

## Is 4-Hydroxynonenal a Predictive Parameter for the Development of Joint Erosion in Patients With Rheumatoid Arthritis?

Gürkan AKGÖL,<sup>1</sup> Hasan ULUSOY,<sup>2</sup> Selda TELO,<sup>3</sup> Arif GÜLKESEN,<sup>1</sup>  
Tülay YILDIRIM,<sup>4</sup> Ahmet Kürşad POYRAZ,<sup>5</sup> Arzu KAYA<sup>1</sup>

<sup>1</sup>Department of Physical Medicine and Rehabilitation, Medical Faculty of Firat University, Elazığ, Turkey

<sup>2</sup>Department of Rheumatology, Medicana International Samsun Hospital, Samsun, Turkey

<sup>3</sup>Department of Biochemistry and Clinical Biochemistry, Medical Faculty of Firat University, Elazığ, Turkey

<sup>4</sup>Department of Physical Medicine and Rehabilitation, Medical Faculty of İnonü University, Malatya, Turkey

<sup>5</sup>Department of Radiology, Medical Faculty of Firat University, Elazığ, Turkey

### ABSTRACT

**Objectives:** This study aims to evaluate serum 4-hydroxynonenal (4-HNE) levels and its clinical and radiological significance in patients with rheumatoid arthritis (RA).

**Patients and methods:** The study included 40 patients (8 males, 32 females; mean age 51.4±11.2 years; range 24 to 72 years) with RA and 30 healthy controls (8 males, 32 females; mean age 53.0±11.7 years; range 24 to 72 years). Serum 4-HNE levels were measured using sandwich enzyme-linked immunosorbent assay method. Patients with disease activity score 28 ≤3.2 and >3.2 were allocated into low and high/moderate disease activity groups, respectively. Additionally, patients were divided into two groups as early RA (disease duration ≤2 years) and established RA (disease duration ≥2 years). Functional disability was evaluated using health assessment questionnaire. Radiographs were scored using the modified Larsen scoring.

**Results:** Serum 4-HNE levels in patients with RA were significantly higher than controls (p=0.001). Serum 4-HNE levels did not correlate with laboratory or clinical parameters of disease activity including erythrocyte sedimentation rate, C-reactive protein, disease activity score 28, and health assessment questionnaire. Serum 4-HNE levels were higher in patients with established RA than patients with early RA (r=0.487, p=0.001). Besides, modified Larsen score which indicates structural damage correlated significantly with serum 4-HNE levels (p=0.001).

**Conclusion:** These results indicate that serum 4-HNE levels may be used as an indicator for structural damage such as erosions in the early stage of RA; however, they are not efficient to monitor disease activity.

**Keywords:** 4-hydroxynonenal; disease activity; modified Larsen score; rheumatoid arthritis.

Rheumatoid arthritis (RA) is a chronic, autoimmune multisystem disease with unknown etiology which affects nearly 1% to 2% of the world population. RA is characterized by chronic inflammation of the synovial membranes of the joints. Pain and swelling of hand joints are generally the first manifestations of the disease. Activated inflammatory mediators infiltrate synovial membranes and exert deleterious effects on bone and cartilage.<sup>1</sup> In recent years, numerous studies have investigated the probable role of

reactive oxygen species (ROS) in the etiology and pathogenesis of RA.<sup>2,3</sup> ROS involve apparently in the pathogenesis of various diseases including RA.<sup>4</sup> ROS are considered as the mediators of tissue damage in patients with RA.<sup>5</sup> Numerous activated neutrophils which produce ROS accumulate in the synovial fluid of inflamed rheumatoid joint.<sup>4</sup> Normally, reactive oxygen radicals are produced in all cells and tissues as a result of relatively low-levels of oxidative process. Under normal conditions, ROS production is controlled

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**Correspondence:** Gürkan Akgöl, MD. Firat Üniversitesi Tıp Fakültesi Fiziksel Tıp ve Rehabilitasyon Anabilim Dalı, 23119 Elazığ, Turkey.

