Estimation of Nitric Oxide, Malondialdehyde, and Adenosine Deaminase in Serum of Hypertensive Patients and Normotensive Individuals in Erbil City

Gulzar I. Ibrahim¹, Saman M. Abdulkareem², Lutfiya M. Hasan¹

¹Department of Chemistry, College of Education, Salahaddin University, Erbil, Iraq
²Department of Biology, College of Education, Salahaddin University, Erbil, Iraq

https://doi.org/10.25130/tjps.v24i3.364

ABSTRACT

This study was aimed to estimate nitric oxide (NO), malondialdehyde (MDA), and adenosine deaminase (ADA) in the serum of hypertensive patients. Fifty patients (25 males and 25 females) age 40-70 diagnosed with hypertension involved in the study. Fifty healthy individuals, who had no hypertension in the last year, were identified as the control group. NO, MDA and ADA have performed accordingly. MDA was higher with aging and gender in hypertensive patients. Serum level of MDA was higher in females compared with males due to oxidative stress more in females than males with aging. ADA was higher among hypertensive with aging, though no significant differences among gender. Serum level of NO was lower with aging with no significant differences among gender.

Introduction

Hypertension sets to become vital factors in health worldwide since it causes the increase in mortality rate and disabled people in many countries, through a global frequency of 40.8% and a controlled percentage of 32.3% [1,2]. Hypertension is often related to metabolic abnormalities like dyslipidemia and diabetes, and therefore the average of those diseases is increasing these days [3]. It is recently hypothesised that the vital role of developing hypertension disease is oxidative stress. A drooping in superoxide dismutase and glutathione peroxidase activity has discovered in recently detected and untreated hypertensive subjects, which are inversely related to blood pressure [4]. Oxidative stress is due to the disproportion present between the generation of reactive oxygen species (ROS) and the antioxidant defence system. The reactive species include superoxide (O₂⁻), hydroxyl (HO·), hydrogen peroxide (H₂O₂), peroxynitrite (ONOO⁻), nitrogen oxide (NO) and hypochlorous acid [5]. Animal studies have usually supported the hypothesis that raised in blood pressure is related to heightened oxidative stress; however, human studies have been disagreeing. Oxidative stress stimulates several abnormal activities including the proliferation of vascular smooth muscle cell and hypertrophy and deposition of collagen, leading to vascular media thickening and reducing the vascular lumen. Furthermore, raised in oxidative stress may promote damage of the endothelium and failure of endothelium-dependent vascular dilatation and raises vascular contractile activity. All these effects on the vasculature may clarify the mechanism of how raised in oxidative stress can precipitate in hypertension [6]. Nitric oxide (NO) is a paracrine signalling factor formed by endothelial cells that have been revealed to increase ROS-mediated oxidative damage [7]. The decline in bioavailability of NO in the vasculature lower hypertension by rising in vasodilation capacity. NO synthase synthesises the NO from oxygen and arginine. NO, in addition to its anti-proliferative and vasorelaxing roles, has a vital role in antagonising the effects of endothelin’s, AT-II, and ROS [8]. Lipid peroxidation is owning a sizeable toxic role of cellular damage by affecting cellular integrity and composition and generating more free radicals. Lipid peroxides are biosynthesised by oxidising of polysaturated fatty acids, which are unstable and disintegrate to form a complex series of compounds [9]. These include reactive carbonyl compounds; the most abundant compound is malondialdehyde [10]. MDA attacks the lysine amino acid in protein which leads to proteolysis. Consequently, MDA readings are broadly used as a pointer of lipid peroxidation and...
oxidative stress [11]. Elevated levels of lipid peroxidation products have been associated with a several of chronic infections in both humans and model systems such as diabetes [12], inflammation [13], β-thalassemia [14], myocardial infarction [15], and cancer [16].

Adenosine deaminase (ADA) is one that marker which is cost-effective, just prepared and is a purine catabolic enzyme, that exactly catalysis irreversible deamination of adenosine to inosine. It participates in the organisation of intracellular and extracellular masses of adenosine and modifies adenosine action on its receptors. Increase in serum ADA activity decreases adenosine concentration. ADA appears to be an essential enzyme for modulating the bioactivity of insulin. The rise in plasma ADA activity is correlated with obesity, insulin resistance, abnormal lipid profile, and hypertension [17, 18, 19].

This study tried to determine variations in the levels of plasma nitric oxide (NO), malondialdehyde (MDA) as an indication of lipid peroxidation, and adenosine deaminase (ADA) in healthy control and patients with hypertension, to point out the alterations in their levels and to find whether correlation exists within each of these variables.

