The comparison of antinuclear antibody positivity and thiol/disulfide levels

Antinükleer antikor pozitifliği ve tiyol/disülfid düzeylerinin karşılaştırılması

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Abstract: Objective: There is also a growing body of evidence showing that an abnormal thiol disulphide homeostasis state is involved in the pathogenesis of certain diseases. In the present study, it was aimed to investigate the relationship among ANA positivity and dynamic thiol/disulphide homeostasis in serum samples

Methods: Serum samples were collected from ANA-positive and ANA-negative individuals. The indirect immunofluorescence antinuclear antibody test (HEp 20-10, EUROIMMUN, Germany) was used. The serum thiol/disulfide levels were measured with the fully automated new method.

Results: No statistically significant difference was detected between thiol/disulfide levels in individuals who were negative and those who were positive for ANA (p>0.05). Besides, among the ANA patterns was not found statistically significant difference for thiol/disulfide levels (p>0.05). However, serum native thiol, total thiol and disulfide levels were decreased with aging. The native thiol, total thiol levels and native thiol/disulfide, total thiol/disulfide, native thiol/total thiol ratio was found statistically significant difference among the different age groups (p<0.05).

Conclusion: Determination of thiol/disulfide homeostasis can not provide valuable information on normal or abnormal biochemical processes in ANA-positive individuals. However, determination of thiol/disulfide homeostasis which could be a contributing factor to the pathogenesis of some age-related diseases.

Keywords: Antinuclear antibody, thiol/disulfide homeostasis, aging

Özet: Amaç: Bazı hastalıkların patogenezinde, tiyol/disülfid seviyelerinde görülen anormalliklerin rolünün olduğu düşünülmektedir. Bu çalışma, ANA pozitif serum örneklerinde görülen tiyol/disülfid seviyelerinin prognoz ile ilgili bir ipucu sağlayıp sağlayamayacağı hakkında fikir elde etmek için planlanmıştır.

Metod: Bu çalışma için ANA test sonucu pozitif ve negatif olan kişilerin serum örnekleri toplanmıştır. ANA testi için İndirekt immünfloresans antinükleer antikor testi (HEp 20–10, EUROIMMUN, Almanya) kullanılmıştır. Serum tiyol/disülfid seviyeleri ise tam otomatik yeni bir yöntem ile ölçülmiştir.

Bulgular: ANA pozitif ve ANA-negatif serum örneklerinde kullanılan tiyol/disülfid düzeyleri arasında istatistiksel olarak anlamlı fark gözlenmemiştir (p>0.05). Ayrıca, ANA paternleri arasında da tiyol/disülfid düzeyleri arasında istatistiksel olarak anlamlı fark gözlenmemiştir (p>0.05). Ancak, serum nativ tiyol, total tiyol ve disülfid seviyelerinin yaşla birlikte azaldığı gözlemiştir.

Sonuç: Çalışmamızda, tiyol/disülfid düzeyleri, ANA pozitif bireylerde normal veya anormal biyokimyasal süreçler hakkında bize ayırt edici bir ipucu sağlamamıştır. Ancak,
elde etmiş olduğumuz veriler ışığında tiyol/disülfid düzeylerinin yaşa bağlı hastalıkların patogenezinde önemli bir faktör olabileceği ve bunun yapılacak yeni ve kapsamlı çalışmalar ile aynalatılabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Antinükleer antikor, tiyol/disülfid, yaşlanma

Introduction

The protecting role of antioxidant system of the organism in pathogenesis of various diseases, apoptosis and in the process of natural aging is actively discussed. The important part of the body antioxidant system is biomolecules, which include sulfhydryl groups [1]. Thiols may undergo oxidation reaction via oxidants and form disulphide bonds [2]. There is also a growing body of evidence demonstrating that an abnormal thiol disulphide homeostasis state is involved in the pathogenesis of certain diseases [3].

Autoimmune diseases are disorders in which the body begins a fight against its own cells and tissues. Antinuclear antibodies (ANA) are immunoglobulins or antibodies that bind to one or more antigens expressed within the nucleus of human cells. ANA test is widely used as a serological marker of autoimmune diseases. The ANA test can be a useful laboratory tool to help confirm or exclude the diagnosis of systemic rheumatic disease. On the other hand, the relatively high prevalence of ANA in other inflammatory disorders, as well as healthy individuals, can make a positive result difficult to interpret [4]. For this reason, determination of dynamic thiol disulphide homeostasis can provide valuable information on normal or abnormal biochemical processes in ANA-positive individuals.

