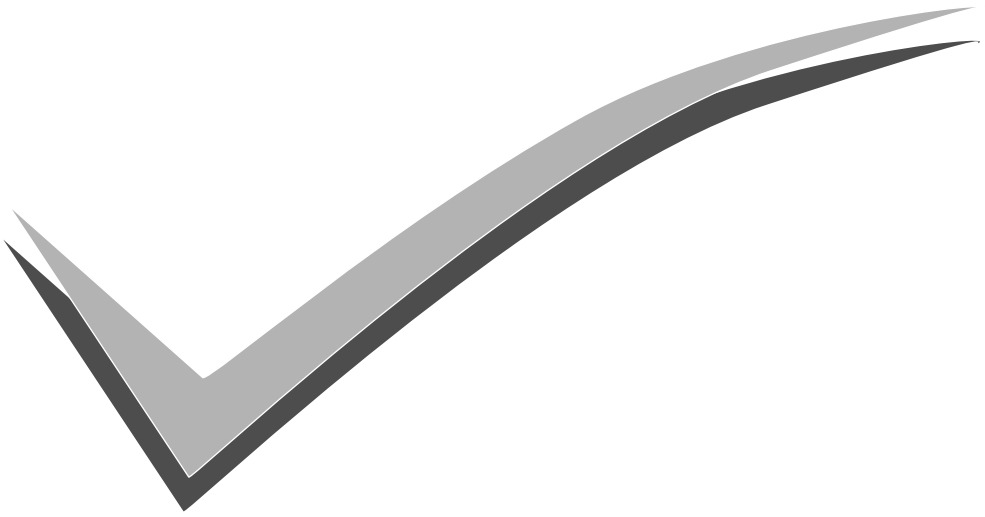

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HIV status and re-activity to RA, ASO, CRP, Syphilis and Facs count among leprosy patients

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Abstract

Twenty five leprosy patient's blood serum compare to control healthy person's blood samples were tested for HIV status and re-activity torheumatoid arthritis, ASO, CRP, VDRL and facs count among leprosy.None of the sample was found to be positive for antibodies to HIV ½. Out of twenty five leprosy samples, three were reactive for Rheumatoid Arthritis factor and seven were positive for antibodies to syphilis as revealed by the test. Further two were reactive for C-reactive protein and one were having titre of Anti-Streptolysin-O. Screening for HIV, Rheumatoid Arthritis and Anti-Streptolysin-O, C-reactive protein and Syphilis (VDRL) were done in order to know the prevalence levels of these infections, as biological markers of risk. Thus, screening the leprosy patients for these would go a long way in early detection of these co-infections. Early treatment, if initiated, would help in further deterioration of the condition of these patients.

Key words : HIV, leprosy, rheumatoid arthritis, ASO, CRP, Syphilis.

Inroduction

India has the largest number of known cases of leprosy and happens to incidentally be endemic for HIV as well. According to Ridley and Jopling (1966) studies done in North and North-Eastern India did not ûnd any association of HIV infection with leprosy patients. A few studies from South Indian states showed a higher prevalence of HIV infection among leprosy patients, but these studies alone do not provide any indication of its association with leprosy (Jayasheela et al., 1994). Leprosy caused by *Mycobacterium leprae*has an unusually long incubation period, and infection with HIV leads to a profound drop in CD4+ T-lymphocyte count and function and compromises the cell-mediated immune response, as well (Miller, 1991; Saha et al., 1993). Earlier studies carried out in this center suggested that per thousand (5/4025:0.124%) of the leprosy patients harbored HIV infection. Follow-up of these patients at an interval of six months, revealed that none of them downgraded into a severe form of leprosy nor developed ARC or AIDS (Hussain et al., 2000). Although this study indicated that leprosy is not a risk factor for developing HIV-1 infection, the HIV surveillance studies on this population was continued with a view to assess the risk and ûnd out the trend in an area where both the infections are prevalent. Some studies shows that the histological features of leprosy also appear to be preserved in HIVinfected patients (Moran et al., 1995; Pereira et al.,2004).

One of the commonly observed complaints among leprosy patients was pain in the joints. Many studies have proven that microbial agents might

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trigger the autoimmune phenomenon and induce rheumatoid arthritis (Albert et al., 1980; Cossermelli-Messina et al., 1997; Gibson et al., 1994). In order to ûnd out if arthritis is present in the HIV-leprosy co-infected patients, the sera from these cases were tested for Rheumatoid arthritis (RA) factor. Many risk behaviors as well as the routes of transmission for HIV infection are identical to those for other sexually transmitted diseases (STDs). For this reason, the leprosy sera samples were tested for Rheumatoid Arthritis andVDRL simultaneously with HIV.

Materials and methods

Leprosy patients, across the spectrum, i.e., tuberculoid (TT), borderline-tuberculoid (BT), mid-borderline (BB), borderlinelepromatous (BL), lepromatous (LL) and neuritic (N) types, classiûed, according to Ridley-Jopling criteria (Ponnighaus et al., 1991), attending the Unit-I of the Outpatient's Department (OPD) of the Central JALMA Institute for Leprosy and other Mycobacterial Diseases (CJILOMD) were included in the study. The leprosy cases in the study were neither newly admitted nor untreated patients, although a few were newly detected cases. For bacteriological determination, the six skin sites used were the two ear lobes and four representative active skin sites, i.e., hand (right arm and left arm), elbow (right and left), back, forehead, and the site of the lesion. In our OPD, four skin sites are routinely used for determination of the bacteriological index (B.I.). The inclusion criteria were: adult leprosy patients between the age group of 16 to 48 yrs. Children and old patients were excluded from the study as it was assumed they were not likely to be sexually active.

Blood samples were collected asceptically from twenty five leprosy patients and twenty five nornal healthyby ante-cubital venipuncture after obtaining pre-informed consent. Samples left at room temperature for 3-4 hours or kept overnight at 4ºC for serum separation. Then serum is separated and collected in eppendrof tubes and labeled properly. The sera samples collected after stored at -20ºC until the assays were performed. ELISA was done using Genedia HIV-1/2 EIA kit (Greencross, Korea). Those found positive were conûrmed by rapid (HIV capillus latex aggregation assay, Trinity Biotech PLC, Ireland) and Western blot assays (WesternBlot, BIO-RAD, NEWLAVBLOT), Nippon Bio-Rad Laboratories, Japan. After post-test counselling, a report was handed over to those found HIV-positive and patient was referred to clinicians for further care and management. To ûnd out any other co-infections, the samples were further tested by HBsAg kit, (Immuno-chromatography test ERBA Hepline, Transasia Bio-Medicals Ltd., Mumbai, India) and VDRL and Rheumatoid Arthritis kits (Carbogen and Rhelax, RF of Tulip Diagnostics (P) Ltd., Bambolim, Goa, India).

Result and Discussion

None of the sample was found to be positive for antibodies to HIV ½ (Fig. 1). Out of twenty five leprosy samples, three were reactive for Rheumatoid Arthritis factor and seven were positive for antibodies to syphilis as revealed by the test. Further two were reactive for C-reactive protein and one were having titre of Anti-Streptolysine-O.

C-reactive protein and Anti-Streptolysin-O rise in acute phase of infections. In the absence of definite diagnosis, much before, appearance of the symptoms of the disease, assessing the levels of reactivity of the C-reactive protein and Anti-Streptolysin-O might be a useful diagnostic tool.

CD4+/CD8+ count declines in leprosy patients, the CD4+ cells counts were found to be were also low i.e. below 500, CD8+ cells count 299 and the CD4+/CD8+ ratio was 1.38.CD4+/CD8+ count declinesin HIV infection, the CD4+ cells counts were found to be were also low i.e. below 100, CD8+ cells count high i.e. up to 500and the CD4+/CD8+ ratio was 0.17. CD4+/CD8+ count declines in in normal healthy control, the CD4+ cells counts were found to be were also high i.e. upto 800, CD8+ cells count high i.e. up to 400and the CD4+/CD8+ ratio was 2.02.

Screening for HIV, Rheumatoid Arthritis and Anti-Streptolysin-O, C-reactive protein and Syphilis (VDRL) were done in order to know the prevalence levels of these infections, as biological markers of

risk. Thus, screening the leprosy patients for these would go a long way in early detection of these co-infections. Early treatment, if initiated, would help in further deterioration of the condition of these patients.

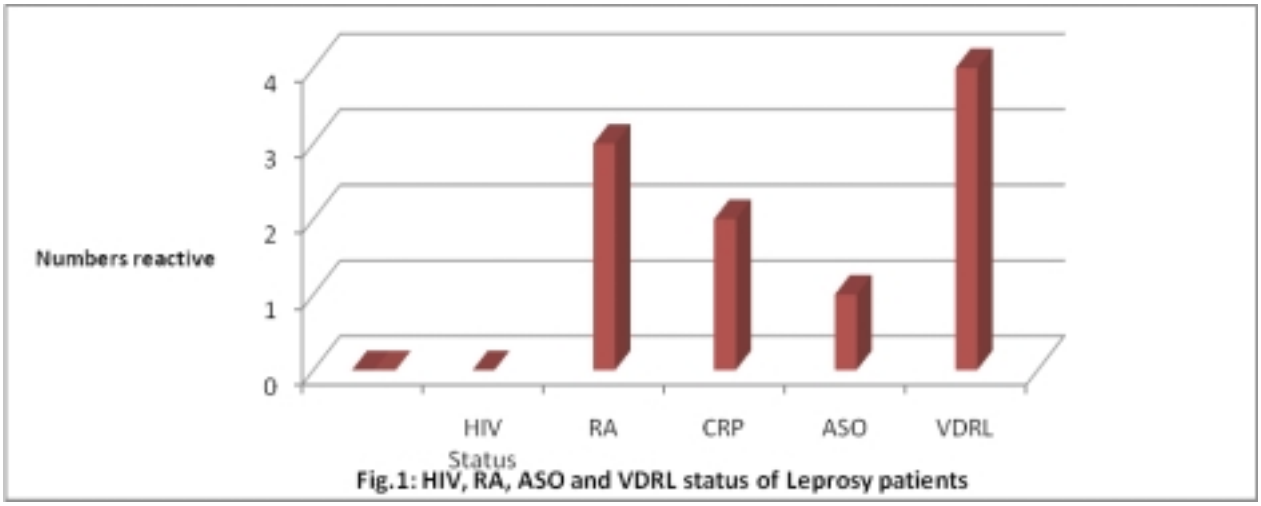

Fig.1: HIV, RA, ASO and VDRL status of Leprosy patients

Table 1. HIV status and FACS counts of leprosy patients, HIV positive and normal healthy controls.

Samples	HIV Status		FACS Count			
	Elisa	Capillus Latex agglutination	CD4+	CD8+	CD3+	CD4+/CD8+ ratio
Leprosy patients	-ve	-ve	315	229	562	1.38
HIV Positive patients	+ve	+ve	91	536	624	0.17
Normal healthy controls	-ve	-ve	857	422	1429	2.02

In normal healthy individuals CD4+ cell counts is higher than CD8+ cells. In HIV infective patients CD4+ cells counts is lower than CD8+ cells. In leprosy patients both CD4+ and CD8+ cells counts is decline than normal value.

