Statistical Analysis of Rheumatoid Arthritis in Association With Demography and Biochemical Investigation

G Y Mythili [1], B. Venkateswarlu[2], T. Subramanyam[3], C. Narayana[4]

[1] Assistant Professor, [2]Senior Assistant Professor, Department of Mathematics, Vellore institute of Technology (VIT), Vellore, Tamilnadu, India, [3]Assistant Professor, Department of Mathematics and Statistics, M.S Ramaiah University of Applied Sciences, Bangalore, India, [4]Associate Professor Department of Mathematics, Sri Harsha Institute of P.G Studies, Nellore, Andhra, Pradesh, India

Abstract

In this paper we discussed the basic statistical analysis of the biological data and associated with the demographic profile and biochemical profile of the samples of arthritis. Arthritis is a multi-factorial disorder consisting of more than 100 types of arthritis[9], it is really difficult to say that a particular disease is causing that disorder. For determination of Rheumatoid Arthritis (RA) we have used biochemical tests of the 70 individual affected with rheumatoid arthritis. The peripheral blood serum and plasma has been collected using vacutainers containing EDTA, the samples have gone through 5 tests for the determining the pattern of arthritis. By using this method pre-diagnostic measure for arthritis, results have shown us that most of the patient have correlative increase and decrease in their serum and plasma level when the patient is affected with RA. Various test such as Hb, Uric acid, serum glucose, serum calcium levels are used to determine the presence of arthritic profile. The results have found completely based on correlation of the demographic data with the arthritic marker, diabetic marker, lipid marker, kidney function marker, liver marker. The results have found that maximum correlation is shown among the liver marker as its maximum correlation with all the arthritic markers and demographic data.

Keywords*: Statistical analysis, Arthritis, Rheumatoid arthritis, demography, biochemical investigations.*

Introduction

Statistical modelling are widely used in science, epidemiology, and health services etc., research to analyse and interpret data,

Obtained from surveys or existing data sources such as claims files and electronic health records. The veracity of such inferences relies not only on the quality and completeness of the collected data, but also on the validity of the researcher's model[14].

Corresponding author:
Dr B. Venkateswarlu,
Department of Mathematics, Vellore Institute of Technology, Vellore, Tamilnadu, India,
Email: venkatesh.reddy@vit.ac.in

Correlation is a statistical method used to evaluate the strength of relationship between two quantitative variables. A high correlation means that two or more variables have a strong relationship with each other, while a weak correlation means that the variables are hardly related. Scatter diagram is the simplest way of graphic representation of a bivariate data, where the given set of 'n' pairs of observations on two variables X and Y say (X_1, Y_1), (X_2, Y_2) …(X_n, Y_n) may be plotted as dots by considering X-values on X-axis and Y-values on Y-axis. By scatter diagram, we can get some idea about the correlation between X and Y. In a scatter diagram, if the points are closer and show either upward or downward trend then there is high degree of correlation.

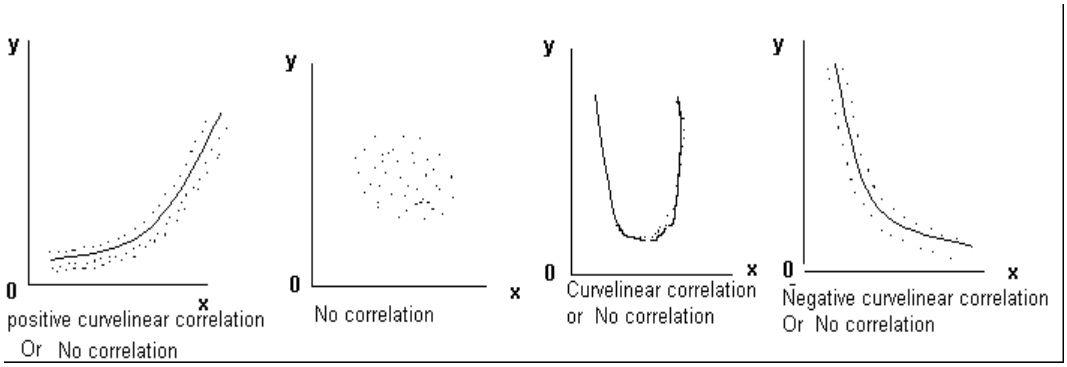

Diagram 1: Scatter diagram shows the direction of correlation

Chi Square Test of Goodness of Fit:

The goodness of fit of a statistical model describes how well it fits a set of observations. Measures of goodness of fit typically summarize the discrepancy between observed values and the values expected under the model in the data. A very powerful test for testing the significance of the discrepancy between theory and experiment was given by prof. Karl Pearson in 1900 is known as Chi-Square Goodness of Fit. It enables us to find if the deviation of the experiment from theory is just by chance or is it really due to the inadequacy of the theory to fit the observed data

$$X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where O = observed value; E = Expected value

For a Chi-square test, a p-value that is less than or equal to significance level indicates there is sufficient evidence to conclude that the observed distribution is not the same as the expected distribution. In this paper we applied Chi square test to find the association between demographic profile and the Rheumatoid Arthritis of the samples and the results obtained is discussed in conclusions. Arthritis the term is derived from Greek arthro- meaning joint and it is meaning inflammation[10]. Arthritis is a multi-factorial disorder consisting of more than 100 types of arthritis, it is really difficult to say that a particular disease is causing that disorder[3]. Humans have been suffering from that disorder almost over a thousands of years and it's still a major concern for the society till date[14]. Majorly arthritis is divided into two parts Osteoarthritis and Rheumatoid arthritis. Rheumatoid arthritis is an autoimmune disorder that affects hands and feet, it's an inflammatory disease and often occurs with severe pain in the joints[4]. Other types include lupus, gout, fibromyalgia, and septic arthritis occurs in 3 % of people and Rheumatoid arthritis occurs in only 0.2% of people, more than 20% of USA population are suffering from rheumatoid arthritis[4]. As of till date no particular reason behind rheumatoid arthritis is found to be genuine as the symptoms vary from person to person. Someone having arthritis may or may not feel the presence of the disease as the joint inflammation is very mild and often it gets worsen as the age progresses[16]. 1 out of 5 people may show a very mild symptoms of rheumatoid arthritis. Blood tests, x-rays helps to diagnose an arthritis patient. Various biochemical tests are involved in determining arthritis. Rheumatoid arthritis contains complex pleiotropic network of inflammatory cytokines and having numerous pathway[7]. The current concept is that inflammation and tissue destruction is related to cellular interaction of rheumatoid synovium[12].

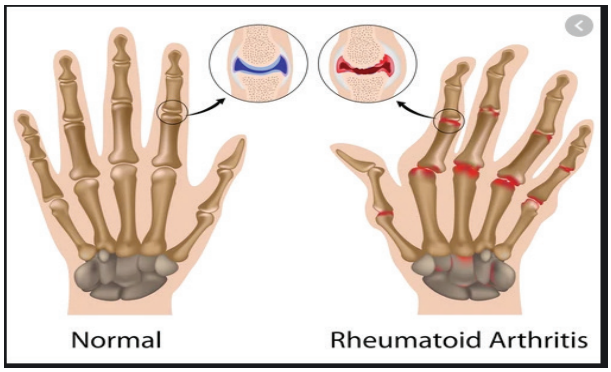

Diagram2: diagramatic representation of Rheumatoid Arthritis

The Progression of Arthritis:

Rheumatoid arthritis begins with the inflammation of the synovial membrane of the joints, especially in the small ones of the fingers and feet and is mostly bilateral. The inflammatory cells, if in inappropriate large amount, destroy body tissue. The synovial fluid accumulates and the joints swell in time and thicken into an abnormal tissue. During the course of time the abnormal tissue erodes the joint's cartilage and possibly scar tissue will be formed, connecting the bone.