Tel: +90 424 - 233 35 55 / 2032 e-mail: drgurkanakgol@gmail.com

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by various antioxidant mechanisms. However, excessive production of ROS and/or inadequate elimination from the environment leads to oxidative stress which may cause severe metabolic functional disorders and damage of the biological macromolecules.<sup>6</sup> Besides, decomposition of peroxidized lipid may induce various end-products containing 4-hydroxynonenal (4-HNE). Increased levels of 4-HNE have been demonstrated in serum (or plasma) and synovial fluid of patients with RA.<sup>7,8</sup>

The 4-HNE is one of the aldehydes specific to lipid peroxidation. 4-HNE is believed to be predominantly responsible from the cytopathologic effects seen during oxidative stress.<sup>9,10</sup> Any factor compatible with stress or activity of antioxidant enzymes may trigger potentially dangerous metabolic pathway of peroxidative damage. Peroxidative damage induced by free radicals has been demonstrated to play role in the pathogenesis of RA, systemic lupus erythematosus, progressive systemic sclerosis, diabetes mellitus type 1, and myasthenia gravis. Increased oxidative stress has been associated with increased lipid peroxidation in these patients. Lipid peroxidation occurs as a result of increased oxidative stress stemming from deranged pro-oxidant/anti-oxidant balance and represents an important pathogenic process in the oxygen toxicity. As a result of lipid peroxidation increases in the levels of conjugated dienes, isoprostanes, 4-HNE and malondialdehyde have been demonstrated.<sup>11</sup> On the other hand, the etiology of structural damage like bone erosion and joint deformity has not been fully understood in RA. Cartilage and bone damage is associated with activation of free radicals, pro-inflammatory cytokines and metalloproteinases. Inflammatory cells as macrophages, T-cells, B-cells and neutrophils accumulate in inflamed synovial membrane.<sup>11</sup> Many researchers have focused on oxidative stress and increased tendency of RA patients to lipid peroxidation for the last few years.<sup>3</sup>

Although various studies have been performed on antioxidant vitamins and enzymes in inflammatory diseases,<sup>6,12,13</sup> limited number of studies have focused on lipid peroxidation status and inflammatory parameters in patients with RA. Determination of the pathologic mechanism has a special importance in the early diagnosis

and treatment of RA. Therefore, in this study, we aimed to evaluate 4-HNE levels and its clinical and radiological significance in patients with RA.

## PATIENTS AND METHODS

The study included 40 patients (8 males, 32 females; mean age  $51.4 \pm 11.2$  years; range 24 to 72 years) with RA who met American Collage Rheumatology classification criteria for RA and who were followed at least for one year at Department of Physical Medicine and Rehabilitation Firat University Hospital, between October 2015 and January 2016 and 30 healthy controls (8 males, 32 females; mean age  $53.0 \pm 11.7$  years; range 24 to 72 years).<sup>14</sup> Patients who were using biologic agents or leflunomide were excluded. As a prerequisite, any change in disease modifying anti-rheumatic drugs (DMARDs) within three months was accepted as an exclusion criterion (apart from changes in drug dosages). Other exclusion criteria were any diagnosis of autoimmune disease other than RA, acute and chronic infections, malignancies, serious pulmonary, hepatic, kidney or endocrinological diseases, smoking, and hypertension (arterial blood pressure  $\geq 140/90$  mmHg or use of anti-hypertensive drugs) or hypercholesterolemia. The study was approved by the local ethics committee and written informed consent forms were obtained from all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Severity of pain, fatigue and patient and physicians global assessments of disease activity were assessed on a visual analog scale. Duration of morning stiffness was noted in minutes. Disease activity was evaluated using disease activity score 28.<sup>15</sup> Patients with disease activity scores 28 of  $\leq 3.2$  and  $>3.2$  were evaluated as having low disease activity and high/moderate disease activity, respectively.<sup>16</sup> Additionally, patients were divided into two groups as early RA (disease duration  $\leq 2$  years) and established RA (disease duration  $\geq 2$  years). Functional disability was evaluated using health assessment questionnaire.<sup>17</sup>

Routine laboratory test results were recorded including erythrocyte sedimentation rate, blood chemistry, whole blood cell count, and urinalysis.