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Seroprevalence of HIV Infection among Leprosy Patients in Agra, India: Trends and Perspective[1]

Tahziba Hussain, Shikha Sinha, K. K. Kulshreshtha, Kiran Katoch, V. S. Yadav, U. Sengupta, and V. M. Katoch[2]

ABSTRACT

This study compares the results of HIV seroprevalence, which was carried out in two phases, i.e., 1989 to 1993 and 1999 to 2004. Although the number of leprosy patients screened for HIV infection in the second phase is less (2125) as compared to those screened during the first phase (4025), a rise in HIV infection from 0.12% to 0.37% is certainly disturbing since this area appears to be endemic for both the infections. During the study period, the Out Patient department attendance of a few types of leprosy patients like borderline and borderline lepromatous have risen, whereas others like borderline tuberculoid and polar tuberculoid have declined in the second phase as compared to that of the first phase. The trend over a decade suggests that HIV infection is low among the leprosy patients when compared with other risk groups. Follow-up of these patients at an interval of six months, revealed that none of them downgraded into a severe form of leprosy nor developed ARC or AIDS. In this study, it appears that neither infection precipitated the other. The occurrence of downgradation as well as reversal reactions and neuritis (both chronic and acute) was not observed among the leprosy patients. None of them developed erythema nodosum leprosum reactions. Similarly, the HIV-positive leprosy cases did not develop either AIDS related complex (ARC) or full blown case of AIDS.

RESUME

Cette étude compare les résultats de séroprévalence du VIH, obtenus en 2 phases distinctes : de 1989 à 1993 et de 1999 à 2004. Bien que le nombre de patients testés pour l'infection par le VIH soit moindre dans la seconde phase (2125) que dans la première (4025), une augmentation de prévalence de 0.12% à 0.37% est préoccupante puisque la région étudiée est endémique pour les 2 infections. Pendant la durée de cette étude, si la seconde phase est comparée à la première, la présentation de patients au service de Consultations Externes a augmenté pour quelques types de patients lépreux comme les patients borderline et borderline lépromateux et diminué pour les patients borderline tuberculoïdes et tubercu-

[1] Received for publication on 21 September 2004. Accepted for publication on 13 February 2005.

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loïdes polaires. La tendance dégagée sur une décennie suggère que l'infection par le VIH est faible chez les patients lépreux, comparés à d'autres groupes à risque. Le suivi tous les 6 mois de ces patients indique qu'aucun d'entre eux n'a rétrogradé en une forme sévère de la lèpre ou n'a développé le complexe associé au SIDA (ARC) ou le SIDA. Dans cette étude, il apparaît qu'aucune de ces infections ne précipite l'autre. Il ne fut pas observé de déplacement vers le bas le long du spectre immuno-pathologique ou de réactions inverses ou de névrites (à la fois chroniques ou aiguës) parmi les patients hanséniens. Aucun n'a développé de réaction de type érythème noueux lépreux. Concomitamment, les cas de lèpre aussi positifs au VIH n'ont développé ni de syndrome ARC ni de SIDA terminal.

RESUMEN

Este estudio compara los resultados de una encuesta sobre la prevalencia del VIH en pacientes con lepra, realizada en dos fases, la primera de 1989 a 1993 y la segunda de 1999 a 2004. Aunque el número de pacientes investigados para VIH fue mayor en la primera fase (4025) que en la segunda (2125), se notó un incremento en la infección por VIH de 0.12% a 0.37%. Esto es preocupante porque sugiere que esta área es endémica para las dos enfermedades. En la segunda fase del estudio, se observó un incremento en el número de pacientes BL/LL que acudieron al Instituto y una disminución en el número de los pacientes BT/TT. Los resultados globales indican que la infección por VIH es baja entre los pacientes con lepra en comparación con la infección en otros grupos de riesgo. El examen de estos pacientes a los 6 meses de seguimiento reveló que ninguno de ellos "se degradó" a una forma más severa de la lepra, ni desarrolló los signos del complejo asociado al SIDA, ni la enfermedad en si. Además, ninguna de las enfermedades precipitó a la otra. Ninguno de los pacientes desarrolló reacciones reversas (neuritis agudas y crónicas), ni eritema nodos leproso (ENL).

India has the largest number of known cases of leprosy and happens to incidentally be endemic for HIV as well. Some of the earlier studies done in North and North-Eastern India did not find any association of HIV infection with leprosy patients ([24]). A few studies from South Indian states showed a higher prevalence of HIV infection among leprosy patients, but these studies alone do not provide any indication of its association with leprosy ([12]). Leprosy caused by *Mycobacterium leprae* has an unusually long incubation period, and infection with HIV leads to a profound drop in CD4+ T-lymphocyte count and function and compromises the cell-mediated immune response, as well ([19, 25]). Earlier studies carried out in this center suggested that 1 per thousand (5/4025 : 0.124%) of the leprosy patients harbored HIV infection. Follow-up of these patients at an interval of six months, revealed that none of them downgraded into a severe form of leprosy nor developed ARC or AIDS ([10]). Although this study indicated that leprosy is not a risk factor for developing HIV-1 infection, the HIV surveillance studies on this population was continued with a view to assess the risk and find out the trend in an area where both the infections are prevalent. This study compares the results of HIV seroprevalence, which was carried out in two phases; first, from April, 1989 to March, 1993 when HIV infection was being detected in India in different risk group populations to assess the risk among leprosy patients, and then from September, 1999 to March, 2004. This is the first report of a decade of HIV screening of leprosy patients in this region of the country and the longest follow-up of HIV-leprosy co-infected cases.

One of the commonly observed complaints among leprosy patients was pain in the joints. Many studies have proven that microbial agents might trigger the autoimmune phenomenon and induce rheumatoid arthritis ([1, 5, 8]). In order to find out if arthritis is present in the HIV-leprosy co-infected patients, the sera from these cases were tested for Rheumatoid arthritis (RA) factor. Many risk behaviors as well as the routes of transmission for HIV, Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infection are identical to those for other sexually transmitted diseases (STDs) ([3]). For this reason, the leprosy sera samples were tested for HBsAg and VDRL simultaneously with HIV.

MATERIALS AND METHODS

Leprosy patients, across the spectrum, i.e., tuberculoid (TT), borderline-tuberculoid (BT), mid-borderline (BB), borderline-lepromatous (BL), lepromatous (LL) and neuritic (N) types, classified, according to Ridley-Jopling criteria ([23]), attending the Unit-I of the Outpatient's Department (OPD) of the Central JALMA Institute for Leprosy and other Mycobacterial Diseases (CJILOMD) were included in the study. The leprosy cases in the study were neither newly admitted nor untreated patients, although a few were newly detected cases. For bacteriological determination, the six skin sites used were the two ear lobes and four representative active skin sites, i.e., hand (right arm and left arm), elbow (right and left), back, forehead, and the site of the lesion. In our OPD, four skin sites are routinely used for determination of the bacteriological index (B.I.). The inclusion criteria were: adult leprosy patients between the age group of 16 to 48 yrs. Children and old patients were excluded from the study as it was assumed they were not likely to be sexually active. In order to ensure that the patients were not screened over and over again, their OPD cards were marked, "HIV-Screened." This helped in excluding the repeat testing of the patients. Blood was collected asceptically from leprosy patients by ante-cubital venipuncture after obtaining pre-informed consent. The sera samples collected after centrifugation at 2500 g were stored at –20°C until the assays were performed. ELISA was done using Genedia HIV-1/2 EIA kit (Greencross, Korea). Those found positive were confirmed by rapid (HIV capillus latex aggregation assay, Trinity Biotech PLC, Ireland) and Western blot assays (WesternBlot, BIO-RAD, NEWLAVBLOT), Nippon Bio-Rad Laboratories, Japan. After post-test counselling, a report was handed over to those found HIV-positive and patient was referred to clinicians for further care and management. To find out any other co-infections, the samples were further tested by HBsAg kit, (Immuno-chromatography test ERBA Hepline, Transasia Bio-Medicals Ltd., Mumbai, India) and VDRL and Rheumatoid Arthritis kits (Carbogen and Rhelax, RF of Tulip Diagnostics (P) Ltd., Bambolim, Goa, India).

RESULTS

The prevalence of HIV-1 infection in leprosy patients was observed in two phases. In phase one, 4025 patients [30 indeterminate (I), 141 polar tuberculoid (TT), 1888 boderline tuberculoid (BT), 409 borderline (BB), 600 borderline lepromatous (BL), 751 polar lepromatous (LL), 200 N] were screened between 1989 and 1993, out of which only 8 were ELISA positive and 5 were Western Blot reactive. Subsequently, in the second phase from 1999 to 2004, 2125 patients (21 I, 19 TT, 646 BT, 332 BB, 610 BL, 324 LL, 173 N) were screened, out of which 8 were ELISA positive and 5 were Western Blot reactive (Table 1). The variation in the results of the two tests correlated well with the titre of HIV-1/2 antibodies in the sera samples. The strongly positive samples having a high absorbance value, ranging between 1.5 and 2.0, measured in terms of O.D. at 450 nm in an ELISA reader had an excellent pattern of reactivity in Western Blot. The samples with weak or moderate positivity in ELISA, with an O.D. ranging between 0.5 and 0.7, did not react with Western Blot. A rise in HIV infection from 0.124% to 0.376% was observed. Two samples were reactive to HIV-2 by Western Blot. Among all the HIV-positive leprosy patients, there were no other co-infections like Hepatitis B, Syphilis and RA. Out of the 8 HIV-leprosy co-infected patients, 2 each were BT and BL types, 3 were BB and 1 was LL type of leprosy.

The predominant clinical features were hypo-pigmented lesions, clawing of fingers and toes, pain, and hand muscle atrophy. Whereas 4 patients had deformity in hands, only one of them reported acute pain. All the patients completed a full course of standard anti-leprosy multi-drug therapy, responded satisfactorily, and were later clinically and bacteriologically negative. The initial bacterial index, prior to treatment, which ranged between 2+ and 3+ became negative on completion of the treatment. Two of the 8 HIV-leprosy co-infected patients (BL, LL) became bacteriologically negative after 6 months and another 2 (BT, BL) became negative after 24 months of treatment (Table 2). We have observed that following treatment, B.I. became negative even in BL and LL cases. The HIV-positive

TABLE 1. *The phase-wise screening of leprosy patients for HIV-1/2 infection.*

I Phase[a] (N = 4025)		HIV status		II Phase[b] (N = 2125)		HIV status	
		EIA	WB			EIA	WB
Borderline-Tuberculoid (BT)	1888 (46.90%)	2	2	Borderline-Tuberculoid (BT)	646 (30.48%)	2	1
Tuberculoid (TT)	141 (3.50%)	1	1	Tuberculoid (TT)	19 (0.89%)	0	0
Indeterminate (I)	30 (0.74%)	0	0	Indeterminate (I)	21 (0.98%)	0	0
Lepromatous-Leprosy (LL)	751(18.65%)	1	0	Lepromatous-Leprosy (LL)	324 (15.24%)	2	2
Borderline-Lepromatous(BL)	600 (14.90%)	1	0	Borderline-Lepromatous (BL)	610 (28.70%)	2	1
Mid-Borderline (BB)	415 (10.31%)	0	0	Mid-Borderline(BB)	332 (15.62%)	2	2
Neuritic (N)	200 (4.96%)	0	0	Neuritic (N)	173 (8.14%)	0	0

[a] denotes I Phase of HIV screening of the leprosy patients which was from April, 1989 to March, 1993.
[b] denotes II Phase of HIV screening of the leprosy patients which was from September,1999 to March, 2004.
EIA = ELISA, WB = Western Blot.

patients are being followed up at six month intervals. On follow-up, to date none of the patients with HIV-1 infection have progressed into a more severe form of the disease. None of the co-infected cases have been lost so far in follow-up. In these co-infected patients, it is difficult to assess which infection occurred first. Our results indicated that HIV-1 infection does not contribute in any way to the precipitation of serious forms of leprosy.

DISCUSSION

It is well recognized that HIV infection constitutes a major risk factor for tuberculosis (TB) and for other mycobacteria, such as *M. avium* and *M. intracellulare*, but there are still uncertainties regarding its association with leprosy. The association between the HIV and tuberculosis and certain other non-tuberculous mycobacterial infections have been established ([20, 21]). Potential effects of HIV infection on leprosy have been suggested and discussed by several authors but, despite expectations, little interaction has been observed uptil now ([9, 17, 22]). Although an association between HIV and leprosy has been described in Zambia ([18]) and in Tanzania ([27, 28]), there is some evidence from studies in Mali ([15]), Ethiopia ([6, 7]) and in other African countries that HIV infection is not a risk factor for leprosy ([14, 16]). On the contrary, a few studies carried out in some African countries to determine the association between leprosy and HIV infection suggest that HIV infection is an important risk factor for leprosy ([4, 18]). Some of these studies had limitations in study design and some found no association between the two diseases ([2, 13]).

