A study of Some Hormones and Antioxidant Systems Disturbances in Older Men

Reem Abdul-Raheem Al-Saadi
College of Medicine, Department of Anatomy & Histology, University of Babylon
drreemalsaad@gmail.com
Dakhel Ghani Al-Watify
Department of Biology, College of Science for Woman, University of Babylon
dakhelghani@gmail.com

Abstract

Ageing is a physiological phenomenon that manifested itself with disturbances of many homeostatic regulating mechanisms of the body. The present study was conducted and employed to investigate two major systems (hormones and antioxidant systems) that can be implicated in progress of aging. The total number of subjects included in the present study was fifty (50) healthy men and classified according to their ages into two groups, the first group included 25 younger men (control group) and their ages ranged between 21 to 30 years old whereas the second group included 25 older men and their ages were between 61 to 70 years old. Data obtained from this study indicated a significant decrease (p<0.05) in the levels of thyroid stimulating hormone (TSH) which is associated with marked elevation (p<0.05) of triiodothyronine (T3) and tetraiodothyronine (T4) of older men when compared with younger men. In regard to levels of cortisol and testosterone hormones were significantly decreased (p<0.05) in aged men when matched with their counterparts of younger men. Concerning concentrations of malondialdehyde (MDA), a final product of lipid peroxidation, confirmed a remarkable elevation (p<0.05) in older men compared to younger men and these results were associated with significant lowering (p<0.05) in the activities of antioxidant components including glutathione peroxidase (GPX), reduced glutathione (GSH) and catalase. In addition, there are no significant correlations (p>0.05) occurring among hormones (testosterone, T3) and glutathione peroxidase and of malondialdehyde. From these results, one can be concluded that with ageing there are many disturbances and fluctuations of hypothalamic-adrenal and thyroid axis that accompanied with drop of essential antioxidant components that may be lead to suppress of defense against free radicals and the present study concluded that the changes occurring in studied hormones have not relations and effects on the antioxidant systems.

Key words: ageing, hormones, antioxidants.

دراسة بعض الاضطرابات الحائطة ببعض الهرمونات والأنظمة المضادة للأكسدة لدى الرجال المسنين

الخلاصة

تتميز ظاهرة التقدم بالعمر بحصول العديد من الاضطرابات خاصة في الميكانيكيات المنظمة للإدامة. وضعت الدراسة الحالية لبحث منظومتين قيماً وثابتة في حصول تقدم العمر، وهما بعض الهرمونات ومضادات الأكسدة. شمل العدد الكلي للأشخاص الدراسة انتخاب خمسون رجلاً من الأشخاص، وقد قسموا حسب نسبتهم إلى مجموعتين شملت المجموعة الأولى خمسة وعشرون رجلاً (عمرهم بين 21-30 سنة), في حين شملت المجموعة الثانية خمسة وعشرون رجلاً (عمرهم بين 61-70 سنة). النتائج المستحصمة من الدراسة الحالية حصلت انخفاض معنوي (p<0.05) في مستوى الهرمون المحفز لمدرقيا (TSH) وبنفس الوقت حصول ارتفاع معنوي (p<0.05) في مستوى هرموني الهرمون المحفز للدراقيا (T3,T4), عند الأشخاص المسنين على مستوى هرموني الهرمون المحفز للدراقيا عند الأشخاص الشباب, وخصوصاً هرموني الهرمون المحفز للدراقيا (Testosterone والكروتيزيول (Cortisol), فقد بيئت النتائج حصول انخفاض معنوي (p<0.05) في تراكيز كلا الهرموني في الرجال المسنين عند مقابلتهم مع الرجال غير المسنين (الشباب). نتجت سجلت في تركيز كلا الهرموني في (MDA) ارتفاعاً معنوي (p<0.05) لدى الرجال المسنين عند مقابلتهم مع الرجال الشباب في حين لوحظ حصول انخفاض معنوي في مستوى العوامل المضادة للأكسدة.
 مثل الكموتاثيون بروكسيداز (GPX) والكموتاثيون المختزل (GSH) والكاثائز (Catalase) لدى الرجال المسنين عند مقارنتهم مع الرجال الشباب. كما بنيت النتائج عدم حصول ارتباط معنوي (p>0.05) بين كل من هرموني T3 و HORM من جهة و مركب MDA وفعالية إنزيم GPX من جهة أخرى.

من النتائج المستحصمة من هذه الدراسة يمكن الاستنتاج بأنه مع تقدم العمر تحصل العديد من الاضطرابات والتقلبات في محاور جسم تحت المهاد والدرقية والكظرية والتي تكون مصحوبة بإفراط المنظومات المضادة للأكسدة والتي قد تودي إلى ارتفاع مستوى الجذور الحرة والتي بدورها تسبب اضطرابات في الوظائف الفسمجية للجسم على مستوى الأجهزة والخلايا.

الكلمات المفتاحية: العمر، الهرمونات، مضادات الأكسدة.