Rheumatoid factor and C-reactive protein levels were measured using nephelometric methods.

Venous blood samples drawn from the patients and controls were centrifuged at 3000 rpm for 10 minutes. The sera were kept at -20 °C until the analysis. For the measurement of 4-HNE levels, sandwich enzyme-linked immunosorbent assay method was applied using commercial kits (Sunred, catalogue No: 201-12-1979, Shanghai Sunred Biological Technology Co., Ltd, Shanghai, China).

Standard plain hand radiographs of the patients which were taken within last six months were examined. Structural damage in hand radiographs were evaluated by a radiologist who was blinded to patients' clinical and laboratory data. Radiographs were scored using the modified Larsen scoring which scores 24 areas in both hands (each area 0 to 5 points, total score 0 to 120 points).<sup>18</sup>

### Statistical analysis

Statistical analysis of the study was performed using SPSS Statistics for Windows version 20.0 program (IBM Corporation, Armonk, NY, USA). The results of the study were evaluated using parametric and nonparametric statistical methods for data with normal and non-normal distribution, respectively. Intergroup comparisons

were performed using independent groups t test for parametric and Mann-Whitney U test for non-parametric values. Correlations between parameters were evaluated using Spearman correlation coefficient. A *p* value of <0.05 was accepted as the level of significance in statistical evaluations. The results were expressed as mean  $\pm$  standard deviation.

## RESULTS

There was no statistically significant difference between patients with RA and controls in terms of age and sex (*p*=0.890 and *p*=0.511, respectively) (Table 1). Mean disease duration was 5.52 $\pm$ 4.56 years and 26 patients (65%) were rheumatoid factor positive. All patients were taking low or moderate dose corticosteroid treatment. Mean prednisolone dose was 7.5 $\pm$ 3.4 (2.5-20) mg/day. Eleven patients (40.7%) were on prednisolone monotherapy, the rest (59.3%) was taking methotrexate (MTX), sulfasalazine and hydroxychloroquine alone or in combination with DMARDs or prednisolone.

Serum 4-HNE levels (*p*=0.001) were significantly higher in patients with RA than those of the controls (Table 1). Serum 4-HNE (*p*=0.649) levels were similar between moderate/