The increase in HIV infection as compared to that of the first phase is disturbing and the mode of transmission appeared to be heterosexual as revealed during the post-test counselling session. None of the co-infected cases admitted to having a homosexual relationship or had a history of blood transfusion. Two of the males had symptoms of STDs at the time of testing.

The trend over a decade suggests that HIV infection is low among the leprosy patients when compared with other risk groups, like TB patients, which is 4.3% (26/600) in Agra (in press). The prevalence and incidence for HIV infection in Agra varies in different groups. Our institute has a Voluntary Confidential, Counselling and Testing Center (VCCTC), a State body of the National AIDS Control Organization (NACO), where screening for HIV infection is carried out routinely from different groups, namely, Volunteers (individuals opting for voluntary HIV testing), HIV-suspected cases referred from different hospitals, female sex workers (FSWs), residents at the Government Protective Home, and cases referred by District Jail and District Magistrate, Agra. The recent annual figures (Jan. through

TABLE 2. *Clinical presentations and bacteriological index among the HIV-leprosy co-infected patients.*

		Clinical findings				
		Skin Lesions	Nerves	Pain	Deformity	
1	BL	>5	5	Pain	Nil	Smear 3+ (Negative after 24 months)
2	BL	>5	4	Nil	Nil	Smear 2+ (Negative after 6 months)
3	BB	>5	1	Nil	Nil	Negative
4	BT	1	Nil	Nil	Nil	Negative
5	LL	>5	4	Nil	Hand	Smear 3+ (Negative after 6 months)
6	BB	1	4	Nil	Hand	Negative
7	BB	>5	6	Nil	Nil	Negative
8	BT	>5	4	Nil	Hand	Smear 3+ (Negative after 24 months)

Dec., 2004) revealed that the local prevalence and incidence of HIV-positivity in the area is, 40.31% (156/387) among Volunteers and 43.39% (46/106) among the Referred cases (communicated).

In the second phase as compared to that of the first phase, the OPD attendance of a few types of leprosy patients has risen during the study phase, whereas others have declined. A striking feature which has emerged during the second phase of the study is that there is an increase in the attendance of BB and BL types of leprosy patients, whereas there is a decrease in the BT and TT types of leprosy patients as depicted in Table 1. This could be one of the reasons for the higher HIV-positivity observed among the BB and BL cases. Another one could be attributed to the better control due to multi-drug therapy (M.D.T.) and decreased transmission of *M. leprae,* with new cases dominated by a long period of incubation, in the lepromatous leprosy cases. Although the number of leprosy patients screened for HIV infection in the second phase is less as compared to those screened during the first phase, a rise in HIV infection is disturbing since this area appears to be endemic for both the infections.

Expansion of the HIV epidemic could have a significant effect on the epidemiology of leprosy. In this study, it appears that neither of the infections precipitated the other. The incidence of downgradation, as well as reversal reactions and neuritis (both chronic and acute), was not observed among the leprosy patients. None of them developed Erythema Nodosum Leprosum (ENL) reactions. The total cases of HIV-positive leprosy patients were only thirteen in both the phases (5 in phase I, and 8 in phase II), which have been followed up very carefully and with special care. We have also observed that reversal reactions and ENL did not occur among any of the HIV-leprosy co-infected cases. If the number of cases were more, then probably one might have noted some reversal or ENL reactions. To resolve the issue, a larger study, with longer follow-up is required. Clinical manifestations of lepromatous leprosy cases might be immunologically mediated and these features could be abrogated by HIV infection.

Similarly, the HIV-positive leprosy cases did not develop either AIDS related complex (ARC) or full blown case of AIDS. None of the co-infected cases have been lost so far in the follow-up. This is the first report of a decade of HIV screening of leprosy patients in this region of the country and the longest follow-up of the largest number of HIV-leprosy co-infected cases. Other studies have reported follow-up of very less number of the co-infected cases ([11, 26]). The underlying mechanism by virtue of which the severity of both the diseases is lowered is not known. The infectious agents and host defences seem to have co-evolved to reach balanced states where virus and host survive. While HIV has not quite yet reached an optimal balance, tuberculosis (TB), leprosy, HBV, HCV in humans or lymphocytic choriomeningits virus (LCMV) in mice have successfully established persistence ([29]).

Although the present study does not show any association between HIV and leprosy, future study is warranted to find out the reasons for cross-protection, if any, at the genetic and molecular level.

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ORIGINAL ARTICLE

Diagnostic utility of anti-CCP antibodies and rheumatoid factor as inflammatory biomarkers in comparison with C-reactive protein and TNF-α in rheumatoid arthritis

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Abstract

Aims and Objectives: Rheumatoid arthritis is a systemic inflammatory disease whose diagnosis is primarily based on clinical manifestations because of lack of suitable diagnostic tests. As substantial joint damage already occurs by the time patient presents clinically, a validated biomarker for the diagnosis is urgently required. **Materials and Methods:** Sera from a total of 68 clinically suspected rheumatoid arthritis patients and 68 age-and sex-matched controls were tested for rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibodies, C-reactive protein (CRP), and tumor necrosis factor-alpha (TNF-α). **Results:** Anti-CCP and CRP were found to be positive in all patients with positive RF; however, TNF-α was present in only two of them. As regards anti-CCP antibodies, out of the 10 samples that showed positive results, RF, CRP, and TNF-α were also present in 4, 5, and 4 cases, respectively. **Conclusion:** The recognition of utility of such markers is essential to gain insight into the activity of this disease, which is important for early treatment that may limit functional disability consequent to the disease.

Keywords: Anti-cyclic citrullinated peptide antibodies, C-reactive protein, rheumatoid arthritis, rheumatoid factor, tumor necrosis factor-alpha

Introduction

Rheumatoid arthritis(RA) is a severe, progressive, comorbid systemic inflammatory disease of unknown etiology. Since timely intervention with new and effective treatments can alter the course of the disease, reduce functional impairment, and lengthen life, better biomarkers for diagnosis and prognosis are needed to identify these patients at an early stage in order to fine-tune therapeutic options to the individual patient.[1]

Rheumatoid factor(RF), an antibody specific for the Fc portion of human IgG, has been historically considered a marker for RA and was one of the diagnostic criteria for RA that was established by the American College of Rheumatology (ACR).[2] The sensitivity and specificity of RF for the diagnosis of RA has been reported in the range of 50-80% and 70-80%, respectively.[3,4] The specificity of the RF test is known to be relatively poor and is often questioned. With around 5% false positivity in the general population, RF is found in many patients with other diseases of infectious or autoimmune origin. Consequently, a search for better diagnostic markers, especially those with improved specificity for RA, ensued. Though due to a low sensitivity and moderate specificity RF has a little diagnostic utility, it has retained its place in practice because of its prognostic capacity and lack of an alternative test.

Citrullination (deimination) of proteins is a chemical reaction which occurs when inflammatory cells release enzymes in local tissues. Citrullination of synovial antigens, especially fibrin, during synovial inflammation probably allows the induction of anti-cyclic citrullinated

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peptide (anti-CCP) antibody in RA patients through an antigen-conducted activation of B cells. Capacity to form antibodies to citrullinated peptides and not citrullination of peptides *per se* seems to be unique to RA; the exact significance of antibodies to these peptides, however, remains uncertain. Greater sensitivity and specificity than IgM RF and probable predictability of erosive disease in RA or the eventual development of undifferentiated arthritis into RA makes anti-CCP antibodies potentially important surrogate markers for the diagnosis and prognosis in RA.[5] The progressive evolution of assays for anti-citrullinated peptide antibody (ACPA) detection has led to a high level of diagnostic accuracy with a specificity of 95-97% and a sensitivity of 67-80%.[6,7] The new 2010 RA Classification Criteria, updated to diagnose RA in an earlier phase, include detection of ACPA as a key item for diagnosing the disease.[8] Anti-CCP guided aggressive treatment at an early stage or correlation of anti-CCP levels with various therapeutic interventions are, however, important areas for research.

Another potential marker for increased risk of RA and disease activity may be C-reactive protein (CRP), since CRP is a sensitive marker of systemic inflammation and is elevated in patients with RA.[9] The acute-phase reactants like CRP are a class of serum proteins whose concentration in the blood increases after various stimuli such as trauma or inflammation. The magnitude of the acute-phase protein response is roughly proportional to the severity of the stimulus and, therefore, measurements of these proteins can be used to monitor the progress of an inflammatory disorder. Though CRP is a part of ACR core data set for measuring disease activity in RA, the quantitative usefulness has been evaluated in many studies with no clear consensus.

Tumor necrosis factor-alpha (TNF-α), yet another marker of disease activity, is one of the key cytokine molecules that causes inflammation in RA and plays a dominant role in rheumatoid synovitis.[10] TNF-α is now recognized as a mediator of a wide variety of effector functions which are recognized components of the RA disease spectrum, including endothelial cell activation and chemokine amplification leading to leukocyte accumulation; osteoclast and chondrocyte activation promoting articular destruction; nociceptor sensitization; impaired cognitive function and metabolic syndrome.[11] All these have led to the potential role of TNF-α inhibitors to induce a rapid and sustained attenuation of disease activity in patients with RA.

The best characterized predictors for rapid progression are the number of swollen joints and the levels of acute phase reactants, as swollen joints indicate synovitis and the acute phase response acts as a biomarker of pro-inflammatory cytokine production. The objective of the study was to compare the diagnostic utility of the two most widely used serological markers of RA, i.e, anti-CCP antibodies and RF, and correlate their potential as inflammatory biomarkers with important markers of disease activity like CRP and TNF-α.

Materials and Methods

This case–control study was conducted in the immunology section of the Department of Microbiology, University College of Medical Sciences and Guru Tegh Bahadur Hospital, Delhi. Sixty-eight patients with RA as per ACR criteria were enrolled in the study. At inclusion, the patients had symptom duration of at least 6 weeks, but less than 6 months, and were not receiving any glucocorticoid or immunosuppressant drug. Each enrolled patient gave written consent prior to being included in the study. Healthy hospital personnel (n = 68) without any history of inflammatory diseases served as controls. Serum samples were obtained from both patients and controls, and were aliquoted and stored at 80°C until assayed.

Detection of RF, anti-CCP antibodies, CRP, and TNF-α was done with the help of commercially available kits following the manufacturers' instructions. RF was detected by RHELAX-RF (Tulip Diagnostic, Goa, India) which is a latex agglutination slide test for the detection of RFs of the IgM class with a sensitivity of 10 IU/ml. Anti-CCP antibodies were analyzed with a commercial enzyme-linked immunosorbent assay (ELISA) IMTEC-CCP-Antibodies (IMTEC Human, Weisbaden, Germany) which is a test system for measuring IgG class autoantibodies against cyclic citrullinated peptides in human serum or plasma. The interpretation of the results was possible by correlating the absorbance of the reference control and the samples. CRP was assayed using RHELAX-CRP (Tulip Diagnostic, India) which is a slide test for detection of CRP based on the principle of latex agglutination with a sensitivity of 0.6 mg/dl. TNF-α was assayed by a commercial solid-phase sandwich ELISA (Diaclone, Besancon Cedex, France) and the sensitivity minimum detectable dose was found to be 8 pg/ml.