Materials and Methods

Participant

The sample was taken from a group of fifty hypertensive subjects (25 men and 25 women) with the age range of 40 - 70 years from Rizgari Hospital in Erbil city. Hypertension is diagnosed when the systolic/ diastolic pressure read 140/ 90 mmHg correspondingly at three random checks. Apart from patients with a complication such as renal, endocrine or hepatic disease, diabetes, obesity, viral and bacterial infections were not taken as a part of the sample collection. Also, a total number of 50, age and sex-matched persons, ranging from 40-70 years; possessing normal and regular blood pressure and no notable diseases, this group identified in this study as a control group.

Participants were guided to fast in food and caffeine-containing drinks for 18 hours before the experiment and refrain from using alcohol or smoking for at least 24 hours.

Tools

Different instruments for this study have been used, including spectrophotometer (LKB, Model 4050), hotplate (Stuart Scientific Co. LTD No.5371 England), centrifuge centra 4, International (IEC), water bath (Memert Gm bH+ Co. KG D 91126).

Chemicals

All the common laboratory chemicals and reagents used in this study were of analar grade unless otherwise specified and were gotten from the following company: Griess reagent, ethanol, vanadium (111) chloride, sodium nitrite, trichloroacetic acid, thiobarbituric acid (TBA) (Merk), 1.1.3.3-tetramethoxypropane, phosphate buffer, adenosine buffer, ammonium sulphate, phenol, nitroprusside, sodium hydroxide, alkaline hypochlorite (Merk).

Collection of blood sample

From the chosen participants (hypertensive and non-hypertensive groups) about 10 ml of blood sample was taken from a forearm vein, standing for few minutes to let the blood sample clotting at room temperature and then centrifuge for 10 minutes at 3000rpm. Serum samples that obtained divided into three parts. Finally, the separated serum was kept in a deep-freezing atmosphere (-18°C) to be used in the later.

Measurement of serum NO

By using the Griess reaction, Serum NO was estimated. The principle of Griess reaction based on that oxygenated solution of NO degraded into two products, one is nitrate (NO$_3^-$), and the other one is nitrite (NO$_2^-$). It was found that the only stable product of oxygenated solution of NO is nitrite (NO$_2^-$) which react with Griess reagent under low pH to form azo dye colour[20].

Cooled ethanol added to the serum samples at 1:2 v/v (0 °C), which were then mixed well by vortex and deproteinized, then centrifuged at 14000 rpm for 5 minutes, then incubated for 30 minutes at (0 °C). A reducing agent Vanadium (III) Chloride was added to reduce nitrate to nitrite in which nitrite reacted with Griess reagent to give azo dye colour, the absorbance read at 540nm by using a spectrophotometer. From the standard curve from 0 to 120μmol/L of sodium nitrite, the concentration of NO in serum was established by measuring the OD of the serum samples[21].

Measurement of serum MDA

Spectrophotometrically oxidative stress evaluated by quantifying thiobarbituric acid (TBA) reactivity as MDA. To each of 0.5 ml of the serum, 0.5 ml of 30% trichloroacetic acid (TCA) added, and centrifugation separated the supernatant at 3000 rpm for 5 minutes. Afterwards, 0.5ml of the supernatant was added to 0.5 ml of TBA (1%) in a boiling water bath for 30 minutes, following each tube was kept for 10 minutes in an ice-cold water bath.

The resulting chromogen absorbance was determined at the wavelength of 532nm at room temperature in opposite to blank reference. The concentration of MDA recorded from a standard calibration curve plotted using 1,1.3,3-tetra-ethoxypropane (TEP). The amount of lipid peroxidation was represented as MDA in n mole/L [22].

Determination of ADA activity

Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further responds react with phenol and hypochlorite in an alkaline medium to produce a blue colour indophenol complex with sodium nitroprusside serving as a catalyst. The intensity of the blue coloured indophenol complex produced is directly proportionate to the amount of ADA present in the sample. The absorbance was read against water at
635 nm utilising a spectrophotometer. One part of ADA described as the amount of enzyme needed to liberate one micromole of ammonia per minute from adenosine at standard assay conditions. ADA activity was expressed as units per litre (U/L) in the serum[23, 24].

Adenosine + H₂O → ADA → Ammonia + Inosine

Ammonia + phenol + hypochlorite → Blue indophenols complex

The data of the present study gave as a mean ± standard deviation, and statistical analysis of the obtained results was done by using students t-test to compare between the two groups which performed by GraphPad Prism 6 software with the level of significance set at p<0.05.

Results

Table 1 shows the age and gender distribution of hypertensive patients and controls in their different age. A total of 50 hypertensive patients were (25 males and 25 females), and a total of 50 healthy and normotensive subjects were (25 males and 25 females) as controls. Most of the subjects were within the age range of 40-70 years. Its demonstrations that there are no notable variances among the groups regarding age and gender.

