Exploring Role of SMAD3 in Serum of Rheumatoid Arthritis Patients

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ABSTRACT
Background: Being an autoimmune and inflammatory illness, rheumatoid arthritis results from the body's immune system attacking healthy cells by mistake, creating inflammation in the affected areas. **Aim of study:** The purpose of this study was to use ELISA to assess the serum concentration level of SMAD3 in RA patients and control groups.. **Methodology:** The research was conducted between October 15, 2023, and January 15, 2024.The study, conducted at the Medical City Hospital under the Baghdad Government, involved male and female participants ranging in age from 18 to over 75. All participants in the trial provided a total of 90 clinical specimens (5 ml of blood): 45 patients and 45 controls. To use ELISA (Enzyme Linked Immune Sorbent Assay) to examine the immunological parameter Smad3, blood was taken from the patients. **Results:** There were statistically significant variations in the means of the concentration levels of the parameter Smad3 in RA patients (12.11±4.91) and controls 1.89 (0.98_4). Expression of Smad3 (ng/ml): For RA patients, the male median was 11.21±5.09 (range: 7.33–15.83), whereas the female median was 12.61±4.83 (range: 8.32–16.29). In Stage 1, Smad3 achieved 7.26±2.75, in Stage 2, 10.25±1.46, in Stage 3, 12.3±4.04, and in Stage 4, 18.97±1.72. **Conclusion:** There were statistically significant variations between the means of the concentration levels of the parameter Smad3 in RA patients and controls. There may be a slight correlation between RA stages and biomarker levels (Smad3), however this relationship is not statistically significant, and the effects are small.

1. Introduction

The common systemic inflammatory disease known as rheumatoid arthritis (RA) is characterized by symptoms, persistent joint inflammation, and the degeneration of bone and cartilage [1]. There is general consensus that FLS play a major role in the degenerative processes of RA. Fibroblast-like synoviocytes. Hyperproliferation, resistance to apoptosis, increased invasiveness, and the generation of inflammatory mediators are among the aggressive features displayed by these RA-FLS [2]. While it's widely acknowledged that the interplay of (environmental, immunological, and genetic factors) contributes to RA development, the precise molecular mechanisms underlying the behavior alterations in RA-FLS remain incompletely understood [3]. Rheumatoid arthritis predominantly affects the joint lining, causing painful swelling that may eventually lead to joint deformity and bone erosion, in contrast to the degenerative damage observed in osteoarthritis [4]. Furthermore, the inflammatory process associated with rheumatoid arthritis can cause damage to other body parts [5]. Rheumatoid arthritis (RA) is one of the chronic autoimmune diseases that is influenced by both genetic and environmental factors. Genetic links suggest the involvement of CD4+ T cells, which express a high number of genes discovered within loci connected to RA, particularly those containing common epitope alleles of the human leucocyte antigen (HLA) [6]. “Polymorphisms in T cell-expressed genes”, including transcription factors, have been identified as risk factors not only for RA but also for other autoimmune diseases [7]. Smad3, a member of the SMAD family, is important for T cell activation and the generation of inflammatory cytokines, according to recent studies.Smad3, a transcription factor that may collaborate with other transcription factors at composite sites within gene promoters, mediates the transcriptional control of gene expression [8]. For articular cartilage to remain intact, the Smad3 signaling pathway prevents the terminal hypertrophic differentiation of chondrocytes and regulates the synthesis of matrix components [9]. Despite its known importance in T lymphocyte transcriptional regulation, the role of “Smad3 gene polymorphisms” in autoimmune diseases like RA remains poorly understood [10]. Therefore, this study was conducted to evaluation the serum concentration level of SMAD3 for RA patients and control groups by ELISA.

2. Materials and Methods

Inclusion Criteria to 45 (case) Patients Diagnosed by Specialist Physicians is Rheumatoid Arthritis.

The study includes both male and female patients and age of patients between 18-75 years suffered from morning stiffness (at least one hour), arthritis in more than three joints' areas (swollen), and confirmed diagnosis of RA by physician specialist and.

Exclusion Criteria Sample were Excluded based on the following criteria.

This study excluded the patients aged less than 18 years or more than 75 years, also excluded the patients suffered from diabetic mellitus, proteinuria, pregnancy females.