In the present study, it was aimed to investigate the relationship among ANA positivity and dynamic thiol/disulphide homeostasis in serum specimens.

Materials and Methods

Ethics committee approval was received for this study from the ethics committee of the Suleyman Demirel University, Medical Faculty.(72867572-050-597)

The study group consisted of 185 participants. Of the 185 participants, 25 were chosen as 4+, 40 were chosen as 3+, 40 were chosen as 2+, 40 were chosen as 1+, 40 were negative for ANA. Amongst the participants, 28 were males and 158 were females (age range: 7–88 years).

The indirect immunofluorescence antinuclear anti-body test (HEp 20-10, EUROIMMUN, Germany) was used in dilution of 1:100. Fluorescence intensity was interpreted semiquantiatively based on negative control (0) and positive control (+4).

Dynamic thiol/disulphide homeostasis was determined totally using a Shimadzu UV-1800 spectrophotometer with a temperature controlled cuvette holder and a Cobas c501 automated analyser (Roche Diagnostics, Germany). The total thiol (–SH + –S–S–) content of the serum sample was measured using modified Ellman reagent. Dynamic disulphide bonds (–S–S–) in the serum sample are reduced to functional thiol groups (–SH) by sodiumborohydrate. The unused reductant remnants were completely removed by formaldehyde. Thus, the total thiol amount could be accurately measured. Mercaptoethanol solutions were used as calibrators. Native thiol content is subtracted from the total thiol content and half of the obtained difference gives the disulphide bond amount. Besides –S–S–/–SH, –S–S–/(–SH + –S– S–), –SH/(–SH + –S– S–) ratios were calculated synchronously [3].

A statistical analysis was performed using IBM SPSS Statistics Version 15.0 (SPSS Inc., Chicago, IL, United States). The data's compliance with normal distribution was tested by One Sample Kolmogorov Smirnov Test. One way ANOVA with Tukey’s post test was used to compare means between groups, and p value < 0.05 was accepted as statistically significant.

Results

Of the 185 participants, 145 were ANA-positive and 40 were ANA-negative. The mean age was 31.5±16.2 in ANA-positive and ANA-negative individuals. ANA patterns were homogeneous pattern (67 individuals), granular pattern (63 individuals), and nucleolar pattern (15 individuals). The primary data, including age, gender and ANA positivity are shown in Table 1.

Comparisons between ANA positivity and thiol/disulphide levels evaluated in this study. No statistically significant difference was detected between thiol/disulphide levels in individuals who were negative and those who were positive for ANA (p>0.05). Besides, no statistically significant difference was found for thiol/disulfide levels amongst the ANA patterns (p>0.05).

Serum native thiol, total thiol and disulphide levels were decreased with aging. The native thiol, total thiol and disulphide levels were found significantly different amongst the different age groups (p<0.05). In
Table 1: Distribution of antinuclear antibody positivity according to age and gender.

| Age   | Gender | ANA –(n) | ANA 1+(n) | ANA 2+(n) | ANA 3+(n) | ANA 4+(n) | Total (n) |
|-------|--------|----------|-----------|-----------|-----------|-----------|-----------|
| 7–17  | Female | 1        | 1         | 2         | 2         | 2         | 8         |
|       | Male   | –        | 1         | –         | –         | –         | 1         |
| 18–35 | Female | 7        | 7         | 13        | 11        | 6         | 44        |
|       | Male   | 3        | –         | 3         | –         | 1         | 7         |
| 36–64 | Female | 17       | 23        | 13        | 18        | 12        | 83        |
|       | Male   | 6        | 1         | 2         | 4         | –         | 13        |
| 65–88 | Female | 4        | 3         | 6         | 5         | 4         | 22        |
|       | Male   | 2        | 4         | 1         | –         | –         | 7         |
| Total (n) |       | 40       | 40        | 40        | 40        | 25        | 185       |

Table 2: The list of the serum thiol/disulfide levels measured in our study group. However, no statistically significant difference was found between female and male groups in terms of thiol/disulfide levels (p>0.05).