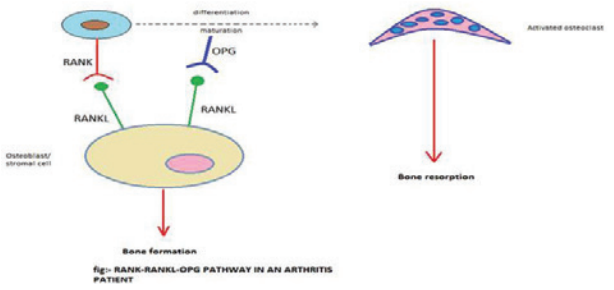

Diagram 3: Rank-Rankl-OPG pathway

Materials and Methods

Materials: For performing biochemical assays the chemical kit were ordered from laboratory and assays were performed in the university laboratory. For performing Immuno-histochemical assays were performed using chemicals such as xylene, ethanol, haematoxylin, PBS, FBS, Antibodies (primary, secondary), blocking solution, DAB substrate solution etc. other reagents Merck Pvt Ltd, Trizol solution, chloroform, DEPC water.

Methods: About 70 patients at the age range of 25-78 were taken for the study of RA. The patient tissue& blood samples were collected from Orthopaedics Dept., Tripura Medical College and Dr B.R Ambedkar memorial Teaching Hospital. The patients suffering from Rheumatoid Arthritis fulfilled the American Rheumatism Association Criteria and were suffering from RA for about 6 months. For the study of control, 60 healthy subjects are chosen. Informed consent was taken from the subjects. Various quantitative Biochemical profiles such as Uric acid levels, glucose levels, calcium levels, cholesterol levels. Qualitative assays includes Rheumatoid factor-Reactive protein. Haematological analysis includes Haemoglobin% and Erythrocyte sedimentation rate (ESR). Patients were diagnose as arthritis, serum samples are taken and biochemical assays were taken. OD values are measured and compared with the normal control patient and hence the values are observed and studied for arthritic profile.

Table 1: Reagent compositions

HAEMOGLOBIN REAGENT 1000 Ml
Potassium Phosphate 2.0 mmol/L Potassium ferricyanide 0.60 mmol/L Potassium cyanide 0.90 mmol/L Sodium chloride 1.4 mmol/L
HAEMOGLOBIN STANDARD 1 x 4 Ml
Cyanmethaemoglobin standard con. 60 mg/dL
URIC ACID:
URIC ACID (S.L) R1 2 x 30 mL / 2 x 50 mL / 2 x 100 mL
Phosphate Buffer (pH 7.0) 180 mmol/L
TOPS 1.10 mmol/L
Amino-4-antipyrine 0.25 mmol/L Uricase> 50 U/L Peroxidase> 100 U/L Preservatives and Stabilizers
URIC ACID STANDARD 1 x 4 mL Uric acid standard concentration 8 mg/dL

GLUCOSE:
REAGENT COMPOSITION
GLUCOSE (S.L)
R1 5 x 100 mL / 1 x 1000 mL
Tris Buffer, (pH 7.40) 92 mmol/L
Phenol 0.3 mmol/L
Glucose Oxidase 15000 U/L
4- Aminophenazone 2.6 mmol/L
GLUCOSE STANDARD 1 x 4 mL
Glucose standard concentration 100 mg/dL

CHOLESTEROL:
REAGENT COMPOSITION
CHOLESTEROL (S.L)
R1 5 x 25 mL / 5 x 100 mL / 4 x 250 mL
Pipes buffer (pH 6.70) 50 mmol/L
Phenol 24 mmol/L
Sodium Cholate 0.5 mmol/L
4-aminoantipyrine 0.5 mmol/L
Cholesterol Esterase > 180 U/L
Cholesterol Oxidase> 200 U/L
Peroxidase> 1000 U/L
CHOLESTEROL STANDARD 1 x 4 mL
Cholesterol std. concentration 200 mg/dL

Results

A significant increase ($p<0.0001$) in the values of biochemical levels among the patients suffering from Rheumatoid Arthritis (RA) comparing with healthy individuals (Table 1). All the results are expressed in mean +sd.