Patients Group

A case control study has been conducted from 15 October 2023 to 15 of January 2024. This study included 45 adult females and male which diagnosed with RA disease by specialist physician, and their ages ranges between 18 to 75 years. Blood was drawn from the patients to investigate the immunological parameter Smad3 by ELISA (Enzyme linked Immune Sorbent Assay).

Control Group

The control group were 45 healthy subject's Iraqi people. The control group was used only for comparing parameters**.** The control samples were approximately similar with the patient samples in terms of number, age ratio and the place of living also urban and rural.

Blood Collection

The blood samples (5ml) withdrawn in this study From each patient diagnosed with RA patients and controls. The patient's hand was sterilized three times using 70% alcohol, and the venipuncture site was then cleansed with 2% iodine. Approximately 5 ml of venous blood was drawn, and the blood samples were put in a Gel tube for serological study. Sera for patients and control groups collected in screw-capped test tubes after centrifugation at 3500 rpms for up to 15 minutes. Sera were transferred into plastic eppendorf and stored on a deep freezer at (-20 C°) to determined Samad3 by ELISA system.

Determination of Smad3 by ELISA

ELISA technology was used to measure the immunological parameter Smad3 according to the instructions and recommendations attached to the kit by the manufacturer using a Human Smad3 ELISA kit from the Chinese company Melsin, Code Number: ED14460.

3. Result and Discussion

Age-dependent comparison of Smad3 in RA patients and controls

Based on the information presented in Table 1, it seemed that patients 12.11±4.91 and controls 1.89(0.98_4) had statistically significant differences in the means of the concentration levels of the parameter Smad3.

Table (1): Age-dependent comparison of Smad3 in RA patients and controls

Variables	RA patients n=45		Healthy control n=45		p-value
	Mean ±SD	Median (IQR)	Mean ±SD	Median (IQR)	
Age (year)	52.22±9.93	51(43.5_60)	51.44±10.69	51(42.5_61.5)	0.721 NS
Smad3 (ng/ml)	12.11±4.91	10.71(8.32_15.7)	2.47±1.75	1.89(0.98_4)	0.0001**

Significant differences at p-value * <0.05, ** <0.01. NS: non-significant. SD: standard division. IQR: inter quarter range.

Effects of gender on clinical biomarker SMAD3 in RA patients as compared with Healthy controls.

Based on Table 2, it appears that the data for Gender and Smad3 expression among male and female individuals for RA patients and Healthy controls are as follows:

Gender:

For RA patients: male: 51.63±8.19 (median: 51, range: 42.75-58.75) while for female: 52.55±10.9 (median: 52, range: 43.5-60.5). For Healthy controls: for male: 49.4±10.16 (median: 48, range: 41-59.5), while for female: 54±11.04 (median: 58, range: 45-63). Smad3 expression (ng/ml): For RA patients: in male reached: 11.21±5.09 (median: 10.65, range: 7.33-15.83) and in female reached: 12.61±4.83 (median: 11.43, range: 8.32-16.29). while for Healthy controls: in male reached: 2.2±1.68 (median: 1.4, range: 0.9-3.85) and in female reached: 2.82±1.82 (median: 2.93, range: 1.05-4.32). The p-values indicate the level of significance for the differences observed between male and female individuals within each variable. In this case, most p-values are not significant (NS). SMAD, is able to mediate the several ways that TGF-β regulates cells by transferring the signal from the cell membrane into the nucleus. Studies have demonstrated that individuals with SMAD3 gene knockouts would exhibit RA symptoms and have noticeably greater serum Smad3 concentration than controls. SMAD3 is a receptor-activated SMAD [11].

Mothers against decapentaplegic homolog 3, or Sma and Mad homologue 3, is a member of the SMAD family. The SMAD3 gene, which has nine exons and eight introns, is found on chromosome 15q21–22 [12]. The aberrant expression of the SMAD3 gene may be one of the hereditary variables impacting RA risk since SMAD3 is the most essential mediator in transforming growth factor-β (TGF-β)/Smad signal transduction pathway and TGF-β plays a key role in the onset and progression of RA [13]. Aref Eshghi et al. [14] referred to that in RA patients, SMAD3 expression was greater in females than in males. This agreed with our results. While Aref-Eshghi [13] mentioned that, compared to men, women with rehumatoid arthritis expressed less SMAD3. This contradicts our results.

Table (2): Effects of gender on clinical biomarkers in RA patients as compared with Healthy controls.