Results

In our study, 50% of patients were between 21 and 40 years of age and 20% were between 41 and 50 years of age. The extremes of age, i.e, less than 20 years and more than 50 years, constituted 30% of the enrolled patients. Fifty-one out of 68 patients, i.e, 75% of patients, enrolled

in the study were females. Male:Female ratio in our study was 1:3 [Figure 1]. The frequency distribution of clinical wards from where the patients were admitted is depicted in Figure 2. Out of a total of 68 patients, 29 patients were from orthopedics ward and 23 from medicine ward. Four patients were from neurology, three from dermatology, two from surgery, and one patient was from pediatrics ward.

Out of 68 patients who were clinically suspected cases of RA, only 4 patients had positive RF while 10 patients had positive anti-CCP. CRP was positive in 9 cases and TNF-α was positive in 14 of the total cases. None of the controls were positive for any of the markers except anti-CCP antibodies which were present in two females who were 22 and 25 years old, respectively [Figure 3].

Table 1 shows the comparative evaluation of RF and anti-CCP antibodies in correlation with CRP and TNF-α. Anti-CCP and CRP were found to be positive in all patients with positive RF; however, TNF-α was present in only two of them. As regards anti-CCP antibodies, out of the 10 samples that showed positive results, RF, CRP, and TNF-α were also present in 4, 5, and 4 cases, respectively. All four markers were simultaneously present in only two cases, whereas combinations of anti-CCP/RF/CRP, anti-CCP/RF/TNF-α, anti-CCP/CRP/TNF-α, and RF/CRP/TNF-α were present in four, two, three, and two cases, respectively.

Discussion

For decades, the diagnosis of RA has been primarily based on clinical manifestations due to lack of reliable alternative tests. Approximately one-third of the RA patients do not fulfill the ACR classification criteria, which makes the diagnosis of this disease difficult in the early stages.[12] Adding to the problem is the fact that substantial irreversible joint damage occurs within the first 2 years by the time the diagnosis can be confirmed by radiological or laboratory parameters.[13,14] Hence, optimization of timely and aggressive disease-modifying anti-rheumatic drug (DMARD) treatment demands prompt and accurate diagnosis and prognostic information. Though RF test has been widely used routinely in the diagnosis of RA, the enhanced specificity and early prediction of joint

Table 1: Correlation of rheumatoid factor, anti-CCP antibodies, C-reactive protein, and TNF-α among the cases (*N*=68)

Inflammatory markers	Rheumatoid factor		Anti-CCP antibodies	
	Positive (*n*=4)	Negative (*n*=64)	Positive (*n*=10)	Negative (*n*=58)
TNF-α				
Positive (*n*=14)	2	12	4	10
Negative (*n*=54)	2	52	6	48
CRP				
Positive (*n*=9)	4	5	5	4
Negative (*n*=59)	0	59	5	54

CRP=C-reactive protein, TNF-α=Tumor necrosis factor-alpha

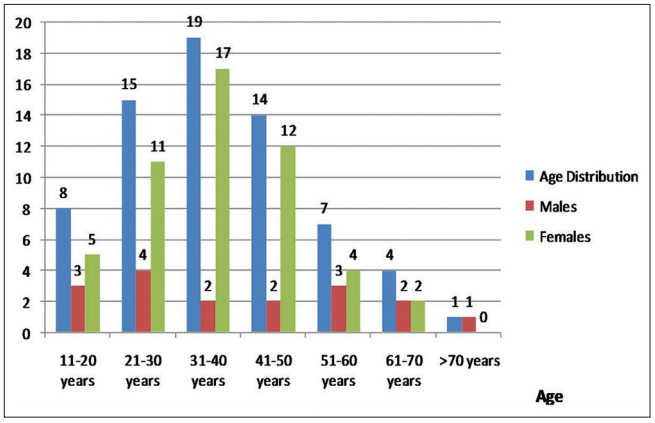

Figure 1: Age and sex distribution of the patients enrolled in the study (*N* = 68)

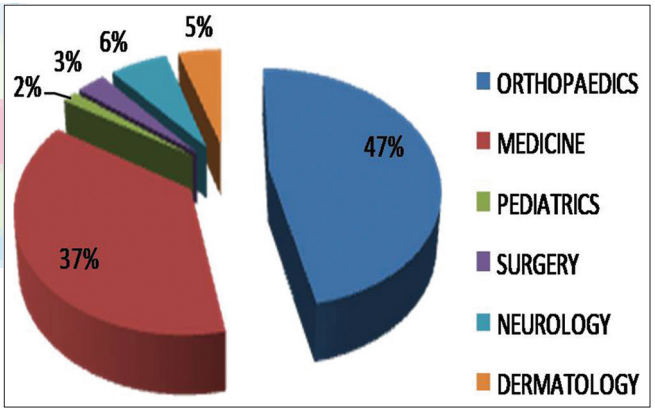

Figure 2: Ward-wise distribution of cases (*N* = 68)

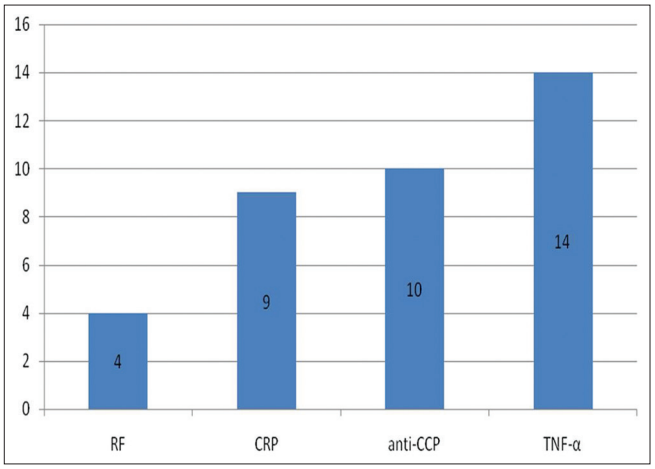

Figure 3: Comparative positivity of inflammatory markers among the cases (*N* = 68)

damage have made the assay for anti-CCP antibodies an attractive option. To better correlate these two markers for the diagnosis of RA and also to evaluate their role in inflammation, this study was planned.

RA, the most common inflammatory arthritis affecting roughly 0.5-1% of the general population worldwide with a male to female ratio of 1:2.5, may appear at any age, but it is most commonly seen among those aged from 40 to 70 years.[15] In our study, 36.76% of the clinically suspected RA patients belonged to this age group; the maximum (70.59%) belonged to 21-50 years age group with an overall male to female ratio of 1:3.

A recent study reports RF positivity in 90% and 40% of anti-CCP positive and negative patients, respectively, compared to a positivity of 40% and 0% in anti-CCP positive and negative patients, respectively, in our study.[14] The same study also found a small but significant correlation between RF and anti-CCP, though no significant correlation was found between anti-CCP and CRP as a marker of disease activity.

Another study reported that 50% of the patients were positive in both tests with 78% of the RF-positive and 40% of the RF-negative RA patients being anti-CCP antibody positive, unlike our study where 100% and 9% of RF-positive and -negative patients, respectively, were positive for anti-CCP antibodies.[3] This study confirms that the diagnostic sensitivity of anti-CCP antibodies in patients with recent-onset RA is the same as that of agglutinating RF and that seropositivity for the two tests correlates significantly. A study reported that 41.4% of patients were positive for RF and 41.7% of patients positive for anti-CCP at baseline, in contrast to 5.9% and 14.7% anti-CCP and RF positivity, respectively, in our study.[16] Kroot *et al*., identified 66% positive RA serum samples with CCP-ELISA, which was only 14.6% in our case.[17] In another study,[18] the results from the seronegative RA patients demonstrated high prevalence of anti-CCP positivity (60%) in the RF-negative RA patients, which was higher than previously published results where prevalence was reported to be between 20%[19] and 43%,[17] 3and in our study was as low as 8.8%. Such a variation in the positivity is not clear though generation of ELISA used, population dynamics, or geographic location could be responsible for this. Also, a study on the prediction of disease course of RA by anti-CCP reports that the proportion of anti-CCP antibody positive patients increases with the number of ACR criteria fulfilled.[3] In another study, both RF and anti-CCP antibody tests were reported to be positive in 40.4% and negative in 28.1% of cases, as compared to our study where they were together positive in 5.9% and negative in 85.3% of RA patients.[20]

Patients with RA show considerable variability in disease activity that can be difficult to predict at the onset of disease. The characterization of acute phase reactants' responses in RA is essential to gain insight into the activity of this disease and to assess the degree of inflammation. A study on the association between acute phase reactant response and the disease activity score concluded that CRP was elevated in RA patients as compared to controls, with a significant correlation observed with the disease activity score.[21] In our study, CRP was positive in 9 out of 68 RA patients and it correlated with RF in 4, anti-CCP in 5, and TNF-α in 4 cases. A previous study observed that CRP was significantly higher in the anti-CCP positive patients than in the anti-CCP negative group and the differences in disease activity measures between IgM RF or IgA RF-positive and-negative patients showed the same tendency as with anti-CCP.[3] In our study, CRP was positive in 5/10 (50%) anti-CCP positive and 4/58 (6.9%) anti-CCP negative patients and in 4/4 (100%) RF-positive and 0/64 (0%) RF-negative patients. One study, however, did not show a significant and convincing trend, contrary to other studies, regarding the use of CRP in RA patients.[22] Soluble TNF receptors are found in high concentrations in the synovial fluid and serum of patients with RA.[23] In our study, TNF-α was positive in 14 out of the 68 suspected cases of RA and the positivity correlated with RF, anti-CCP, and CRP in 2, 4, and 4 cases, respectively. In another study on 242 RA patients, anti-CCP antibodies positively correlated with higher erythrocyte sedimentation rate (ESR), CRP, swollen joint count, and worse physician global assessment ratings. If other possible causes of alteration in these surrogate inflammatory markers values are closely monitored before interpretation, the diagnostic utility of this measure should further improve.

Though RA is a disease defined by well-accepted criteria, the clinical presentation and molecular pathogenesis of this disease are varied and complex due to which prioritizing diagnostic tests or predicting treatment responsiveness is often not so easy. Our study addresses the important issue of the status of serological markers present in RA, which may predict the development of disease or prognosticate the damage that has occurred. The recognition of utility of such markers is important, as early detection of the disease will allow for early treatment, which may limit functional disability consequent to the disease.

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Research Article
Biological Sciences

IMMUNOLOGICAL AND SERIOLOGICAL PROFILE OF ARTHRITIS

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ABSTRACT

Arthritis is one of the common diseases. in this present study twenty patients with symptoms of arthritis were included is as control another twenty patients without arthritis are included. The serum samples of these patients were collected and left for RF(or) RA which detects the immunoglobulin of the class IgM, IgA and IgE and careful prognostic marker of RA ie IgM is detected. Different reagents like RHELAX RF reagent, positive control, and negative control are used. Mean while interpretation of rest results were observed. An enhanced immuno turbiclimetric test i.e CRP test was conducted (c-reactive protein Turbilaten), which represents a useful laboratory test for diction of acute infection as well as for monitoring inflammatory processes also in acute rheumatic and gashroin testinal diseases. To detect the presence of antibodies in blood that are sensitive to temperature changes cold agglutinins test is performed, finally serum sample was tested for cryoprecipitation to detect immune complex. Different types of joints and their classification is included in this present study. And types of movements at synovial joints, classification of Arthritis like osteoarthritis, rheumatoid arthritis, neuropathic arthropathy, metabolic arthritis etc where studied, out of twenty arthritis patients rheumatoid factor was detected in ten samples, c-reactive protein detected in nine samples, cold agglutination detected in one sample, and immuno compleres were detected in two samples of sera both rheumatoid factor and c-reactive proteins were detected. In two RF, CRPS, Ic. In 4at synorial fluid Rheumatoid factor and c-reactive proteins were detected. Our study includes that most of arthritis observed in females and mainly Rheumatoid arthritis. Serologically c-reactive protein detected in most of the patients.

KEY WORDS

Arthritis Synovial fluid Joints, Rheumatoid Arthritis, Immunological, serological.