Introduction
With age, most if not all body homeostatic mechanisms become disturbed and tend to be progressively declined of their important and vital functions necessary to make the body systems act normally. The loss of efficiency of essential functions renders the body unable to adapt for different exogenous and endogenous stressful factors that finally lead to aggravate and exaggerate of many chronic diseases (Kumar et al., 2017). Ageing is a life phenomenon of all organisms especially mammals, this problem progress step by step and finally tend to damage several essential activities of body such as network of hormones, metabolism, nutrition and growth (Jonas and Boelaert, 2015). Among systems affected with ageing is neuroendocrine system, the hypothalamic–pituitary axis and other peripheral endocrine glands become more affected with progress of age, the primary responses and sensitivities of target receptors that localized on many cells become inactive and down regulation (Puzianowska-Kuznika, 2012). The hypothalamic-pituitary-adrenal is responsible for maintaining and regulation of many fluctuations and stressful conditions via variable levels of glucocorticoids secreted from adrenal cortex (McEwen, 1998). In addition, hypothalamic-pituitary-thyroid axis is also subjected to many alterations during advanced age, the thyroid hormones undergo from dysregulation and fluctuations in their levels (Costa and Rosenthal, 1996; Slemer et al., 2014). On the other hand, the reactive oxygen species (ROS) can be originated from either exogenous or endogenous factors, furthermore, there are many theories indicated that production of those free radicals resulted from metabolic pathways, in particular, mitochondria are implicated in deterioration effects of living tissues with progress of age (Adkins et al., 2017). The body has specific systems specialized in scavenging and delay the harmful effects of free radicals, these systems are composed from enzymatic and non-enzymatic antioxidant components (Lenas, 1998; Zsnagy, 2001).

Materials and Methods
Subjects of the study
The present study was carried out in college of science for women of Babylon university and Marjan hospital from the period ranged from December 2016 to June 2017. The study included two different age groups of younger and older men. The total number of the subjects was 50 men and subdivided according to their ages into two groups, the ages of the first group of men (25 men) were ranged between 21 to 30 years old and were used as control group and the ages of the second group (25 men) were 61 to 70 years old. All subjects were volunteered and recruited from Babylon university and public health laboratory. All subjects of the study were selected carefully to exclude the subjects (men) who complain from common chronic diseases and other bad habits such as smoking and drug abuse. The principle
questions were directed to the subjects about essential their health criteria and lifestyle to explain the subjects have a complete fitness to include them in the present study.

**Collection of blood samples**

All blood samples were collected during morning between 8 to 10 a.m. Before collection, the subject asked to rest on a chair for at least 10 minutes, and then the antecubital vein of left arm are mostly employed. The site of collection were cleaned with alcohol (70%) and wormed to improve blood circulation and engorge the targeted vein. A tourniquet was applied at 7 cm above site of collection. A syringes with 23 gauge were used and adequate blood samples withdrawn and then transferred to gel plain tubes (without anticoagulants) to permit the blood to coagulate. After completing coagulation, the samples transferred for centrifugation at 3000 rpm for 15 minutes and then serum was aspirated by micropipettes and kept by eppendroff tubes. The serum samples were kept in a deep freeze (-20 °C) to perform the future biochemical analyses.

**A- Estimation of hormones**

Measurements of thyrotropin hormone (TSH), triiodothyronine hormones (T3), tetraiodothyronine hormone (T4), testosterone hormone and cortisol hormone these hormones are determined by ELISA and Minividas technique (according to kits of Biomerixue company).

**B-Determination of antioxidants**

1- **Assay of catalase activity**

Catalase is an enzyme that catalyses the decomposition of hydrogen peroxide molecules (H2O2) to give final products (H2O and O2). Catalase activity is measured according to previous method reported by Aeobi (1974). The decomposition of H2O2 is followed directly with decrease in the absorbance of samples at 240 nm wave length. The difference in absorbance per unit of time represented the measurement of catalase activity.

2- **Activity of glutathione peroxidase**

Glutathione peroxidase is determined according to method invented by Paglia and Valetin (1967).

3- **Concentrations of reduced glutathione**

5.5-dithiobis (2-nitrobenzoic acids) (DTNB) is a disulfide chromogen that is immediately reduced by the presence of sulfahydral group of reduced glutathione (GSH) in serum sample to produce intensely yellow color. The absorbance of reduced chromogen is estimated at 412 nm that directly proportional to GSH concentrations (Burtis and Ashwood, 1999).

4- **Level of malondialdehyde (MDA) concentrations**

The concentrations of MDA is based on the ability of MDA to react with thiobabituric acid (TBA) producing final product (MDA-TBA2) and its absorbance is read at 532nm. (Guide and Shah, 1989)
C- statistical analysis
All results of the present study are showed as means ± standard deviation (SD) and the results are analyzed by using SPSS program. The differences among different groups are performed by using Student t- test and p< 0.05 represented the lowest significant limit (Daniel, 1999).