**Table 1.** Comparison of demographic, clinical and laboratory parameters between patient and control groups

|  | Rheumatoid arthritis (n=40) |                   |           | Controls (n=30) |                 |            | <i>p</i> |
|--|-----------------------------|-------------------|-----------|-----------------|-----------------|------------|----------|
|  | n                           | Mean $\pm$ SD     | Min-Max   | n               | Mean $\pm$ SD   | Min-Max    |          |
| Age (years)                              |                             | 51.4 $\pm$ 11.2   | 24-72     |                 | 53.0 $\pm$ 11.7 | 24-72      | 0.890    |
| Sex                                      |                             |                   |           |                 |                 |            | 0.511    |
| Female                                   | 32                          |                   |           | 22              |                 |            |          |
| Male                                     | 8                           |                   |           | 8               |                 |            |          |
| Duration of the disease (years)          |                             | 5.5 $\pm$ 4.6     | 1-18      |                 |                 |            |          |
| Morning stiffness (min.)                 |                             | 78.4 $\pm$ 65.9   | 10-240    |                 |                 |            |          |
| Pain (0-100 mm VAS)                      |                             | 50.6 $\pm$ 24.7   | 12-94     |                 |                 |            |          |
| Fatigue (0-100 mm VAS)                   |                             | 55.1 $\pm$ 24.1   | 21-100    |                 |                 |            |          |
| Patients global (0-100 mm VAS)           |                             | 51.0 $\pm$ 24.7   | 8-100     |                 |                 |            |          |
| Physicians global (0-100 mm VAS)         |                             | 46.7 $\pm$ 30.6   | 5-96      |                 |                 |            |          |
| Number of the swollen joints (0-28)      |                             | 1.2 $\pm$ 2.2     | 0-10      |                 |                 |            |          |
| Number of the tender joints (0-28)       |                             | 4.1 $\pm$ 4.4     | 0-17      |                 |                 |            |          |
| Erythrocyte sedimentation rate (mm/h)    |                             | 30.8 $\pm$ 15.3   | 6-68      | 19.9 $\pm$ 6.8  |                 | 3-29       | 0.001    |
| C-reactive protein (g/dL)                |                             | 23.5 $\pm$ 23.5   | 3-109     | 3.8 $\pm$ 0.6   |                 | 3.36-4.87  | 0.001    |
| Rheumatoid factor (IU/mL)                |                             | 157.2 $\pm$ 241.8 | 10-949    | 9.8 $\pm$ 0.6   |                 | 8.69-10.30 | 0.001    |
| Serum 4-HNE (pg/mL)                      |                             | 1.4 $\pm$ 0.3     | 0.98-2.01 | 1.1 $\pm$ 0.1   |                 | 0.78-1.40  | 0.001    |
| Disease Activity Score 28                |                             | 4.1 $\pm$ 1.5     | 1.96-7.03 |                 |                 |            |          |
| Health Assessment Questionnaire 20 (0-3) |                             | 1.3 $\pm$ 0.8     | 0.00-3.00 |                 |                 |            |          |
| Modified Larsen Score (0-120)            |                             | 30.3 $\pm$ 23.6   | 8-114     |                 |                 |            |          |

SD: Standard deviation; Min: Minimum; Max: Maximum; VAS: Visual analog scale; 4-HNE: 4-hydroxynonenal.

**Table 2.** Comparisons of patients with high/moderate and low disease activity

|                                       | High/moderate disease activity (n=23) |           |           | Low disease activity (n=17) |          |           | p     |
|---------------------------------------|---------------------------------------|-----------|-----------|-----------------------------|----------|-----------|-------|
|                                       | n                                     | Mean±SD   | Min-Max   | n                           | Mean±SD  | Min-Max   |       |
| Age (years)                           |                                       | 50.9±12.8 | 24-72     |                             | 52.1±8.8 | 37-67     | 0.157 |
| Sex                                   |                                       |           |           |                             |          |           | 0.631 |
| Female                                | 19                                    |           |           | 13                          |          |           |       |
| Male                                  | 4                                     |           |           | 4                           |          |           |       |
| Duration of the disease (years)       |                                       | 5.5±3.8   | 1-16      |                             | 5.5±5.5  | 1-18      | 0.441 |
| Prednisolone (mg/day)                 |                                       | 7.7±3.6   | 2.5-20    |                             | 6.5±2.5  | 2.5-10    | 0.261 |
| Methotrexate (mg/week)                |                                       | 11.9±2.1  | 10-15     |                             | 10.4±2.0 | 7.5-15    | 0.092 |
| Erythrocyte sedimentation rate (mm/h) |                                       | 37.1±15.6 | 17-68     |                             | 22.2±9.9 | 3-33      | 0.004 |
| C-reactive protein (g/dL)             |                                       | 35.7±24.3 | 8-109     |                             | 7.0±5.4  | 3-22      | 0.001 |
| Serum 4-HNE (pg/mL)                   |                                       | 1.4±0.3   | 0.99-2.01 |                             | 1.4±0.3  | 0.98-1.79 | 0.649 |

SD: Standard deviation; Min: Minimum; Max: Maximum; 4-HNE: 4-hydroxynonenal.

high and low disease activity RA subgroups (Table 2). Besides, serum 4-HNE levels were higher in established RA patients than early RA patients ( $1.52\pm 0.26$  vs.  $1.20\pm 0.16$  pg/mL,  $p=0.001$ ) (Table 3).