INTRODUCTION

Free and active movements of various parts of the body like limbs and head are due to formation of joints between different bones at their terminal ends. Stability to these joints is provided by Muscle and ligaments (Rahu et al., 2003).Inner side of joints is maintained smooth by synovial membrane .synovial fluid lubricates the surface and prevents friction during movement.

The rigid nature and mode of growth of skeletal tissue requires that the skeleton consists of multiple osseous elements, each joined to its neighbours by a variety of structural arrangements. All such unions are grouped as arthroses (synonyms: articulations, junctrurae (classical); joints, articulations, junctions (Anglicized). (Allander et al., 1974). Arthorses are concerned with differential growth, transmission of forces (tensile, compressive, shear and torsion) and movement (from consolidation and complete rigidity at one extreme, through to relatively free but controlled movement at the other). Which of these attributes predominates varies with site and age,

often changing markedly with the latter. The scientific study of the fuctional topography and temporal variation of arthroses is Arthrology.

Arthroses are classified in a number of ways, with different criteria and degrees of quantitative accuracy being adopted by different groups of workers, Hence, sources limited to a single classification should be considered with respect to the intended audience, varying from a simplified introductory grouping, through a more detailed vocational grouping, to greater mensural information-used by specialist kinesiologists. All these approaches to classification are given here, initially as a synopsis of principal heading and terms; in later pages and (where indicated) elsewhere, these are defined and described in greater detail. Where the morphology is mixed, or changes radically with time, and in some exceptional situations (e.g. the costal cartilages and larynx), additional classificatory groups have been included. Although unusual, this confers a more complete logic to the frameworks employed **(**Roy et al., 2004).

Joint classifications

Joints are classified structurally, based of their anatomical characteristics, and functionally, based on the type of movement they permit.

The structural classification of joints is based on two criteria: (1) the presence or absence of a space between the articulating bones, called a Synovial cavity, and (2) the type of connective tissue that binds the bones together, structurally, joints are classified as one of the following types:

Fibrous joints: The bones are held tighter by fibrous connective tissue that is rich in collagen fibers: they lack a Synovial cavity.

Cartilaginous joints The bones are held tighter by cartilage, they lack a Synovial cavity.

Synovial joints: The bones forming the joints have a Synovial cavity and are united by the dense irregular connective tissue of an articular capsule, and often by necessary ligaments.

The functional classification of joints relates to the degree of moment they permit. Functionally, joints are classified as one of the following types Synarthrosis Sin'-ar THRO-sis-= together): An immovable joint. The plural is synarthroses.

Amphirthrosis (am'-fe-ar THRO-sis = movable joint); A freely movable joint. The plural is diarthroses.

Diarthroses: (di – ar. THRO – sis = movable joint) A freely movable joint. Plural id diarthruses. Ass diarthroses are Synovial joints. They have a variety of shapes and permit several different types of movements (Uppal et al., 2003).

Types of Fibrous joints:

Sutures

A suture (SOO-chur; suture- =seam) is a fibrous joint composed of a thin layer of dense fibrous connective tissue that unites only bones of the skull.

Some sutures, although present during childhood, are replaced by bone in the adult. Such a suture is called a synostrisis**.**

Syndesmoses

A slyndesmosis (sin' – dez- OM-sis; syndesmo - =band or ligament) is a fibrous joint in which, compared to a suture, there is a greater distance between the articulating bones and more fibrous connective tissue.

Types of Cartilaginous Joints:-

Synchondroses:

A synchondrosis (sin'-kon-DRO-sis chondro - = cartilage) is a cartiliaginous joint in which the connecting material is hyaline cartilage.

Symphyses

A symphysis (SIM – fi-sis = growing together) is a cartilaginous joint in which the ends of the articulating bones are covered with hyaline cartilage, but a broad, flat disc of fibrocartilage connects the bones. All symphyses occur in the midline of the body .

This type of joint is also found at the intervertebral joints between the bodies of vertebrae.

Types of Synovial joints:-

Synovial joints (si-NO-ve-al) have certain characteristics that stinguish them form other joints. The unique characteristic of Synovial joint is the presence of a space called a synovial joint) cavity between the articulating bones (Laham et al., 1982).

Synovial cavity allows a joint to be freely movable; hence, all Synovial joints are classified functionally as diathroses. The bones at a synovial joint are covered by **articular cartilage,** which is hyaline cartilage. The cartilage voers the articulating surface of the bones with a smooth, slippery surface but does not bind them together. Anrticular cartilage reduces friction between bones in the joint during movement and helps to absorb shock (Tanaka et al., 2004).

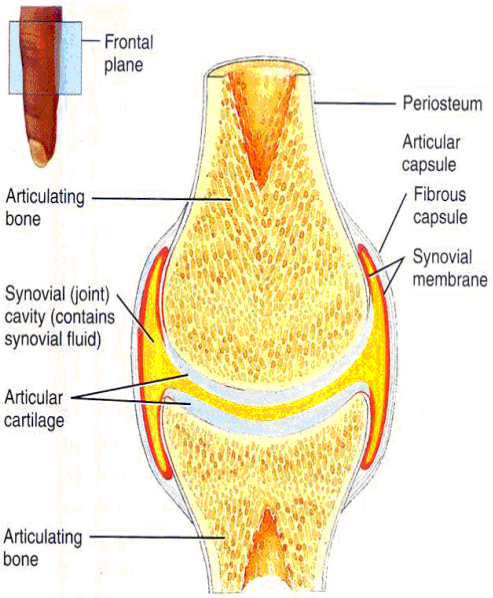

Figure 1: Structure of a typical synovial joint.

Articular Capsule

A sleeve like **articular capsule** surrounds a synovial joint, encloses the synovial cavity, and unites the articulating bones. The articular capsule is composed of two layers, an outer fibrous capsule and an inner synovial membrane.

Synovial Fluid

The synovial membrane secrets **synovial fluid** (ov- = egg), which forms a thin film over the surfaces within the articular capsule. This viscous, clear or pale yellow fluid was named for its similarity in appearance and consistency to uncooked egg white (albumin). Synovial fluid consists of hyaluronic acid, secret by fibroblast –like cells in the Synovial membrance, and interstitial fluid filtered from blood plasma.

Synovial fluid also contains phagocytic cells that remove microbes and the debris that results from normal wear and tear in the joint (Singer et al., 1974).

Other Types of Synovial Joints:

1. Planar Joints: - The articulating surfaces of bones in a planar joint are flat or slightly curved planar joints primarily permit side-to-side and back-and forth gliding movements.

2.Hinge Joints :In a hinge joints, the convex surface of one bone fits into the concave surface of another bone As the name imlies, hinge joints produce an angular, opening-and –closing motion like that of a hinged door. Hinge joint is monaxial because they typically allow motion around a single axis.

3. Pivot Joints: In **pivot** joints, the rounded or pointed surface of one bone articulates with a ring formed partly by another bone and partly by a ligament. A pivot joint is monaxial because it allows rotation around its on longitudinal axis only.

4. Condyloid Joints: In a condyloid joint (KON-di-loyd; condyl-=knuckle) or ellip soidal joint, the convex oval-shaped projection of one bone fits into the oval-shaped depression of another bone. A condyloid joint is biaxial because the movement it permits is around two axes.

5. Saddle Joints: In a saddle joints, the articular surface of one bone is saddle shaped, and the articular surface of the other bone fits into the "saddle" as sitting rider would sit. A saddle joint is a modified condylod joint in which the movement is somewhat freer. Saddle joints are biaxial, producing side-to-side and up-and-down movements.

6. Ball-and-Scoket Joints: A ball-and-socket joint consists of the ball-like surface of one bone fitting into a cuplike depression of another bone. Such joints are multiaxial (polyaxial) because they permit movement around three axes plus all directions in between.

Arthritis:

Classification of Arthritis

Inflammation of a joint is known as arthritis; clinically arthritis may be classified as:

Osteoarthritis (degenerative)

Primary

Secondary

Rheumatoid arthritis

Seropositive

Rheumatoid arthritis

Juvenile Rheumatoid arthritis

Scronegative

Ankylosing spondylitis

Reiter's disease

Psoriatic arthritis

Enteropathic arthritis

Neuropathic arthropathy

Metabolic arthritis

Gout

Pseudogout

Alkaptonuric arthritis

Arthritis in systemic disease –

Haemophilia

Others:

Villonodular synovitis

Synovial chondromatosis.

1. Degenerative Osteoarthritis (Osteo-arthrosis):

Osteoarthritis is a degenerative disease of the joints. It is a pure degenerative pathology without any inflammatory affliction of the joint. Inflammation of the synovium may occur late in disease and is certainly not the cause of it. The term osteoarthritis is therefore a misnomer. The appropriate term is osteoarthritis. In this condition the joints are constantly subjected to cyclical loading of forces which result in constant stresses acting at the joint surfaces. Osteoarthritis is of two types; (i) Primary, and (ii) secondary.

1. Primary osteoarthritis: - This is due to the wear and tear occurring in the joints with aging. The exact cause is not known, however, obesity, hormonal and genetic factors have been blamed to predispose this condition. It commonly affects the weight bearing joints like hip and keen. However, it is also in spine, carpometacarpal join of the thumb and distal inter phalangeal joints of the fingers.

2. Secondary osteoarthritis: - This is due to the wear and tear occurring in an abnormal joint. The abnormality may be due to:

a) Incongruous joint surfaces as in intra-articular fractures.
b) Abnormally oriented joint surfaces as in juxta-articular fractures, of in congenital maldevelopment.
c) **c**.Previous disease or infection destroying the articular cartilage.
d) Deformity of one of the constituent bone as in osteohondrosis, genu valgum or varum.
e) A vascular necrosisi of the femoral head (Gerad et al., 1992).

Movement of joints restricted in the following condition

Trauma may cause tear of an alignment, fracture of the bones involved is joints, and diglocation of joints. With advancing of age the Synovial fluid gets depleted and joints surface becomes dry. Movement of such a joint will be painful and restricted.

Joints also become swolles and painful whenever there is inflammation involving bones are Synovial membranes or both. Inflammation of joint is called Arthritis. It is due to infection by micro organisms it is called petic arthritis.

Arthritis canal so occur due to immunological reactions. One of such is rheumatoid Arthritis which is the commonest especially among middle aged females. Another non infective type of arthritis is ankylosing spondylitis.

In about 50% of patients with rheumatoid arthritis, denatured globulin called Rheumatoid factor is detected in the blood.

In any inflammatory conditions including Arthritis a protein called C - reactive protein can be demonstrated in blood samples of some patients.

Another evidence of immunological mechanism in Arthritis cases can be detection of immune complexes in the blood and Synovial fluid of the patients (Cecil et al., 1932).

2. Rheumatoid Arthritis: Rheumatoid arthritis is a systemic disease. It involves system/organs other than the joints. However, only the orthopaedic aspects of this disease will be discussed here.

1. Seropositive Rheumatioid Arthritis: Seropositive rheumatoid arthritis is a systemic inflammatory disease. It is an autoimmune disease mainly affecting the connective tissue. Hence the greatest effect is seen in the parts with more of connective interstitium.

2. Rheumatiod Arthritis: Rheumatoid arthritis is a chronic inflammatory systemic disease of young or middle-aged adults, characterized, by destructive and proliferative changes in Synovial membrane, periarticula structure, skeletal muscle, and perineural sheaths, skeletal muscle, and perineural sheaths. Eventually, joints are dwstroyed, ankylosed, and deformed (Heller et al., 1954).

3 Juvenile Rheumatoid Arthritis (JRA):

Juvenile rheumatoid affects adolescents. It is commonly seen among females. It can manifest in three forms:

1. **Systemic onset (Still's disease):** High fever, rashes, lymphadenopathy, splenomegaly, carditis and arthritis.
2. **Pauciarticular onset:** It involves than 4 joints in the body. It is the most common presentation. It is seen in 40-50% of the cases.
3. **Polyarticular onset:** This is seen in 30-40% of patients. It involves four or more joints.
4. **Neuropathic arthropathy**: Neuropathic osteoarthropathy can be defined as bone and joint changes that occur secondary to loss of sensation and that accompany a variety of disorders. Charcot first described the relationship between loss of sensation and arthropathy in 1868.