Table 2: Measures of metabolic markers in rheumatoid arthritis (RA) subjects.

Parameter	Rheumatoid arthritis (n= 70)	Rheumatoid arthritis control (n=60)
Age	47.53	46.06
	11.93	13.91
Haemoglobin (%)	9.18	13.24
	1.48	1.43
Uric Acid (mg/dl)		3.98
		0.7
	-7.84	
	1.74	
Glucose (mg/dl)	117.87	90.16
	29.45	7.84
Calcium (mg/dl)	8.15	9.88
	1.14	1.68

Cont ... **Table 2: Measures of metabolic markers in rheumatoid arthritis (RA) subjects.**

This study was carried out using 70 RA ($p<0.0001$) the demographic data for the patient are shown in Table3.

Biochemical investigations:

Table 3: Individuals demographic data

Biochemical Markers	Rheumatoid arthritis	RA control	P value
Age	46.32±11.9	46±13.76	<0.0001
Hb %	10.2±1.62	14.24±1.410	<0.0001
ESR (mm/hr)	79.04±20.72	23.2±1.22	<0.0001
RF	65%	-	<0.0001
CRP	8.67±14.28	0.58±0.33	<0.0001
Uric Acid (mg/dl)	7.036±1.047	3.98±0.68	<0.0001
DAS 28	2.18-8.29	4.21±1.51	<0.0001
GAS (0-100)	5-70	26.81±18.61	<0.0001

In this study we have seen age plays a crucial role among the RA patients as in the middle aged population (>30 years) have a higher level of CRP (p<0.0001). The BMI (p<0.0001) value gets decreased more in the middle-aged population. In other studies have seen that Disease Activity Score (DAS 28) and Global assessment score (GAS) (p<0.0001) is directly proportional with the duration of RA and also with the middle aged population it increases. Here we excluded all the patients who were under anti-rheumatic drug treatments. Biochemical investigations found that inflammatory markers i.e., Hb%, ESR, RF, CRP, Uric Acid have a correlation with age, lower level in Hb% (p<0.0001) among the middle aged population. Also found that ESR mm/hr (p<0.0001) gets increased (79.04+20.72) in rapid amount among the middle aged and old aged population. Other study include RF which was investigative found that 65% of RA patient is RF positive. CRP (p<0.0001) serum levels is much higher compared to the control subjects. The serum uric acid (p<0.0001) level is significantly increases in RA patient compared to control subjects shown in table 4. Matrix correlation for demographic data and inflammatory markers are shown in fig-1.

Figure 1: matrix correlation of demographic data and inflammatory markers

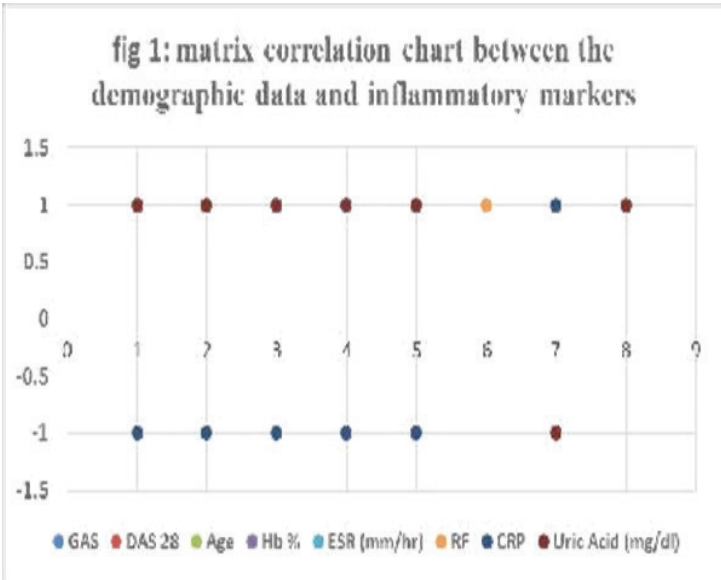

Table 4: Inflammatory markers of patients with rheumatoid arthritis (mean +SD)

