SURVIVIN, A PROGNOSTIC MARKER FOR RHEUMATOID ARTHRITIS - A PROSPECTIVE COHORT STUDY

Contributors

1. Arumugam Hemalatha,MDS Senior Lecturer - Tagore Dental College and Hospital
2. Gunasekaran Nandhini,MDS Reader - SRM dental college and hospital
3. Ajay Ashwini, MDS - SRM dental college and hospital
4. V.S Jayant, MDS - SRM dental college and hospital
5. L.S.Makesh Raj,MDS, Professor - Tagore Dental College and Hospital
6. Krishnan Rajkumar, MDS HOD and Professor - Tagore Dental College and Hospital

Department(s) and institution(s)
Department of Oral and Maxillofacial Pathology, SRM dental college and hospital
Corresponding Author:
Dr.A.Hemalatha
No.5, Nehru Street,
Kamaraj Nagar,
Chrompet,
Chennai – 600044
9789059908
E-mail – HEMA.333HEMU@GMAIL.COM

ABSTRACT

INTRODUCTION: Rheumatoid arthritis (RA) is a chronic inflammatory disorder of autoimmune origin that may affect many tissues and organs but principally attacks the joints. Analysis of pannus tissues in RA patients discloses characters of transmuted cells living for longer duration with, apoptotic tolerance, oncogenes expression and somatic mutations. Survivin belongs to the Inhibitory Apoptotic Protein family which is suggested to play a role in the pathogenesis of RA, and is recently emerging as a biomarker for Rheumatoid Arthritis.

AIMS AND OBJECTIVES: The objectives of this study were to estimate and compare the salivary survivin concentration with serum survivin concentration and to correlate with the treatment.

METHODS: 30 newly diagnosed Rheumatoid Arthritis patients who were undergoing same type of therapy were enrolled for the study. Saliva and Serum samples were collected before

the treatment; three months and six months after treatment and Human survivin levels were estimated using ELISA.

RESULTS: The findings of the current study showed a significant difference in survivin concentrations with respect to the treatment and it was more in RA patients before treatment and it showed gradual reduction in serum and salivary survivin levels after 3 months and 6 months in response to the treatment. Survivin levels were detected in higher levels in serum. There was a poor positive correlation between salivary and serum Survivin levels.

CONCLUSION: From the current study, salivary survivin levels can be used as an adjuvant non-invasive prognostic tool in Rheumatoid Arthritis patients. Further studies with increased sample size might prove its reliability.

KEYWORDS: Rheumatoid Arthritis, Survivin, Serum, Saliva

INTRODUCTION:

"Rheumatoid arthritis (RA) is a chronic inflammatory disorder of autoimmune origin that may affect many tissues and organs but principally attacks the joints, producing a non-suppurative proliferative and inflammatory synovitis".[1] RA is an inflammatory disease chronic in nature with lining inflammation involving, T and B lymphocytes, fibroblasts, dendritic cells, macrophages and chondrocytes which subsequently cause destruction of the adjacent bone and cartilage.[2] Pain and stiffness in many joints are the typical presentation in RA. Systemic symptoms such as low-grade fever, weight loss, and fatigue occur in accordance with the active disease.[3]

Apoptosis in general is a physiologic process mediating the death of selected cells. Necrosis occurs due to toxic or non-specific cell injury, whereas apoptosis is a process initiated by ligand-receptor interactions which are more regulated and coupled to the

phagocytosis of apoptotic cells.[4] In RA, the proliferation of synovial fibroblasts and their invasive growth are due to impairment in cell cycle regulation. Analysis of pannus tissues in RA patients discloses characters of transmuted cells living for longer duration with, apoptotic tolerance, oncogenes expression and somatic mutations. Numerous conflicts through apoptosis mechanism were noticed in RA patients. The apoptosis signals are abrogated by the family of Inhibitory Apoptotic Proteins(IAPs).[5]

Survivin is one of such protein which is the smallest member of IAP protein family. Survivin is a mammalian IAP which is defined as proficient of regulating both cell production and cell death by apoptosis by inhibiting the activity of caspase 3, caspase 7 and caspase 9. Survivin can thereby down regulate, directly or indirectly, both death-receptor-mediated and mitochondria-mediated pathways of apoptosis. It has also been reported to be associated with erosiveness of the rheumatoid arthritis. The serum and the synovial survivin levels were increased in RA patients, which are related with early synovial joint impairment and reduced prognosis to the therapy. Also, survivin transforms the synovial fibroblasts to their invasive form which causes synovial tissue proliferation. Studies have found that survivin has a major role in the pathogenesis of RA.[6] Till date many bio markers have been estimated from many of the body fluids. The blood and saliva are being widely studied for estimation of many of the bio markers. In RA, the patients will be suffering from severe pain due to the disease even though collection of blood is less invasive, it contribute to certain level of pain in RA patients. In an attempt to avoid this we are trying to determine whether saliva can be used as a diagnostic and prognostic tool in RA. Hence this study uses survivin to find out whether it can serve as an effective diagnostic and prognostic marker in RA patients by evaluating the serum and salivary survivin levels before treatment, 3 months after treatment and 6 months after treatment.

