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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).

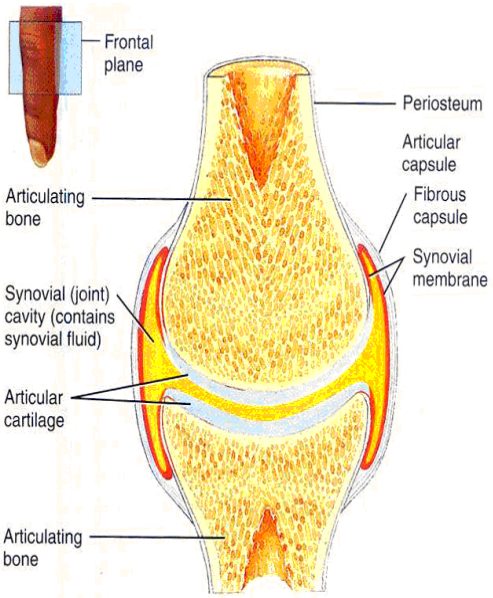

Figure 1: Structure of a typical synovial joint.

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

Lars – Olof Hanson et al. Current opinion in infect. Diseases 1997 10 196-201.

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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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