The radiographic changes include destruction of articular surfaces, opaque subchondral bones, joint debris, deformity, and dislocation. Neuropathic arthropathy poses a special problem in imaging when it is associated with a soft tissue infection.

Pathophysiology: The pathophysiology of neuropathic arthropathy is debatable. The general consensus is that the loss of proprioception and deep sensation leads of recurrent trauma, which ultimately leads to progressive destruction, degeneration, and disorganization of the joint, Another theory postulates that neurally mediated vascular reflex results in hyperemia, which can cause osteoclastic bone resorption.

Causes of neuropathic arthropathy include the following:

Diabetes

Use of steroids

Alcoholism

Trauma

Infection

Amyloidosis

Leprosy

Connective disorders, such as rheumatoid arthritis and scleroderma

Thalidomide embropathy(congenital arthropathy in offspring of exposed mothers)

Paraneoplastic sensory neuropathy

4. **Metabolic arthritis**:-

Metabolic arthritis is mainly 3 types

1. Gout:

Gout is a metabolic disorder characterized by abnormally high levels of the byproduct, uric acid, in the blood and tissues, in gout, crystals of uric acid are deposited in the joints, where they cause gouty arthritis. They also may be deposited in the kidneys, where they can cause kidney stones. In some patients, the high levels of uric acid are triggered by a diet rich in chemicals called purines, which are found in anchovies, nuts and organ foods such as liver, kidney and sweetbreads. In other patients, the body's own production of uric acid is simply too high regardless of their diet. This also may occur in certain inherited genetic metabolic disorders, leukemia and cytotoxic treatment for cancer. Lastly, gout also can happen when the kidney's excretion of uric acid is too low. This occurs in some forms of kidney disease, in starvation and with alcohol intake. For some patients, it is a combination of these factors that leads to excess uric acid in the body and subsequent gout **(**Buschmann et al., 2004.)

Some of the major risk factors for gout include obesity or sudden weight gain; a purine-rich diet; alcohol use, especially binge drinking, high blood pressure, especially if treated with diuretic drugs such as hydrochlorothiazide, a family history of gout; trauma or major surgery; and certain types of cancer or cancer treatments. About 90 percent of patients with gout are men older than 40. Gout is quite rare in younger women and typically occurs in women many years after menopause.

2. Pseudogout:

Pseudogout (pronounced soo-doe-gowt) is a type of arthritis that is caused by the build of calcium (pronounced cal-see-um) in the body.

The calcium forms crystals that deposit in the joints between bones. This causes swelling and pain in the area. This is called inflammation.

The calcium deposits and inflammation can cause parts of the joints to get weak and break down.

Cartilage is the tough elastic material that covers and protects the ends of bones. Which pseudo gout bits of cartilage may break off and cause more pain and swelling in the joint.

Over time the cartilage may wear away entirely, and the Bones rub together.

Pseudogout results form a buildup of calcium crystals (calcium pyrophosphate dehydrates) in a joint. The joint reacts to the calcium crystals by becoming inflamed. The calcium deposits and chronic inflammation can cause parts of the joint structure to weaken and break down. Cartilage, the tough elastic

material that cushions the ends of the bones, can begin to creak and get holes in it, Bits of cartilage may break off into the joint space and irritate soft tissues, such as muscles, and cause problems with movement.

Much of the pain of pseudogout is a result of muscles and the other tissues that help joints move (such as tendons and ligaments) being forced to work in ways for which they were not designed, as a result of damage to the cartilage. Cartilage itself does not have nerve cells, and therefore cannot sense pain, but the muscles, tendons, ligaments and bones do. After many years of cartilage erosion, bones may actually rub together. This grinding of bone against bone adds further to the pain. Bones can also thicken and form growths, called spurs or osteophytes, which rub together **(**Gladman et al., 2004).

The word 'pseudogout' actually means 'fake' or 'imitation gout,' like the disease gout, pseudogout can come on as sudden, recurrent attacks of pain and swelling in a single joint. Gout is also caused by the build-up of crystals within a joint. However, gout is caused by the build-up of uric acid crystals, rather than the calcium crystals. Gout usually attacks the big toe, while pseudogout most often attacks the knee.

3. Alkaptonuric Arthritis:

Alkaptonuria is a congenital disorder of amino acid metabolism affecting the joints. The breakdown of the amino acid tyrosine does not go beyond the stage of homogentic acid due to the absence of its oxidizing enzyme. Hence homogentistic acid appears in the urine. This condition is rare.

Clinically, the first phase of the condition is simple *alkaptonuria* ; the child passes urine which on exposure to air turns black. In the second phase, there is a deposit of 'ocher' coloured deposirs in the bone, cartilages, tendon sheaths and in the pinna of the ear. The cartilage of the pinna of the ear. The cartilage of the pinna of the ear appears darkly pigmented. This stage is called *'ochronosis'*. The sclera show characteristic slate coloured slit like patches on either side of the cornea. In the stage of *alkaptomuric arthritic* occurring in the 4^{th} or 5^{th} decade, the patient develops generalised pain and stiffening of the spine, peripheral joints like hip and knee.

Movement of joints gets restricted in the following condition:-

Trauma may cause tear of alignment, muscle, fracture of the bones involved in joints and dislocation of joints with advancing of age the synovial fluid gets depleted and joints surface the comes dry. Movement of such a joint will be painful and restricted.

Joints also become swollen and painful whenever there is inflammation involving bones or synovial membranes or both. Inflammation of joint is called Arthritis if it is due to infection by micro organisms it is called septic arthritis.

Arthritis can also occur due to immunological reactions. One of such is Rheumatoid Arthritis which is the commonest especially among middle aged females. Another non infective type of arthritis is ankylosing spondylitis **(**Chun-Jen et al., 1978)**.**

In about 50% of patients with rheumatoid arthritis, a denatured globulin called rheumatoid factor is detected in the blood.

In any inflammatory conditions including Arthritis a protein called C-Reactive protein can be demonstrated in blood samples of some patients.

Another evidence of immunological mechanism in Arthritis cases can be detection of immune complexes in the blood and synovial fluid of the patients.

MATERIALS AND METHODS

Immunological and serological profile of arthritis

Perform of patient sheet

Name: **Age:** **Sex:**

Present Symptoms with duration:

Any H/O Injury

Clinical Diagnosis:

Tests Performed

Blood	Synovial fluid
1. RA Test	**1.RA Test**
2. CRP Test	**2.CRP Test**
3. Cold agglutination	**3.Cryprecipition**
4. Cryprecipition	

Reports of the test:

Interpretation in Diagnosis:

Patients attending Orthopedic department of N.R.I. General Hospital formed the subjects for the present statement.

Only patients giving the History justify of Arthritis were selected after clinical examination only non infective cases of arthritis for including the statement. Blood and synovial fluid samples were collected from this patients.

The blood was allowed to clot and serum were separated and stored in the incubator. Synovial fluid was physical examined to detect the colts' poor net nature and stored in the incubator.

TEST FOR RF (or) RA

SLIDE TEST FOR RHEUMATOID FACTORS

Sometimes autoantibodies are produced by the human body against self antigens. The precise role that this aberrant immunity plays in the pathogenesis of certain rheumatic diseases in unknown. However the presence of these auto antibodies serve as credible marker of the disease.

In rheumatoid arthritis, diagnostically useful auto antibodies termed as "Rheumatoid factors" (RF) can be detected which are immunoglobulin of the class IgM, IgG, IgA and IgE. Practically, IgM class RF with specificity to human IgG (Fc) is the most useful prognostic marker of RA . The clinical significance of RF determinations consists in differentiation between rheumatoid arthritis, in which RF of modified IgM class have been demonstrated in the serum of approximately 80% of the cases examined and rheumatic fever, in which RF are almost always absent. The agglutination test is most frequently used because of its greater sensitivity and simplicity. RHELAX RF is a latex agglutination slide test for detection of rheumatoid factors of the IgM class (Caporali et al., 2004)

REAGENT

1. RHELAX RF reagent: A uniform suspension of polystyrene latex particles coated with suitably modified Fc fraction of IgG. The reagent is standardized to detect = 10 IU/ml of RF or more.

2. Positive control, reactive with the RHELAX RF reagent.

3. Negative control, non-reactive with the RHELAX RF reagent. Each batch of reagents undergoes rigorous quality at various stages of manufacture for its specificity, sensitivity and performance.

REAGENT STRORAGE AND STABILITY

1. Store the reagents at 2-8 C DO NOT FREEZE.

2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

PRINCIPLE

RHELAX RF side rest for detection of rheumatoid factors is based on the principle of agglutination. The test specimen is mixed with RHELAX RF latex reagent and allowed to react. It RF is present within detectable levels then a visible agglutination is observed. If RF is absent below detectable levels then no agglutination is observed.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. All the reagents derived from human source have been tested for HBsAg and Anti-HIV antibodies and are found to be non-reactive. However handle the material as if infections.

3. Reagent contains 0.1% Sodium acide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. The reagent can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagent be verified with positive and negative controls provided with the kit.
5. Shake the RHELAX RF latex reagent well before use to disperse the latex particles uniformly and improve test readability.
6. Only a clean and dry glass slide must be used. Clean the slide with distilled water and wipe dry.
7. Accessories provided with the kit only must be used for optimum results.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection by approved techniques. Only serum should be used for testing. Should a delay in testing occur, store the sample at 2-8 C. Samples can be stored for upto a week. Do not use hemolysed serum.

MATERIAL PROVIDED WITH THE KIT Reagent

Rhelax RF latex reagent, Positive control, Negative control.

Accessories

Glass slide with six reaction circles. Sample dispensing pipettes. Mixing sticks, Rubber teat.

ADDITIONAL MATERIAL REQUIRED

Stopwatch, Test tubes, A high intensity direct light source, Isotonic saline.

TEST PROCEDURE

Bring reagent and samples to room temperature before use.

Qualitative Method

1. Pipette one drop of serum onto the glass slide using the disposable pipette provided with the kit.
2. Add one drop of RHELAX RF latex reagent to the drop of serum on the slide. Do not let the dropper tip touch the liquid on the slide.
3. Using a mixing stick, mix the serum and the RHELAX RF latex reagent uniformly over the entire circle.
4. Immediately start a stopwatch. Rock the slide gently, back and forth, observing for agglutination macroscopically at two minutes.

Semi Quantitative Method

1. Using isotonic saline prepare serial dilutions of the serum sample positive in the qualitative method 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and so on.
2. Pipette each dilution of the serum sample onto separate reaction circles.
3. Add one drop of RHELAX RF latex reagent to each drop of the diluted serum sample on the slide. Do not let the dropper tip touch the liquid on the slide.
4. Using a mixing stick, mix the sample and the latex reagent uniformly over the entire circle.
5. Immediately start a stop watch. Rock the slide gently, back and forth, observing for agglutination macroscopically at two minutes.

INTERPRETATION OF TEST RESULTS

Qualitative Method

Agglutination is a positive test result and indicates the presence of rheumatoid factors in the test specimen. No agglutination is a negative test result and indicates the absence of rheumatoid factors in the test specimen.

Semi Quantitative Method

Agglutination in the highest serum dilution corresponds the approximate amount of rheumatoid factors in IU/ml present in the test specimen.

To calculate the RF in IU/ml, use the following formula:

RF (IU/ml) = S .D

Where S = Sensitivity of the reagent i.e. 10IU/ml.

D = Highest dilution of serum showing agglutination.

TEST FOR CRP

C-REACTIVE PROTEIN (CRP)Turbilatex

Method

Particle enhanced immunoturbidimetric test

Clinical Significance

CRP is an acute phase protein present in normal serum, which increases significantly after most forms of tissue injuries, bacterial/viral infections, inflammation and malignant neoplasia (Aubin et al., 2004).