Serum 4-HNE levels correlated significantly with disease duration in RA ( $r=0.613$ ,  $p=0.001$ ). However, they did not correlate with any other laboratory or clinical parameters of disease activity. Serum rheumatoid factor levels did not correlate with 4-HNE levels. However, a significant correlation was detected between modified Larsen scores and serum 4-HNE levels ( $r=0.487$ ,  $p=0.001$ ).

Serum 4-HNE levels were similar between patients taking prednisolone alone or combined with MTX ( $p=0.153$ ) (Table 4). Similarly, serum 4-HNE levels were comparable between patients MTX and other DMARDs ( $p=0.864$ ).

## DISCUSSION

Our study revealed that 4-HNE levels were higher in patients with RA compared to healthy individuals and higher in established RA than early RA. Moreover, 4-HNE levels were associated with joint damage assessed by modified Larsen scores.

Rheumatoid arthritis is an important cause of morbidity. The role of ROS in the pathophysiology of the inflammation has been well established.<sup>19,20</sup> Aberrant oxidative stress has been considered to have an important role in the pathogenesis of autoimmune diseases by impairing immunological tolerance, inducing apoptotic cell death, and increasing inflammation.<sup>21</sup> Selley et al.<sup>8</sup> showed markedly higher 4-HNE levels in patients with RA when compared with osteoarthritic patients. Remarkably higher levels of end products related

**Table 3.** Data for early and established rheumatoid arthritis

|  | Early RA (<2 years) (n=13) |           |           | Established RA (>2 years) (n=27) |           |           | p     |
|--|----------------------------|-----------|-----------|----------------------------------|-----------|-----------|-------|
|  | n                          | Mean±SD   | Min-Max   | n                                | Mean±SD   | Min-Max   |       |
| Age (years)                              |                            | 51.2±11.6 |           |                                  | 51.6±11.5 |           | 0.917 |
| Sex                                      |                            |           |           |                                  |           |           | 0.237 |
| Female                                   | 9                          |           |           | 23                               |           |           |       |
| Male                                     | 4                          |           |           | 4                                |           |           |       |
| Serum 4-HNE (pg/mL)                      |                            | 1.2±0.2   |           |                                  | 1.5±0.3   |           | 0.001 |
| Morning stiffness (min.)                 |                            | 82.3±81.7 | 10-240    |                                  | 76.5±58.5 | 10-240    | 0.673 |
| Pain (0-100 mm VAS)                      |                            | 45.1±28.2 | 12-92     |                                  | 53.2±22.9 | 12-94     | 0.259 |
| Patients global (0-100 mm VAS)           |                            | 45.1±27.4 | 8-90      |                                  | 53.9±23.2 | 13-100    | 0.272 |
| Physicians global (0-100 mm VAS)         |                            | 40.6±35.1 | 5-96      |                                  | 49.6±28.4 | 10-92     | 0.319 |
| Erythrocyte sedimentation rate (mm/h)    |                            | 34.2±19.6 | 6-68      |                                  | 29.1±12.8 | 11-61     | 0.506 |
| C-reactive protein (g/dL)                |                            | 24.4±31.5 | 3-109     |                                  | 23.0±19.2 | 3-69      | 0.427 |
| Disease Activity Score 28                |                            | 3.8±1.7   | 1.96-6.66 |                                  | 4.2±1.4   | 2.07-7.03 | 0.319 |
| Health Assessment Questionnaire 20 (0-3) |                            | 1.1±0.8   | 0.00-2.12 |                                  | 1.4±0.7   | 0.25-3    | 0.332 |
| Modified Larsen Score (0-120)            |                            | 19.8±6.6  | 8-30      |                                  | 35.3±27.1 | 8-114     | 0.112 |

RA: Rheumatoid arthritis; SD: Standard deviation; Min: Minimum; Max: Maximum; VAS: Visual analogue scale; 4-HNE: 4-hydroxynonenal.