AIMS AND OBLECTIVES:

The objectives of this study were to estimate and compare the salivary survivin concentration with serum survivin concentration, to correlate with the treatment and also find the correlation between serum and salivary survivin levels.

METHODOLOGY:

The study was approved by Ethical committee and Institutional review board of SRM Dental College, SRM institute of science and technology, Chennai. The present study comprised a total of 30 patients according to the inclusion and exclusion criteria and according to 2010 American College Rheumatology/ European League Against Rheumatism classification criteria for Rheumatoid Arthritis. Salivary and serum samples were obtained from all the patients before treatment, 3 months and 6 months after treatment.

Method of Data Collection:

The study was conducted in the time period of two years. Informed consent was obtained from all the individuals included in the study. Convenience sampling technique was used for selection of the participants. Full case histories, both written and verbal consent were acquired from all patients and enrolled in the study. After complete oral examination was done; the salivary flow rate, TMJ status and periodontal health status were recorded. Salivary and serum samples were obtained from all the patients before treatment, 3 months and 6 months after treatment.

Inclusion criteria:

This study included patients who were newly diagnosed Rheumatoid Arthritis cases before treatment. No age, sex or geographical location was used as limitation for inclusion for a patient into the study. Treatment according to American College of Rheumatology was also an important inclusion criteria for bring enrolled into the study.

Exclusion criteria:

Patients with other underlying systemic diseases and who are under any other form of treatment for RA are not included in the study. Patients whose treatment was changed during the follow-up period were also excluded from the study.

Sample collection and processing:

Serum

After explaining the purpose of the study and obtaining consent, blood sample (2.0ml) was collected by venepuncture from all the participants. The samples were collected in a non – coated 10ml threaded centrifuge tube. The sample was allowed to clot in a slant at room temperature for about 30 minutes. The blood was centrifuged at 3000 rotations per minute for 15 minutes at a temperature of 4°c and the serum was separated, immediately aliquoted and stored at a temperature of - 80°C freezer for further analysis using ELISA.

Saliva

Whole unstimulated saliva sample was used for the study. The patients were instructed to wash the mouth using water and whole unstimulated saliva was collected. Each individual expectorated 1 ml of saliva without any stimulatory conditions in a silent room into a sterile uri-cup. Samples were collected by requesting the patients to swallow the saliva first, incline their head frontward and expectorate the saliva into the uri-cup for duration of 10 minutes without swallowing. After collection, the sample was transferred into a 10ml threaded centrifuge tube and centrifugation was done immediately in a cooling centrifuge at 3000 rotations per minute for duration of 15 minutes at a temperature of 4°C to eliminate cell debris and squamous cells. Then, the resulting supernatant was separated and aliquoted and

stored at a temperature of - 80°C freezer for further analysis using Enzyme Linked Immuno-Sorbent Assay (ELISA).

Method

Human Survivin Elisa Kit Cat.No.E1612Hu, (Bioassay Technology Laboratory, India) was used for the analysis. Samples are added to the wells which were pre-coated with monoclonal antibody for SURVIVIN. (Fig1A) After incubation a "biotin-conjugated anti-human SURVIVIN antibody" is added which will bind to SURVIVIN present in the samples. After incubation the unbound "biotin-conjugated anti-human SURVIVIN antibody" is washed away during the washing step. Streptavidin-HRP was added which bind to the "biotin-conjugated anti-human SURVIVIN antibody". After incubation, in the second washing step the unbound Streptavidin-HRP is washed away. On adding the Substrate solution a colour change occurs according to the amount of human SURVIVIN in the sample. (Fig 1B) The reaction is finished by addition of acidic stop solution (Fig 1C) and the optical density is determined by measuring the absorbance at 450 nm.

Statistical Analysis:

Statistical analysis was done with SPSS (version-16) software for the obtained data. The descriptive statistics such as mean and standard deviations [SD] were calculated for the individual intervals. The comparison of salivary and serum Survivin levels prior to treatment, 3 and 6 months after treatment was done using Repeated measures ANOVA. The comparison between serum and salivary Survivin levels was done using Pearson's correlation test.