It is elevated up to 500mg/l in acute inflammatory processes associated with bacterial infections, post-operative conditions or tissue damage within 6 hours reaching a peak 48 hours. The measurement of CRP represents a useful laboratory test for detection of acute rheumatic and gastrointestinal diseases. CRP testing shows various advantages in comparison to the erythrocyte sedimentation rate (ESR) and the leukocyte count. In fact, it is more sensitive, the increase occurs earlier and its levels return to the reference range more rapidly after healing. In recent studies it has been CRP concentrations and the risk for developing coronary heart disease (CHD) (Beaton et al., 2004).

Principle

The assay is based on photometric measurement of antigen-antibody-reaction. In this kit, CRP present in patient's sample is reacted against anti-CRP coated micro latex and the values are measured photo metrically.

Reagents

Reagent 1: Diluent

Tri Buffer 20 mmol/L,

Sodium Azide 0.95g/LpH 8.2

Reagent 2: Latex Reagent

Suspension of latex particles coated.

Which anti human CRP.

Spdium azide. 95g/L

Reagent 3: CRP Calibrator

Lyophilized Human serum.

CRP concentration on label.

Calibration

The assay is calibrated to the Reference Material CRM 470/RPPHS. Other commercial calibrators are not recommended.

Reagent Preparation & Stability

Working Reagent: **G**ently mix the contents of the Latex Reagent vial. Prepare working reagent as follows (See Note).

1 ml Latex Reagent + 9 ml Diluent .

This working reagent is stable for 30 days at 2 – 8 C.

NOTE: It is recommended that each Laboratory prepares a weekly requirement of Latex Working Reagent based on its workload. The Reagent may be prepared on a 1: 10 ratio, or by taking lower multiples of the above mentioned volume.

CRP Calibrator: Reconstitute the contents of the vial with 1.0 ml of Distilled Water. Wait for 10 minutes before use.

Stable for 30 days at 2 – 8 C or 3 months at – 30 C.

Stability: All kit components are stable up to expiry dates printed on them. Do not freeze reagents. Present of visible particulate matter indicates deterioration of reagents.

DO NOT FREEZE REAGENTS.

Samples

Fresh Serum is preferred, though samples stored for 8 days at 2 – 8 C or 3 months at – 20 C may also be used.

Centrifuge samples showing visible particles.

Hemolysed or lipemic samples should not be used.

Equipments Required

Photometer,

Pipetting system

Dispense	Calibrator	samples
Working Reagent	500uL	500ul.
Calibrator	3uL	-
Sample	-	3uL

Blank Instrument with Distilled water. Pipette W.R and Calibralor/Sample as per the pipetting system. Measure Absorbance immediately (A1) and exactly after 2 minutes (A2) of sample addition.

Calculation

$$\frac{(A_2\text{-}A_1)\text{Sample}}{(A_2\text{-}A_1)\text{ Calibrator}} \times \text{Calibratorconcentration}$$

Reference Values

Adults : Up to 6mg/L

New borns up to 3 weeks : < 4.1 mg/L

Infants 7 Children : 2.8 mg/L

Liquid dispensing systems

General laboratory equipments.

Procedure

Assay protocol

Wavelength : 540 nm (530 – 550nm)

Blank : Distilled Water

Temperature : 37°C

Cuvette path Length : 1cm

Auto Analyzers may be suitably programmed in Fixed time mode with a nominal delay of 10 sec and a read interval 0 120 sec. Specific procedure guidance is available from the company.

Performance Characteristics

Linearity:

Up to 100mg/L, under the described assay conditions. The linearity may be increased by decreasing sample volume, though this will compromise sensitivity of the test. When values exceed this range the samples should be diluted 1 + 1 with NaCl solution (9g/l) and the result should be multiplied by 2**. For better linearity, sample volume can be reduced to 3 ul.**

Detection Limit: Values less than 2 mg/L yielded non-reproducible results.

Prozone Effect: No prozone effect was detected up to 800 mg/L.

Interferences

Bilirubin up to 20mg/dl; Rheumatiod Factors up to 300 IU/ml; Hemoglobin up to 5g/L & Lipids up to 20g/L do interfere.

Literature

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Precision (n = 20)

Intra- assay Precision	Mean (mg/L)	SD (mg/L)	CV [%]
Sample1	6.6	0.3	4.7
Sample2	20.4	0.6	0.3
Sample3	88.5	3.1	3.5

Intra- assay Precision(daily calibration)	Mean (mg/L)	SD (mg/L)	CV [%]
Sample1	7.3	0.4	5.9
Sample2	22.1	0.6	2.6
Sample3	95.0	1.2	1.3

3. Cold Agglutination:-

The cold agglutinins test is performed to detect the presence of antibodies in blood that are sensitive to temperature changes. Antibodies are proteins produced by the immune system in response to specific disease agents; autoantibodies are antibodies that the body produces against one of its own substances. Cold agglutinins are autoantibodies that cause red blood cells to clump, but only when the blood is cooled below the normal body temperature of 98.6 F (37 ^{0}C). The clumping is most pronounced at temperatures below 78 F (25.6 ^{0}C).

The cold agglutinins test is used to confirm the diagnosis of certain diseases that stimulate the body to produce cold agglutinins. The disease most commonly diagnosed by this test is mycoplasmal **pneumonia,** but mononucleosis, **mumps, measles, scarlet fever,** some parasitic infections, cirrhosis of the liver, and some types of hemolytic anemia can also cause the formation of cold agglutinins. Hemolytic anemia is conditions in which the blood is low in oxygen because the red blood cells are breaking down at a faster rate than their normal life expectancy of 120 days. In addition to these illnesses, some people have a benign condition called chronic cold agglutinin disease, in which exposure to cold causes temporary clumping of red blood cells and consequent numbness in ears, fingers, and toes.

Clinical Diagnosis:

Antibodies that cause clumping of red blood cells when the blood temperature falls below normal body temperature (98.6 F/37 C) (Mickelson et al., 2000.)

4. **Cryoprecipitation:-**

Done to detect immune complexes 1ml of Serum was diluted and suspension was over night in refrigerator. It was observed for **precipitation** (Leonard et al., 1997).

RESULT

In the present study twenty patients with symptoms of arthritis were included. As control another twenty patients without arthritis were included. Serum samples from all the patients and controls were tested for Rheumatoid factor. C - reactive protein, cold agglutination and immune complexes. From ten patients with arthritis synovial fluid was tested for Rheumatoid arthritis and C - reactive protein.

Out of twenty arthritis patients Rheumatoid factor was detected in ten samples. C - reactive protein detected in nine samples, cold agglutination defected in one sample, and immuno complexes were detected in two samples of sera both Rheumatoid factor and C-Reactive proteins were detected. In two RF, CRPS, IC. In four samples of synovial fluid Rheumatoid factor and C - reactive protein were detected. In the same positive for Rheumatoid factor and C - reactive protein.

Out of the total positive serum samples, eight were female patients and two were male patients. In the two positive male patients both serum and synovial fluid Rheumatoid factor and C - reactive protein were detected.

Out of eight positive female patients, in tow both serum and synovial fluid were positive for Rheumatoid factor and C - reactive protein. Immune complexes were detected in two female patients in whom serum and synovial fluid were positive for Rheumatoid factor and C - reactive protein.

DISCUSSION

Among non septic arthritis cases the common cause is autoimmune mechanism resulting in Rheumatoid arthritis, ankylosing spondylitis. Even though Rheumatoid factor detection is one of the important tests indicative of autoimmune mechanism, its positivity in only 405 of cases limits its usefulness.
Detection of immune complexes can be made by many sensitive methods such as PEG 6ooo prescription method, CI fixation and cryoprecipitation. A combination of these tests would yield more positive results compared to cryo precipitation. CRP is doubtful value as it can be detected in any inflammation. Its positivity in control group may be due to other underlying inflammatory condition.

All the twenty serum samples from control group were negative for Rheumatoid factor. In two female controls C - reactive protein was detected.

SUMMARY

I took blood & synovial fluid samples from men & women.

First I took blood & following tests were done; 1) R.A, 2) C.R.P, 3) cold agglutination 4) cryo precipitation.

Secondly I took synovial fluid & the following tests were done. 1) R.A, 2) C.R.P, 3)cryo precipitation.

Finally I observed some members were suffering from arthritis.

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A radiographic evaluation of temporomandibular and hand (Metacarpophalangeal) / wrist joints of patients with adult rheumatoid arthritis

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Abstract

Go to:

Background:

A review of literature revealed that, although the involvement of temporomandibular joint (TMJ) in rheumatoid arthritis (RA) patients is not uncommon, variation in presentation persist. Comparative studies of bony changes in the right and left TMJ with the right and left peripheral hand (Metacarpophalangeal-MCP)/wrist joints have not been done, to the best of our knowledge.

Materials and Methods:

In this cross-sectional study, the temporomandibular and hand (MCP) and wrist joints of fifteen rheumatoid arthritis patients were evaluated with questionnaires, clinical and lab assessment and radiographically using conventional radiographs and computed tomography. Students *t*-test was applied for the statistical analysis of the data obtained and a *P* value of 0.05 was considered as statistically significant.

Results:

Comparisons between the right TMJ with right MCP/wrist joint and left TMJ with left MCP/wrist joint did not reveal statistically significant results. Radiographically, flattening and erosions were the common manifestations. MCP joints were more affected than the wrist, but whenever the wrist was involved, it was more likely to be bilaterally affected.

Conclusions:

Although the TMJ showed osseous changes of a higher grade than the hand (MCP) and wrist joints radiographically, it was observed that patients were more aware of the peripheral joint discomfort. There were no significant differences between TMJ and peripheral joints on both right and left sides.

Keywords: Diagnostic imaging, hand joints, rheumatoid arthritis, temporomandibular joint

INTRODUCTION

Go to:

The temporomandibular joints (TMJ) or the craniomandibular joints are one of the important joints in the body which may be subjected to disease processes like degeneration and inflammation.

The commonest of the inflammatory joint diseases is Rheumatoid Arthritis (RA) which is a chronic multisystem disease. The disease is characterized by a pathologic immune response that affects the synovial cells, cartilage and bone resulting in joint destruction and permanent disability.

It is not specific to any particular race or region, the prevalence being 1-2% of the population world wide. Females are three times more prone for affliction than males. Although, it can occur at any age, the peak incidence is during the fourth and sixth decades of life. RA often affects the peripheral small joints like the fingers and toes, eventually involving other joints like knee and shoulder.[1,2,3,4] Studies of the incidence of TMJ symptoms in RA have shown wide range of figures of involvement varying from 2-86%.[2,3,4,5]

The position and relative anatomy of TMJ and its surrounding structures requires a skillful and accurate technique of imaging for a detailed study. The first reported formal study of TMJ in RA was done by Cadenat and Blanc (1958) in fifteen patients, which revealed radiographic changes in the TMJ, as well as other joints. The frequency of radiologic findings in the TMJ has been reported to be between 19% and 84% in patients with RA.[6] Also, studies thus far have revealed an involvement of TMJ in RA with many patients having significant changes radiographically, but less clinical signs and symptoms when compared to the peripheral joints.

With this in mind, a study was undertaken to evaluate radiographically the extent of TMJ involvement as compared to hand (MCP)/wrist joints in RA patients by using conventional and modern imaging techniques. Also, an assessment of the TMJ's and peripheral joints on the right and left sides was done, to ascertain, if certain joints were more prone to disease processes, based on functional inequalities.

MATERIALS AND METHODS

Go to:

This study was approved by the ethics committee of SDM College of Dental Sciences, Karnataka, India. Fifteen patients in the age group of 20-60 years (11 females and 4 males) were selected for this study, out of a referral pool of patients from the Oral Medicine, Rheumatology and Orthopaedics clinics. Informed consent was obtained from all the patients.