| Thiols | Age   | Mean (μmol/L) | Std. Deviation |
|--------|-------|---------------|----------------|
| Native thiol | 1–17 | 308.52        | 64.06          |
|         | 18–35 | 287.58        | 57.35          |
|         | 36–64 | 228.70        | 55.80          |
|         | 65–100 | 180.18        | 55.89          |
| Total thiol | 1–17 | 355.80        | 57.84          |
|         | 18–35 | 328.54        | 56.51          |
|         | 36–64 | 268.74        | 58.86          |
|         | 65–100 | 215.28        | 64.61          |
| Disulfide | 1–17 | 23.63         | 6.54           |
|         | 18–35 | 20.48         | 6.67           |
|         | 36–64 | 20.02         | 5.97           |
|         | 65–100 | 17.55         | 7.49           |

**Discussion**

ANA was discovered in association with Systemic Lupus Erythematosus (SLE) but several other autoimmune diseases were found after to be associated with them, almost of which fall under the rubric of rheumatology such as systemic sclerosis, Sjögren’s syndrome, and mixed connective tissue disease. ANA positivity is frequently seen in rheumatoid arthritis but the disease is not considered to be an ANA associated illness [5]. ANA positivity will be seen in a range of conditions where it is not diagnostically helpful. Non-autoimmune diseases such as chronic infection, viral hepatitis and cancer, and also various autoimmune disorders such as multiple sclerosis or thyroid disease where the presence or absence of ANA does not play an important role in diagnosis or prognosis [6].

A pre-existing clinical suspicion of systemic rheumatic illness is critical to enhance the clinical benefit of a positive ANA test result [7]. On the other hand, in the absence of clinical or laboratory markers supporting a diagnosis of rheumatic disease, a positive ANA is rarely useful [4]. Low-intensity ANA is available in up to 40% of healthy individuals [8]. To solve this problem, almost laboratories should be set a cut-off for reporting a positive ANA test result that excludes the most of these low-intensity and clinically insignificant results. ANA test can be done early in the diagnostic evaluation of patients with suggestive clinical symptoms to better direct further investigations as a sensitive, but non-specific, marker of some systemic rheumatic illness [4].

Proteins are one of the main targets for oxidative damage. They are major components of most tissues, cells and plasma and exhibit rapid rates of reaction with many oxidants. This oxidized proteins are known to cause major physiological disorders, including loss of structure or function. Changes in the amino acid sequence or structure may generate neo-epitopes from self proteins, causing aggressive autoimmune attack [9,10].

Diseases in which oxidative damage has been implicated include autoimmune diseases and malignancy [11,12]. Most clinical studies focus on the measurement of oxidative damage by using bio- markers- oxidants and antioxidants. Thiols are considered the biggest and most frequently antioxidants in plasma.

The SH group levels were found to be significantly decreased in SLE patients compared to normal controls [13]. This supports the role of oxidative stress in the pathogenesis of SLE. However, no statistically significant difference was detected between thiols levels in SLE patients who were negative and those who were positive.
In this study, serum thiol/disulfide levels were measured with the fully automated new method. To our knowledge, this was the first study conducted on the association between thiol/disulfide homeostasis and ANA positivity with a new automated assay. Our findings suggest that serum thiol/disulfide homeostasis were not correlated with ANA positivity. We showed that the native thiol, disulfide, and total thiol levels in ANA-positive or ANA-negative individuals were not significantly different. Additionally, serum thiol/disulfide homeostasis were not correlated for ANA patterns and gender in this study.

Our findings suggest that the serum thiol, total thiol, and disulfide levels correlated negatively with aging. The progressive accumulation of damaged biomolecules could be a factor in the adverse physiological changes occurring with aging. Some studies have revealed an age-related shift of plasma thiol/disulfide levels which could be a contributing factor to the pathogenesis of some age-related diseases including cardiovascular diseases, rheumatoid arthritis, and neurological disorders [15–17].

Consequently, determination of thiol/disulfide homeostasis can not provide valuable information on normal or abnormal biochemical processes in ANA positive individuals. However, determination of thiol/disulfide homeostasis which could be a contributing factor to the pathogenesis of some age-related diseases.

**Conflict of Interest:** The authors have no conflict of interest.

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