Characteristic	Range	Mean ±SD
Age	27-48	47.53 ±11.90
Duration	0.8-17.5	4.01 ±3.86
BMI	17-30	22.69 ±2.79
CRP	0.99 -19.14	8.67 ±14.28
DAS 28	2.18 -8.29	4.21 ±1.51
GAS (0-100)	5-70	26.81 ±18.61

(p<0.0001) gets increased (79.04+20.72) in rapid

Diabetic marker that is glucose shows a positive correlation ($p<0.0001$) with Hb, Uric acid, GAS, DAS 28 (+1). As above mentioned data, the arthritic profile of patients by comparing all the biochemical profile we have seen a major correlation among the increase and decrease among the biochemical values with related to patient age, bmi and other factors. Some changes in the serum biochemical values varies in a particular fashion when the person is affected with arthritis rather than the control patients (subjects).The peripheral venous blood collected from patients with vacutainers with EDTA for serum collection and plasma collection using plasma sample collecting vacutainers. Dietary manipulation is a way for patients to assume some control over their own health and management of their disease. Patients should be able to expect advice from their doctors regarding dietary therapies for which there is a high level of evidence. Some parameters commonly measured in joint diseases together with others which have been claimed to represent better markers of disease activity in RA. It was not surprising that the group with RA had a high percentage of abnormal results for RF, CRP, Uric acid. Other biochemical marker include glucose levels, Cholesterol increases with age among patient suffering Arthritis.

Conclusion

The goal of statistical modelling is to summarize a test's results in such a way that, based on the samples we observed that demography variables like Age, BMI are highly correlated with the disease Rheumatoid Arthritis and also biochemical factors like glucose, uric acid shows significance with the disease present Rheumatoid arthritis plays a major concern role in the life of an affected individual, the lifestyle and livelihood is mostly traumatized and it affects the confidence level in a whole[10-12]. Definite workability in an individual is very much hampered and it affects their social life as well. RA it affects the major population nearly 25% of Indian population is affected by arthritis. To determine its severity have done clinical tests in the middle aged population[19]. The metabolic profiles in the population saw a huge changes according to their ages and severity is also seen and extremely drastic changes can be observed. After taking the correlation graph have seen a massive changes in the levels among control and arthritic patient. By going through all the sugar, Haemoglobin etc. other profiles have seen that according to age those metabolic profiles tends to change if the person has arthritis compared to control subjects. The patients suffering from arthritis have a particular pattern according to their respective ages and by understanding their profile we can clinically diagnose that patient and can determine the presence of arthritis in a patient in the early stages. The validity of these results could be questioned because individuals did not have disease of equal severity[5]. However, all patients had disease severe enough to warrant hospital attention and were selected by virtue of persistent symptoms. It was not possible to match the disease groups for age and sex because they characteristically affect different ages and have varying sex distributions. After going through all the tests and matching the correlation among all the metabolic profiles, we have seen that liver function is much correlated and matched with the arthritic profile compared with other metabolic function. Thus it can be used as a detector marker for Rheumatoid arthritis and can be used as an early diagnostic purpose for Rheumatoid Arthritis.