Inclusion criteria

Patients with a diagnosis of RA according to the American Rheumatism Association's Revised Criteria for Rheumatoid Arthritis.[7] Only patients over the age of 18 were included.

Exclusion criteria

Patients with joint involvement other than the TMJ/hand (MCP) and wrist joint were not included. Patients with myofascial pain, TMJ ankylosis, headache, patients with known history of previous trauma, cervical spondylosis and pregnant females were not included.

Detailed case history as per the questionnaire was taken. As the patients were known RA patients, the primary focus was to ascertain the onset, duration, site, type of symptoms like pain, limited/altered movement of the hands and jaw etc. Visual Analog scale was used. Patients were also questioned on psychosocial history and any prior treatments undertaken. Clinical assessment of TMJ for function and range (deviation/limitation) of movement, pain in the joint proper or while biting, audible or palpable clicking of joints were recorded. Examination of the mouth was carried out at the same time as the clinical examination of the joint. Any occlusal discrepancies were noted. Examination of hand/wrist joints for soft tissue swelling, pain, limitation of movement, tenderness to palpation, difficulty in performing simple functions like writing or picking up objects and deformities was done. In all cases, only abnormalities present at the time of examination were recorded.

Laboratory tests involving erythrocyte sedimentation rate (ESR-westergren's method) and RA test using the Rhelax Rf slide test kit (Tulip, India) was used in this study for the determination of rheumatoid factor.

Radiographic evaluation of the TMJ was carried out using the conventional projections, transcranial and OPG and advanced computed tomography (CT). Posteroanterior view (PA) of the hand and wrist joints was also taken.

Transcranial views of right and left TM joints of all patients were taken using Siemens Vertix 100 extraoral radiographic machine with a setting of 70-72 mAs and 74-82 KVp on an average.

Orthopantomographs were taken using the Villa rotograph machine with a machine setting of 70-75 KVp and 60 mAs.

Ten patients were subjected to CT scans using the Toshiba 2D CT scanner model- 300 S and with a machine

setting of 150 KVp and 250 mAs on an average. Slices of 2 mm thickness were taken in both the coronal and axial sections. The remaining five patients were not willing for CT examinations.

PA views of the hand and wrist were made with the Siemens Vertix 100 extra oral radiographic machine with a setting of 55 KVp and 6-8 mAs on an average.

Detailed analysis of the hand and wrist radiographs was performed based on Larsen's gradings[8] [Table 1]. For the TMJ, the gradings were restricted to the condyle proper, as the glenoid fossa could not be clearly visualized in all the radiographs. Since the anatomy of the TMJ is different from that of the hand and foot joints, a modification of the grading system proposed by Larsen *et al*.[8] was made for the TMJ [Tables 2 and 3].[9] These radiographs were assessed individually by a medical and oral radiologist. During the process of deliberation the conclusion arrived at by the examiners were noted.

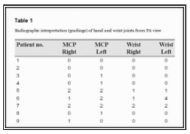

Table 1

Radiographic interpretation (gradings) of hand and wrist joints from PA view

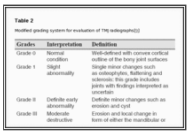

Table 2

Modified grading system for evaluation of TMJ radiographs[9]

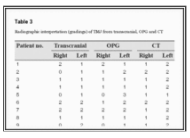

Table 3

Radiographic interpretation (gradings) of TMJ from transcranial, OPG and CT

Student's *t*-test was applied for the statistical analysis of the data obtained.

RESULTS

Go to:

The study was carried out in the proposed manner and the data was statistically analyzed.

Of the 15 patients taken randomly, 11 (73.3%) were females and 4 (26.6%) were males. Ten patients (67%) were seropositive for rheumatoid factor. Values higher than 20 mm for ESR have been taken as abnormal i.e. 13 patients (87%), thereby showed abnormal values.

Comparison of the TMJ with the hand (MCP) and wrist on the right and left side did not reveal any statistically significant results. The results were not significant when the individual joints were compared on the right and left sides [Tables 4 and 5].

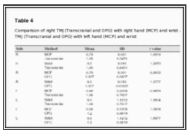

Table 4

Comparison of right TMJ (Transcranial and OPG) with right hand (MCP) and wrist and comparison of left TMJ (Transcranial and OPG) with left hand (MCP) and wrist

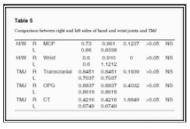

Table 5

Comparison between right and left sides of hand and wrist joints and TMJ

It was however noticed that hand (MCP) joints were more frequently affected when compared to wrist joints and when the wrist was involved, it was bilaterally involved [Figure 1].

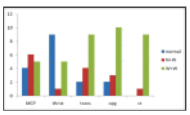

Figure 1

No of patients with unilateral/bilateral/normal joint involvement of the hand-MCP and Wrist and TMJ- as visualised from Transcranial (trans), OPG and CT

Comparison of the mean gradings of the TMJ and MCP and wrist joints on the right and left side indicated a higher grade on left side [Figure 2].

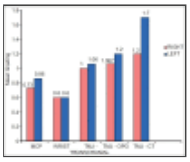

Figure 2
Bar graph showing the radiographic mean gradings across TMJ's and peripheral joints

DISCUSSION

Go to:

Rheumatoid arthritis is a chronic destructive inflammatory disease process known to commonly affect the peripheral joints. This results in joint effusion, swelling, pain, limitation in movement and deformities. The TMJ like the peripheral hand and wrist joints can be a site for chronic synovial inflammation. TMJ involvement in RA results in clinical symptoms like pain, swelling, crepitation, stiffness and limitation in jaw movement.[10]

Radiography is a frequently used diagnostic aid to detect and assess disease severity and response to treatment. The radiographic findings ascribed to RA in the TMJ are erosion and flattening of the condylar head, limitation of movement, marginal proliferations and sclerosis.

In this study, the temporomandibular joints of 15 patients (30 joints) with definite RA were compared with their hand (MCP) and wrist joints to assess the degree of involvement in each case using the transcranial radiograph, OPG and CT scans. Of the 15 patients, 11 (73%) were females and 4 (23%) were males, in the ratio of 2.7:1. This incidence rate indicates that RA affects women approximately three times more than men and is consistent with the well documented findings of other authors.[11,12,13,14]

The mean age of incidence of rheumatoid arthritis in this study is 34.5 years. This finding is comparable with that of the age of patients (35) in the study conducted by Mc Carthy[11] but not in agreement with that of 55 years of Ogus[12] and 56.4 years of Syrjanen.[3] This disparity could be because of the difference in sample size, the higher age group and also the racial differences in other studies.

It was observed that although all the patients presented with complaints of either swelling, morning stiffness or limitation of movement of the MCP and wrist joints, the TMJs were symptomatic in five patients only (33%). TMJ symptoms were less noticed by the patient who has more stiffness and pain with visible inflammatory signs in other joints. Also, patients often attributed TMJ symptoms to tooth related problems. In this study, other symptoms of TMJ involvement like restriction in mouth opening was not observed which is consistent with previous reports. [5,12]

Most studies indicate that TMJ lesions found radiographically vary in frequency from 50% to 80% in RA.[15] In this study, conventional methods revealed that 13 RA patients (87%) had radiographic evidence of TMJ abnormalities (from slight to marked) in one or both TM joints, which is in consonance with the previous studies. [12,16] However, bilateral presentation was observed in 9 (60%) and 10 (67%) patients with the transcranial and OPG, respectively. Symmetric involvement of both right and left joints have been reported in previous studies as well.[12,13]

CT scans were performed to assess whether any additional radiographic changes could be observed in the TMJ and to confirm the accuracy of the findings obtained with transcranial and OPG. It was found that all the patients exhibited radiographic abnormalities, with 9 (90%) patients showing bilateral presentation.

The present study shows that flattening [Figure 3] and bone erosion of the condyle was the most common radiographic manifestations. Of the 15 patients i.e. 30 joints evaluated, transcranial view revealed involvement of 9 TM joints (30%) as erosions and 16 joints (44%) as flattening. This was followed by osteophytes/marginal proliferations seen in 3 joints (10%) and subcortical cyst in 1 joint (3%). Sclerosis and ankylosis could not be visualized from this technique.

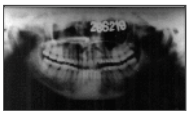

Figure 3
OPG showing flattening of the temporomandibular joint on the left side with normal right TMJ

In OPG, 9 joints (30%) revealed bone erosions, flattening in 14 joints (46%), osteophytes in 2 joints (6%) and cyst in 1 joint (3%). No other radiographic abnormalities could be detected. The low frequency of erosions in the present study is comparable with the finding of less involvement of 24% of the joints in the study by Syrjanen, 1985.

CT scans of all the ten patients showed either mild or moderate involvement of the temporomandibular joint. The higher incidence of positive findings like erosions and joint space narrowing observed in this study is comparable with other studies [Figures 4 and 5].[17,18]

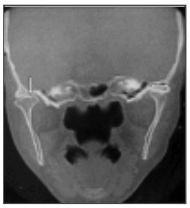

Figure 4
Coronal CT showing erosive changes and decreased joint space of right and left TMJ's

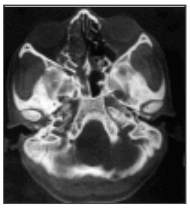

Figure 5
Axial CT shows subchondral cyst in the left TMJ

The analysis of the data revealed possibility of a relationship between TMJ abnormalities and the duration and severity of disease. It was observed that in long standing cases, there was a corresponding increase in degree of structural changes as evidenced in radiographs. In this study, 4 of the 5 patients with TMJ symptoms had duration of disease exceeding 3 years. This may be attributed to the fact that as the disease progresses the chronic inflammatory process results in increased joint destruction. The most common radiographic findings are erosion and flattening of the head of the mandible and articular fossa and reduction of the joint spaces, usually noticed 5 to 10 years after the onset of symptoms.[19,20]

The hand (MCP) and wrist joints of all the patients exhibited radiographic abnormalities on both right and left sides [Figure 6]. Interestingly, it was noted that MCP joints were more affected than the wrist, but whenever wrist was involved, it was more likely to be bilaterally affected and showed a greater grade on radiographs. The most common findings were erosions, flattening and decreased joint space. However, one patient presented with ankylosis of the wrist joints on the left side. This patient aged 60 years had suffered from the disease for more than 10 years.

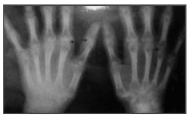

Figure 6
PA view of the hands shows erosive changes in the MCP and wrist joints of both hands

Upon evaluation of the data obtained from this study, it was found that the TMJ and the hand (MCP) and wrist joints of patients with rheumatoid arthritis exhibited similar radiographic abnormalities on both right and left sides. This finding is consistent with the findings of Akerman *et al*., in 1991[21] where they found no significant differences between the changes in TMJ and hand (MCP) and wrist joints.

Functional inequalities, if any, owing to the use of the right side over the left in the peripheral joints does not seem to have any significance with regard to the extent of disease progression. In the TMJ, functional movements like chewing on the right or left side more than the other also does not cause a corresponding increase in disease activity as evidenced by radiographic findings.

CONCLUSION

Go to:

The results of the present study showed similar changes in the TMJ and MCP and wrist joints of patients with RA. Flattening and erosions were the common manifestations seen on radiographs. Though, 87% of patients had definite radiographic changes in the TMJ, only 33% of them had clinical symptoms such as pain on biting followed

by tenderness to palpation.

Few studies have compared radiographically the bony changes in right and left TMJ's with the right and left hand (MCP) and wrist joints in Rheumatoid Arthritis patients. Although, statistically insignificant results and sample size limits this study, it is worthwhile to note that most RA patients exhibit similar TMJ bony changes along with peripheral joints.

Footnotes

Go to:

Source of Support: Nil

Conflict of Interest: None declared

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