**Table 4.** Comparison of patients with rheumatoid arthritis using prednisolone alone or in combination with methotrexate

|                                       | Prednisolone (n=11) |           |           | Methotrexate + Prednisolone (n=16) |           |           | p     |
|---------------------------------------|---------------------|-----------|-----------|------------------------------------|-----------|-----------|-------|
|                                       | n                   | Mean±SD   | Min-Max   | n                                  | Mean±SD   | Min-Max   |       |
| Age (years)                           |                     | 54.3±12.4 | 25-70     |                                    | 51.7±10.0 | 37-72     | 0.797 |
| Sex                                   |                     |           |           |                                    |           |           | 0.296 |
| Female                                | 10                  |           |           | 12                                 |           |           |       |
| Male                                  | 1                   |           |           | 4                                  |           |           |       |
| Duration of the disease (years)       |                     | 5.2±3.8   | 1-10      |                                    | 5.5±4.9   | 1-16      | 0.980 |
| Prednisolone (mg/day)                 |                     | 8.4±4.4   | 5-20      |                                    | 6.3±2.4   | 2.5-10    |       |
| Methotrexate (mg/week)                |                     | 11.1±2.0  | 7.5-15    |                                    |           |           |       |
| Erythrocyte sedimentation rate (mm/h) |                     | 45.8±16.8 | 22-68     |                                    | 22.0±9.7  | 6-40      | 0.001 |
| C-reactive protein (g/dL)             |                     | 42.7±31.6 | 6-109     |                                    | 11.8±12.8 | 3-52      | 0.003 |
| Serum 4-HNE (pg/mL)                   |                     | 1.4±0.3   | 1.02-2.01 |                                    | 1.4±0.3   | 0.99-1.81 | 0.928 |

SD: Standard deviation; Min: Minimum; Max: Maximum; 4-HNE: 4-hydroxynonenal.

to lipid peroxidation have been documented in patients with RA than healthy control populations in previous studies.<sup>5,22-24</sup> However, contrary results have also been reported.<sup>25</sup>

In our literature survey, we did not encounter any study which assessed the association between 4-HNE levels and disease activity in patients with RA. Available data only suggested a possible relationship between lipid peroxidation products, such as malondialdehyde, and disease activity in patients with RA.<sup>2,26-28</sup> Based on our findings, we may assume that serum 4-HNE levels in RA patients are not directly correlated with inflammatory activation in patients with RA. Controversial results in the literature may stem from complex mechanism of action of lipid peroxidation involving more than one step in the pathogenetic process of inflammation.

In our study, a significant correlation was found between serum 4-HNE levels and modified Larsen scores which demonstrates possible association with joint damage. To the best of our knowledge, this association has not been assessed previously. A previous study by Bohanec Grabar et al.<sup>29</sup> showed a correlation between oxidative stress and joint erosions. However, the authors did not use an objective or validated scoring system like modified Larsen method to assess erosions. Our results may shed light on our current understanding of the interaction between ROS and inflammatory processes.<sup>4,5,7,8</sup>

Our results also documented similar levels of 4-HNE in patients taking prednisolone alone or in combination with MTX as well as MTX vs. other DMARDs. The data are highly limited

regarding the potential interaction between lipid preoxidation end-products and anti-rheumatic drugs, requiring further researches.

The main limitation of our study is its cross-sectional design and relatively small sample size. On the other hand, the wide range of our exclusion criteria prevented inclusion of more patients into the study.

In conclusion, our study demonstrated increased serum levels of 4-HNE in patients with RA compared to healthy controls. We did not demonstrate an association between disease activity and 4-HNE; however, we showed a relationship between disease activity and joint damage. Our findings indicate the possible role of 4-HNE levels in RA disease process and possibly joint damage.

#### Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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