RESULTS:

The present study included a total of 30 patients, out of which 23 females and 7 males with an age range of 27 - 73 years and a mean age of 49.8± 11.41. Females were the most

commonly affected gender and the patients who were under the age group of 40 to 60 years were commonly involved. The mean serum survivin level before treatment was **153.67±10.9 ng/L**, **129.33±11.89 ng/L** 3 months after treatment and **95.20± 14.94ng/L** 6 months after treatment. (Table 1) The mean values showed a gradual decrease in values of survivin concentration from before treatment to 6 months after treatment. On comparison, the difference between the mean serum survivin levels with respect to the treatment was significant statistically. (P= 0.0001). The mean salivary survivin level before treatment was **127.15±16.67 ng/L**, **91.56±19.65 ng/L** 3 months after treatment and **49.51± 17.49 ng/L** 6 months after treatment. The mean values showed a gradual decrease in values of survivin concentration from before treatment to 6 months after treatment. On comparison, the difference between the mean salivary survivin levels with respect to the treatment was also significant statistically. (P= 0.0001). The correlation between salivary and serum survivin levels at all the three instances were weakly correlated and was not significant (p> 0.05). (Fig 2, Table 2)

DISCUSSION:

The term Arthritis means “inflammation of the joints” which may be caused due to variety of causes. One of the most common causes is the autoimmune reaction in which the antibodies are targeted towards the normal lining of the synovial joints leading to a disease named, Rheumatoid Arthritis. The triggering factors of RA cumulatively result in impairment of the apoptosis. Apoptosis mechanism in the RA patients shows many disturbances and inhibition of apoptosis has a vital role in the progression of experimental arthritis. Survivin is is an anti-apoptotic protein involved in the severity of the RA and is expressed in higher amounts with respect to the severity. Previous studies have shown high titres of serum and synovial Survivin levels in RA patients in comparison with control subjects without any disease.

Developing foetus express high levels of survivin whereas very minimal levels are expressed among normal adult tissues. Conversely, survivin was overexpressed in many malignancies. Survivin has been already proposed as a Biomarker in various diseases and disorders such as OSCC, PMODs[7,8], Salivary gland malignancies [9], Leukaemia, malignancies of internal organs including lung, brain, liver, pancreas, gastrointestinal tract, uterus, prostate and also in inflammatory conditions like Rheumatoid Arthritis and Multiple sclerosis.[10]

The current research was done to study the relationship between serum and salivary survivin levels before and after treatment in which 30 newly diagnosed RA patients were enrolled.

The mean values showed a gradual decrease in values of survivin concentration in serum from before treatment to 6 months after treatment. This result was in accordance with the study done by **Adrian Levitsky et al** who found anti-rheumatic treatment resulted in an overall decrease of serum survivin levels.[11] This result was also in accordance with the study done by **Mahmoud M. Mahfouz, et al.** who found survivin was negatively correlated with all drugs taken and was significantly correlated with Methotrexate.[12]

The levels of survivin in RA are influenced mainly by the treatment taken. DMARDs are the common drugs chosen to treat RA and among them Methotrexate is the most commonly used drug. Studies have shown that more than any other drug Methotrexate has higher influence on Survivin levels denoting the prognosis of the disease.[12]

Although serum is the most commonly used biological fluid for diagnostic purposes, it has been widely accepted that saliva also could be used as a non-invasive potential diagnostic tool for such investigations due to its distinctive advantages over other body fluids. Studies have shown Serum Survivin levels to be more in RA when compared to controls, but its level in saliva is still not known. In an attempt to find the salivary concentrations of

survivin in RA patients our study has been conducted. According to our knowledge no clinical studies were done to analyse the salivary survivin concentration in RA patients and our study is the first of its kind.

The mean values showed a gradual decrease in values of survivin concentration in saliva from before treatment to 6 months after treatment which was similar to the serum concentrations. This shows that Salivary Survivin concentrations can be used as an **Effective non-invasive Prognostic tool** as an alternative to serum in RA patients.

Even though there was no statistical significant correlation in between serum and salivary concentration of survivin prior to treatment, 3 and 6 months after treatment; but the gradual decrease in the concentrations of Survivin was similar between serum and saliva.

CONCLUSION:

Even though there was no statistical significant correlation in between serum and salivary concentration of survivin prior to treatment, 3 and 6 months after treatment; but the gradual decrease in the concentrations of Survivin was similar between serum and saliva with which it can be stated that Salivary Survivin could be an Effective non-invasive Prognostic tool in Rheumatoid Arthritis patients. Further Studies with increased sample size might prove its reliability.