	Gender	RA patients n=45	Healthy control n=45	Univariate p-value
Age (year)	Male	51.63±8.19 51(42.75_58.75)	49.4±10.16 48(41_59.5) [a]	0.862 NS
	Female	52.55±10.9 52(43.5_60.5) [a]	54±11.04 58(45_63) [a]	
Smad3 (ng/ml)	Male	11.21±5.09 10.65(7.33_15.83) [a]	2.2±1.68 1.4(0.9_3.85) [a]	0.0001**
	Female	12.61±4.83 11.43(8.32_16.29) [a]	2.82±1.82 2.93(1.05_4.32) [a]	
p-value		0.367 [NS]	0.239 [NS]	

Significant differences at p-value * <0.05, ** <0.01. NS: non-significant. SD: standard division. a: **Median (IQR)** ***inter quarter range.***

Comparison for Smad3 Expression, and Age in Various Stages of Rheumatoid Arthritis

The mean values for age and Smad3 expression at various stages of RA are showed in Table (3). The data shows the means and standard deviations for every variable for each of the four RA stages (stage 1, stage2, stage3 and stage 4), as well as the associated p-values for statistical significance.

There are four stages of rheumatoid arthritis, and each stage requires different treatment options [15]: Stage 1 or called early stage: The joint lining or synovial membrane becomes inflamed; but the bones remain healthy. However, expansion of the surrounding tissue often occurs, and this leads to stiffness and pain in the joint. Stage 2, or called intermediate: Inflammation damages the cartilage, which is the elastic material that protects the ends of the bones, and the inability to move the joint due to stiffness and thus loss of range of motion.

Stage 3 or called Serious stage:

Inflammation causes erosion of the cartilage and bones surrounding the joints. The joints may become unstable, the bones begin to shift, and deformities become apparent. Consequently, the patient feels edema and discomfort and thus loses the ability to move. Stage 4 or called the end stage The damage continues even after the inflammation stops. At this stage there is a possibility that the joint will break and the patient will not be able to move, because the joint will be hard, painful and swollen as well. The muscles may be weak and therefore the patient may need to have the joint replaced.

According to following our results, the means for each variable at various RA stages:

Age (40.2±3.97), Smad3 (7.26±2.75) for Stage 1.

Age (47.75±5.43), Smad3 (10.25±1.46) for Stage 2.

Age (56.08±4.46), Smad3 (12.3±4.04) for Stage 3.

Age (64.6±4.99), Smad3 (18.97±1.72) for Stage 4.

Furthermore, at the 0.0001 level of significance, all of the p-values related to the variations in means between the phases are significant. Based on this information, we propose that the various phases of RA exhibit notable variations in age, and Smad3 expression. Age, and Smad3 expression are seen to grow as the stages go on, suggesting possible links between these factors and the development of the RA illness. SMAD3 is an important mediator in the transforming growth factor b (TGF-β) signaling pathway, which is critical for cartilage regeneration and joint homeostasis. However, mutations/SNPs that cause dysregulation of the TGF-β pathway may also have an impact on SMAD3 gene expression. The Arg287Trp mutation renders the protein unable to form SMAD3 homo- or heterodimers with SMAD4, thereby attenuating TGF-β signaling. This may lead to a greater reduction in functional SMAD3, leading to more severe disease manifestations. The possibility that SMAD3 and STAT3 control SMAD3 function raises the possibility that the outcome of their cooperation may be influenced by one or more unidentified elements [16]. According to Sardana et al. [17], SMAD3 may be the most diagnostically capable of classifying individuals with rheumatoid arthritis into its four stages.

Table (3): Age, and Smad3 Expression Comparison at Various RA Stages

Stages of RA	Mean ± SD	
	Age (year)	Smad3 (ng/ml)
Stage 1	40.2±3.97 A	7.26±2.75 A
Stage 2	47.75±5.43 A	10.25±1.46 AB
Stage 3	56.08±4.46 B	12.3±4.04 B
Stage 4	64.6±4.99 C	18.97±1.72 C
p-value	0.0001**	0.0001**

Different letters Significant differences at p-value * <0.05, ** <0.01. between RA stages. SD: standard division.