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Controls</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-50</td>
<td>25</td>
<td>5</td>
<td>6</td>
<td>36</td>
</tr>
<tr>
<td>51-60</td>
<td>11</td>
<td>12</td>
<td>11</td>
<td>34</td>
</tr>
<tr>
<td>61-70</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1: Age (years) and gender distribution of hypertensive patients and controls.

Table 2: Age-wise difference of serum MDA levels in cases and controls

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Cases</th>
<th>Controls</th>
<th>Male</th>
<th>Male</th>
<th>Female</th>
<th>Female</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-50</td>
<td>2.91±2.41</td>
<td>3.05±3.21</td>
<td>1.69±2.31</td>
<td>1.71±4.45</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>4.41±5.67</td>
<td>4.61±8.22</td>
<td>1.79±7.35</td>
<td>1.78±9.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61-70</td>
<td>4.09±6.53</td>
<td>4.83±6.64</td>
<td>1.89±1.34</td>
<td>1.80±5.46</td>
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<td></td>
</tr>
</tbody>
</table>

It was observed no differences among the groups regarding the gender of hypertensive patients in Table 3 for NO level. Serum NO levels were significantly lower (p<0.001) in the patients with age (40-50), for male (3.80±8.21) and female (3.74±6.11) compared with male control (4.67±2.19) and female control (4.70±3.31). Both sexes were compared for NO showed no statistically significant difference in the level of NO was observed between the two genders with different ages. With age (51-60), the serum level of NO was significantly lower (p<0.001) with male (35.62±3.11) and female (35.28±4.54) compared with control male (43.19±2.19) and female (43.21±4.23). It was observed that the level of NO was significantly lower (p<0.001) at the age of (61-70), for male (33.81±5.59) and female (33.56±2.31) compared with control male (42.45±4.35) and female (42.45±1.21) respectively.

Table 3: Age-wise difference of serum NO levels in cases and controls

Serum ADA levels were significantly higher (p<0.001) in the patients with age (40-50) in both male (56.37±6.27) and female (56.47±6.27) compared with control male (40.78±7.34) and female (40.93±4.55). Both sexes in Table 4 were compared for ADA statistically significant difference in the level of ADA was observed between the two genders with different age. With age (51-60), the serum level of ADA levels for patients was significantly higher (p<0.001) for both male (58.31±6.08) and female (59.11±5.82) compared with control male (41.09±0.67) and female (41.15±7.21). It was noticed that the level of ADA levels was significantly higher (p<0.001) for patients at the age of (61-70). Serum level of ADA was higher among male (63.12±3.51) and female (64.21±8.53) compared with control male (42.85±7.3) and control female (43.23±3.5) respectively.

Table 4: Age-wise difference of serum ADA levels in cases and controls

Discussion

In the current study, MDA was significantly higher among cases of hypertension patients compared with control, that was in harmony with that obtained by Yildirim et al. [25]. Another finding of Bednarek-Tupikowska et al. [26], reported that MDA was significantly higher in hypertension patients compared to control. Essential hypertension is
connected with the increased production of ROS that tendency to increase lipid peroxidation which has a significant effect on cell degradation. An increase in free radical production mainly superoxide ions or a decrease in nitric oxide production may cause any easy develop of a sudden muscular contraction in the arterial [27]. MDA can diminish the activity of superoxide ion in the cell membrane that can impair catalase enzyme activity resulting in decrease converting of hydrogen peroxide, that cause in increase H₂O₂ concentration which effects SOD activity, leading to increasing MDA level [28], suggesting that oxidative stress is important in the pathogenesis of hypertension.

The significant positive correlation established between MDA and age indicates that oxidative stress in favoring with increasing age in hypertensive patients from this study. As it was observed that serum MDA level increases among female compared with a male that because of inflammation and oxidative stress act together in the pathogenesis of hypertension [29]. Inflammation could be due to the primary immune response to eliminate pathogens while innate immune cells, such as neutrophils and macrophages, produce ROS such as superoxide and hydrogen peroxide in order to kill pathogens[30].

With age (40-70) years females going through both pre- and post-menopausal females. Even if the differences between pre- and postmenopausal females are not significant, increasing in the generation of reactive oxygen species (ROS) lead to a rise in MDA, and this due to too much oxidative damage caused in females. Many other important biomolecules containing membrane lipids can be oxidized by using oxygen species. Similar reports of raised MDA levels are recounted in patients with PCOS [25]. The estrogen turns as an important signal in gene control of antioxidant mRNA expression which acts to control redox balance, Menopause relate with increasing plasma malondialdehyde [26].

This study showed that NO level was significantly lower among hypertensive patients compared with control. There was no significant difference in NO level between male and females belongs to different ages group. We found that the plasma nitric oxide availability is impaired with advancing age in hypertensive individuals. Male and female aged (40–50) years have significantly higher NO levels compared to older men in the age group (61-70). It was in contrast to the studies by Ghasemi et al.[31], and others reported that NO level is changed among male and female [31, 32, 33].