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Figure1: (A) SAMPLES INTO THE ELISA PLATE;

(B) COLOUR CHANGE AFTER ONE HOUR INCUBATION;

(C) COLOUR CHANGE AFTER ADDING STOP SOLUTION

Figure 2: COMPARISON OF MEAN SURVIVIN CONCENTRATION IN SERUM AND SALIVA PRIOR TO TREATMENT, 3 AND 6 MONTHS AFTER TREATMENT

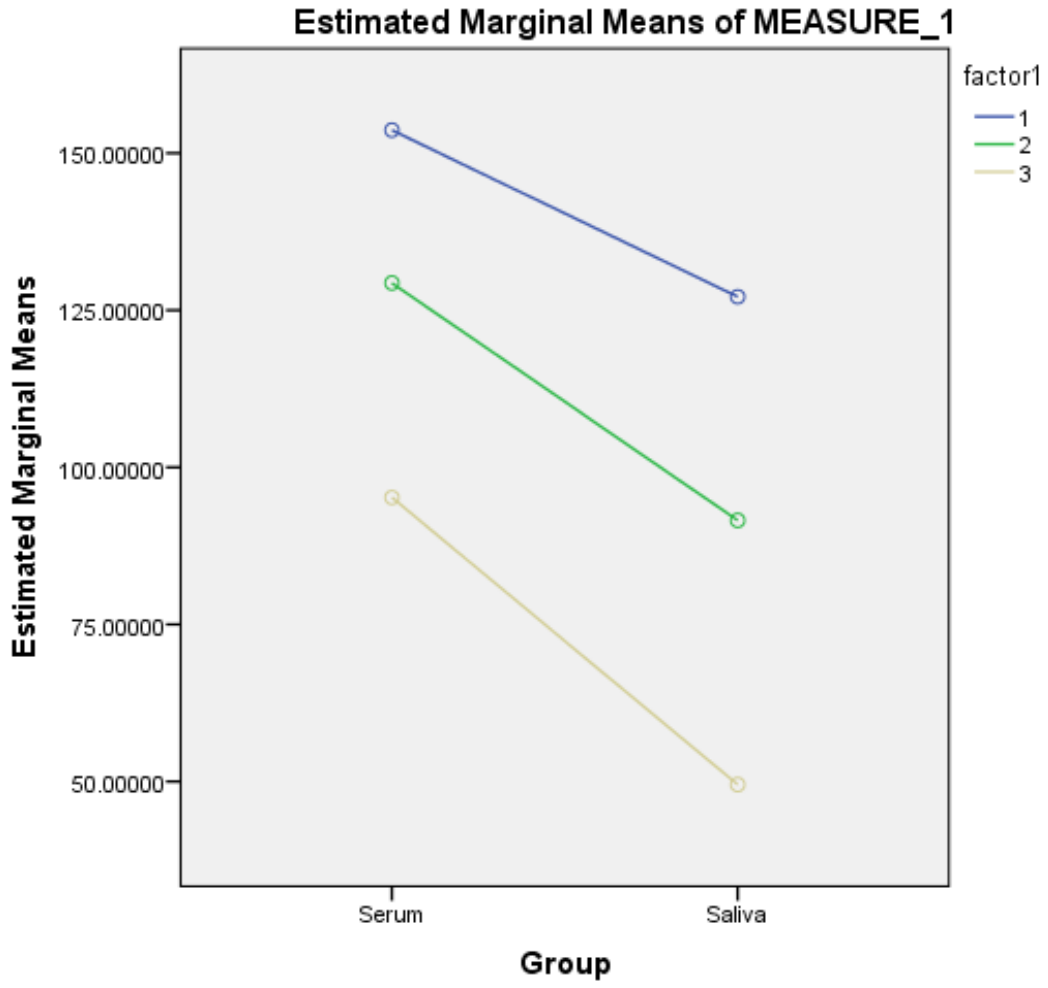

TABLE1: COMPARISON OF SURVIVIN IN SERUM AND SURVIVIN PRIOR TO TREATMENT, 3 AND 6 MONTHS AFTER TREATMENT

	Interval	N*	Mean	Standard Deviation	p Value
Serum	Before treatment	30	153.667	10.895	0.0001
	3 months after treatment	30	129.333	11.885	
	6 months after treatment	30	95.203	14.931	
Saliva	Before treatment	30	127.154	16.672	0.0001
	3 months after treatment	30	91.561	19.652	
	6 months after treatment	30	49.512	17.491	

*N – Sample Size

TABLE2: PEARSON'S CORRELATION BETWEEN SURVIVIN IN SERUM & SALIVA BEFORE TREATMENT, 3 AND 6 MONTHS AFTER TREATMENT

Sample collection Interval	Sample Type	N*	Mean	Standard deviation	Pearson's Correlation Sig. (2 - tailed)	p Value
Before Treatment	**Serum**	30	153.6666667	10.89550871	-0.050	0.794
	Saliva	30	127.1544707	16.67163704	-0.050	
3 Months Treatment	**Serum**	30	129.3333333	10.89550871	0.112	0.556
	Saliva	30	91.5609750	19.65252287	0.112	
6 Months Treatment	**Serum**	30	95.2032520	14.93073897	-0.027	0. .886
	Saliva	30	49.5121947	17.49145229	-0.027	

*N – Sample Size