Smad3 Level Regression Analysis by Age in RA Patients and Healthy Controls

Smad3 (ng/ml) is the dependent variable and Age (year) is the independent variable of interest in this regression model (Table 4). The model looks at the correlation between Smad3 levels and age in both healthy controls and RA patients. The results was as following

Regarding Rheumatoid Arthritis sufferers' patients: With a p-value of 0.0001, the constant term (effect size), which is -9.387, is statistically significant. The constant's 95% confidence interval is between -13.865 and -4.909. With a p-value of 0.0001, the Age (year) coefficient estimate is 0.412, indicating statistically significance. The coefficient's 95% confidence interval falls between 0.327 and 0.496. This implies that Smad3 levels and age have a positive and substantial correlation for RA patients, meaning that as age rises, when Smad3 levels rises.

Regarding the healthy controls is: With a p-value of 0.0001, the constant term (effect size) is -3.912. This result is statistically significant. For the constant, the 95% confidence interval spans -5.639 to -2.185. With a p-value of 0.0001, the Age (year) coefficient estimate is 0.124 and statistically significant. The coefficient's 95% confidence interval falls between 0.091 and 0.157. These results indicate that Smad3 levels increase as people get older, meaning that Smad3 levels and age have a positive and significant association with healthy controls as well. The findings therefore show that Smad3 levels in both patients with Rheumatoid Arthritis and controls subjects are substantially correlated with age. The positive correlation implies that, in both groups, older people typically have higher levels of Smad3 than younger people. This discovery emphasizes the possible influence of aging on Smad3 levels and might have consequences for comprehending age-related modifications in disease processes or treatment responsiveness.

Table (4): Smad3 Level Regression Analysis by Age in Rheumatoid Arthritis Patients and Healthy Controls

Model [a] Dependent Variable: Smad3 (ng/ml)		**B**	**Std. Error**	**t**	**Sig.**	**95.0% CI**
RA patients	(Constant)	-9.387	2.220	-4.228	0.0001	-13.865 to -4.909
	Age (year)	0.412**	0.042	9.854	0.0001	0.327 to 0.496
Healthy control	(Constant)	-3.912	0.856	-4.569	0.0001	-5.639 to -2.185
	Age (year)	0.124**	0.016	7.614	0.0001	0.091 to 0.157

Multiple Nominal Test Regression equivalent to assess the magnitude change of RA stages on SMAD3 Marker.

Assessment of multiple nominal regression for Rheumatoid Arthritis, it means that estimate the effect size of Rheumatoid Arthritis stages on Smad3 levels in RA patients. The results that emerge from using the multinomial regression analysis in order to compare biomarker Smad3 of Rheumatoid Arthritis (RA) of various phases are illustrated in table number 5. The equivalent coefficient estimates for Stages 2, 3, and 4 are 0.039, 0.058, and 0.070, respectively. P-values greater than 0.05 indicate that these coefficients are not statistically significant. For Stages 2, 3, and 4, the chances ratios vary from 1.04 to 1.072, which indicates a little increase in likelihood for each unit rise in RA stage, although they are not statistically significant. Therefore, the current study's findings imply that RA stages and biomarker levels (Smad3) may be somewhat related, although the effects are negligible and not statistically significant in this analysis. When evaluating these data, it's critical to take the clinical relevance and other aspects into account.

Table (5): Regression of Multi-Nominal Test to estimate the impact size of RA stages on biomarker SMAD3.

Biomarkers	Stage of RA [a]	B	p-value	OR	95% CI	
					Lower Bound	Upper Bound
Smad3 (ng/ml)	Stage 2	0.039	0.334	1.04	0.96	1.126
	Stage 3	0.058	0.136	1.06	0.982	1.143
	Stage 4	0.070	0.065	1.072	0.996	1.155

a. The reference category is: Stage 1. B: effect size. OR: odds ratio. CI: Confidence Interval

4. Conclusions

Patients and controls had statistically significant differences in the means of the concentration levels of the parameter Smad3. The various phases of RA exhibit notable variations in age, and Smad3 expression. Age, and Smad3 expression are seen to grow as the stages go on, suggesting possible links between these factors and the development of the RA illness. Smad3 levels and age have a positive and substantial correlation for RA patients, meaning that as age rises, when Smad3 levels rises. RA stages and biomarker levels (Smad3) may be somewhat related, although the effects are negligible and not statistically significant in this analysis. When evaluating these data, it's critical to take the clinical relevance and other aspects into account.

5. Ethical Standards

The Department of Medical Laboratories/ College of Health and Medical Technologies in Kufa, Baghdad Health Department/Russafa, and the Training and Development Center all provided their approval for the current study. Additionally, all participants in both groups provided their informed written consent.

6. Finance

The research was funded by self-efforts

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