NO is estimated in blood by its synthesis, degradation and clearance, utilisation of water and food may also affect NO level in plasma. Nitric oxide is synthesised from L-arginine by NO synthase, mostly present in blood that is initially coming from endothelial and smooth [34]. Hypertension can generate a toxic effect on human endothelium that diminishes the release of NO from vascular endothelial cells, may contribute to lower plasma NO level in a patient with hypertension. Low level of NO in plasma may affect intracellular calcium level which helps to the activity muscle cells of NO synthase. Superoxide anion increase at hypertension which causes degradation of NO and increases lipid peroxidation [35].

ADA was significantly higher among cases compared with control. There was no significant difference in ADA level between male and females belong to different age’s group. It is reported that the ADA levels are elevated whenever cell-mediated immunity is stimulated and thus reflects the activity of stimulated T-lymphocytes. The results of the present study showed highly significant mean levels of ADA in hypertensive patients when compared to controls.

The elevated levels of ADA reflect the changes in the immune response in the pathogenesis of hypertensive patients with getting old. Elevated levels of ADA have also been reported in other diseased conditions like tuberculosis [36], acute nephrotic syndromes [37], leukaemia, Behcet's Disease [38], typhoid [39], and in patients with renal transplants. Another function of adenosine that is the regulation of blood pressure levels. Adenosine will decrease the release of renin secretion from juxtaglomerular cells and thus regulates blood pressure levels. Enzymes that are responsible for adenosine activity includes 5-nucleotidase, adenosine deaminase and adenosine kinase. In adipose tissue, adenosine degraded by ADA, so more synthesized of adenosine lead to increase ADA, whereas the formation of extracellular adenosine depends on an enzyme cascade for metabolism of ATP, ADP, and AMP, that sound to be the greater mechanism that leads to elevated extracellular adenosine [40].

Conclusion

Based on the results from this study, it can be concluded that oxidative stress-mediated tissue damage in hypertension patients. The balancing of oxidative stress changes with aging and elderly people by lowering NO level in serum and high plasma level of MDA and ADA. MDA and NO have no significant relation. Analyzing the level of MDA in serum with different gender helps in diagnosis first degree of disease and prevents the chronic complication of hypertension. Plasma NO levels found to be noteworthy lower in hypertension subjects, may assist in the understanding the duty of NO in regulating blood pressure. This work requires more support to study to clarify the link between oxidative stresses, age, gender and hypertensive. Further clarification of the role of impaired NO bioactivity and increased MDA and ADA level in hypertension could have important implications for the management of hypertension.
References


المستعرض المائي، والاندوسينيدايميننز في مصل دم مرضى المصابين بارتفاع ضغط الدم مقترنة بالصحة في مدينة أربيل، كلازام اسماعيل إبراهيم1، سامان محسن عبدالكريم2، لطفية محمد حسن2

فصل الجسم، كلية التربية، جامعة صلاح الدين، أربيل، العراق

فصل علم الحياة، كلية التربية، جامعة صلاح الدين، أربيل، العراق

المستعرض المائي هو تقدير أوكسيد النيتريك (NO)، مالونديالديه (MDA)، والاندوسينيدايميننز (ADA) في مصل الأشخاص المصابين بارتفاع ضغط الدم مقترنة بالصحة. استُخدمت دراسة عينات من 120 مريضاً (50 ذكور و 70 أنثى) في مدينة أربيل. تم استخراج عينات الدم من جميع المرضى، بعد الانتظار 40 دقيقة من التوتر. تم قياس مستويات MDA، NO، و ADA في عينات الدم الجلدية. كان نتائج الدراسة أن MDA مستويات أعلى في المريضين بالصحة، بينما مستويات NO والADA أعلى في المرضى المصابين بارتفاع ضغط الدم. وكان تقدم العمر و نوع الجنس في المرضى العاديين كان أعلى عنصر في الدين مراقبة بالصحة. كان مستويات NO أقل مع تقدم العمر في مرضي المصابين بالصحة. كان مستوي المصل NO أقل مع تقدم العمر مع عدم وجود فروقات بين الجنسين. كان مستوي المصل NO أقل مع تقدم العمر مع عدم وجود فروقات بين مرضى المصابين بارتفاع ضغط الدم. وكان تقدم العمر و نوع الجنس في المرضى العاديين كان أعلى عنصر في الدين مراقبة بالصحة. كان مستويات NO أقل مع تقدم العمر مع عدم وجود فروقات بين الجنسين. كان مستوي المصل NO أقل مع تقدم العمر مع عدم وجود فروقات بين الجنسين. كان مستوي المصل NO أقل مع تقدم العمر مع عدم وجود فروقات بين الجنسين.