Ethical Clearance: Approval for the conduction of the study was obtained from the Institutional Ethics Committee of Vellore Institute of Technology

Source of Funding: Self

Conflict of Interest: Nil

References:

1. Romas E, Sims NA, Hards DK, Lindsay M, Quinn JW, Ryan PF, Osteoprotegerin reduces osteoclast numbers and prevents bone erosion in collagen-induced arthritis. Am J Pathol, 2012
2. Abu-Amer Y, Abbas S, Hirayama T. TNF receptor type Irregulates RANK ligand expression by stromal cells and modulates osteoclastogenesis. J Cell Biochem 2004; 93:980–9.
3. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, 3rdco, Rheumatoid arthritis classification criteria: an American College of Rheumatology/ European league against rheumatism collaborative initiative. Ann Rheum D, 2010, 69:1580–8.
4. El Meidany Y, El Gaafary M, Ahmed I, Cross-cultural adaptation and validation of an Arabic Health Assessment Questionnaire for use in rheumatoid arthritis patients. Joint Bone Spine, 2003, 70:195–202.

5. Aletaha D, Smolen JS. The Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI). A review of their usefulness and validity in rheumatoid arthritis. Clin ExpRheumatol 2005; 23(5 Suppl. 39):S100–8.
6. Scott DL, Wolfe F, Huizinga TW, Rheumatoid arthritis. Lancet 376, 2010, 1094–108.
7. Carmona L, Cross M, Williams B, Lassere M, March L Rheumatoid arthritis. Best Pract Res ClinRheumatol, 2010, 24, 733–45
8. Avina-ZubietaJA,ChoiHK,SadatsafaviM,Etminan M, Esdaile JM, Lacaille D, Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. Arthritis Rheum 59, 2008 1690–7.
9. Gonzalez A, Maradit Kremers H, Crowson CS et al. The widening mortality gap between rheumatoid arthritis patients and the general population. Arthritis Rheum 56, 2007, 3583–7.
10. Fatima F, Rao U, Moots R, Goodson N, Raised traditional cardiovascular risk factors in Indians with rheumatoid arthritis. Arthritis Rheum 60, 2009, (S10), 948.
11. Salminen E, Heikkila S, Poussa T, Lagstrom H, Saario R, Salminen S, et al. Female patients tend to alter their diet following the diagnosis of rheumatoid arthritis and breast cancer. Prev Med 2002; 34:529-35.
12. Vanderborght A, Linsen L, Thewissen M, Geusens P, Raus J. Stinissen P, Osteoprotegerin and receptor activator of nuclear factor kappa B ligand mRNA expression in patients with rheumatoid arthritis and healthy controls. J Rheumatol, 2004, 31(8):1483–90
13. Friedman SF, Martin P, Munoz JS, Laboratory evaluation of the patient with liver disease. Hepatology, a textbook of liver disease. Philadelphia; Saunders publication, 2003, 1: 661-709.
14. Government of India, Census. Government of India: Office of the Registrar General and Census Commissioner (Ministry of Home Affairs). Office of the Registrar General and Census Commissioner, 2011, New Delhi.
15. P Conaghan, P Bird, et.al, The EULAR–OMERACT rheumatoid arthritis MRI reference image atlas: the metacarpophalangeal joints, Annals of the Rheumatic Diseases (ARD), 2005, 64 (Suppl 1): i11–i2 doi: 10.1136/ard.2004.031815
16. Seibel MJ. Biochemical markers of bone turnover; part I: biochemistry and variability. Clin Bio-chem Rev. 2005, 26: 97-
17. Van staa TP, guesens P, Bijlsm JW, Leufkens HG, cooper C, Clinical assessment of the long term risk of fracture in patients with rheumatoid arthritis Rheum, 2006, 54: 3104-3112.
18. Manrique F, Gamardo J, de Elguezabal K, Abnormalities of bone mineral density and bone mineral density and bone metabolism in Venezuelan patients with rheumatoid arthritis. Clin-2003
19. Calvo MS, Eyre DR, Gundberg CM, Molecular basis and clinical application of biological markers of bone turnover. Endocr Rev., 1996, 17:333-368.