Results of the study
The results that are obtained from the present study are illustrated in the following table which explained below and these results have a significant increase (p<0.05) in the levels of thyroid hormones(T3 and T4) associated with decrease levels of TSH in older men in a comparison with younger men. In regard to levels of cortisol and testosterone hormones were significantly drop (p<0.05)in older men. Furthermore, values of antioxidants ( GPx,GSH, Catalase) showed a remarkable fall (p<0.05) in older men. In contrast, concentrations of(MDA), a final product of lipid peroxidation, indicated a significant increase(p<0.05) in aged men higher than of younger men.

Table :- Shows the results of hormone levels (thyroid stimulating TSH MIU/ml, Triiodothyronine T3ng/ ml, Tetraiodothyronine T4 ug/ml, Cortisol ng/ml, Testosterone ng/ml ) and oxidant- antioxidants activities (Malondialdehyde MDA umol/ml ,Glutathione peroxidase GPX U/L , Reduced glutathione GSH mg/dl, and Catalase K/ml) in younger and older men.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Younger men (21-30 years)</th>
<th>Older men (61-70 years)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH ( MIU/ml )</td>
<td>2.66± 0.33</td>
<td>1.65 ±02*</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>T3 ( ng/ml )</td>
<td>0.947± 0.03</td>
<td>1.75 ±0.35*</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>T4 (µg/ml)</td>
<td>10.4 ± 1.33</td>
<td>12.18 ± 0.61*</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Cortisol ( ng/ml )</td>
<td>115.45± 4.16</td>
<td>95.22±3.85*</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Testosterone( ng/ml)</td>
<td>11.66±1.27</td>
<td>7.84± 1.01*</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>MDA (µmol/ml)</td>
<td>14.99±2.42</td>
<td>39.80±5.98*</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>GPX ( U/L)</td>
<td>234.82±4.86</td>
<td>176.20±15.09*</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>GSH ( mg/dl)</td>
<td>46.96±4.15</td>
<td>24.69±2.76*</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Catalase ( K/ml)</td>
<td>3.77±0.96</td>
<td>1.24 ±0.55*</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

-All values are means ± standard deviation
-Means with mark * are significantly different(p<0.05).
-The following figures refer to correlations among T3 and testosterone hormones with MDA and GPX in both younger and older men:-
The results obtained from the present study indicated the following data:
1-There is a negative non-significant (p>0.05) correlation between testosterone and GPX activity in younger men.
2-There is a positive non-significant (p>0.05) correlation between testosterone and MDA concentration in younger men.
3-There is low negative non-significant (p>0.05) correlation between T3 and GPX activity in younger men.
4-There is a low positive non-significant (p>0.05) correlation between T3 and MDA concentration in older men.
5-There is positive non-significant (p>0.05) correlation between T3 and MDA concentration in younger men.
6-There is no correlation between T3 and GPX in older men.
7- There is low negative non-significant (p>0.05) correlation between testosterone and MDA level in older men.
8- There is a negative non-significant (p>0.05) correlation between testosterone and GPX in older men.

Discussion
Despite increase health care programs and requirements of all modern life style such as nutritional demands and several facilities of life affairs particularly in developed countries, the ageing phenomenon or ageing problem remains the fate of all humans. The exact and causative factors responsible for this problem remain obscure. Many theories and suggestions tried and offered multiple explanations, but, most if not all of previous and recent efforts brought more attentions toward oxidative stress, so that the present study involved the investigations to estimate of some hormones that fluctuated throughout the age and perhaps their relations with antioxidant systems of aged men compared to their counterparts of younger men.

Thyroid hormones
In this study, it found that the levels of T3 and T4 are significantly (p< 0.05) higher in older men than of younger men, but on the other hand, the levels of TSH are significantly (p< 0.05) decreased in aged men.
In elderly human, the thyroid gland functions are subjected to many changes in their hormone production, metabolism and activities, moreover, there are a wide reference ranges have been found in the levels of both thyrotropin (TSH) and thyroid hormones (T3 and T4) between younger and aging population (Aggarwal and Razi, 2013). As individuals become aged, their body systems begin from cells and their cellular components have undesirable changes that they target especially endocrine organs, those abnormal changes can affect synthesis or secretion of hormones as well as target tissues having their receptors that are specialized to those hormones. In addition, it was found that FSH and LH hormones seem more affected and most of these changes were found to be associated with peripheral degradation or resistance to thyroid hormones (World Population Prospects, 2006).
Some previous results confirmed that ageing is implicated to increase prevalence of sub-clinical hypothyroidism that associated with increased levels of TSH and free T4 particularly in individuals over 60 years old (Biondi and Cooper, 2008).

The present results are also inconsistent with previous study of Margi et al. (2002) who showed that thyroid gland appears normal and with no significant abnormalities in its size and appearance, but on other hand, they confirmed increased tissue density although the iodine trapping mechanism remains with normal limits, finally, these observations indicated increase of TSH levels associated with down regulation of T3 and T4, these observations apparently conflict with results obtained from this study and may be affected because of the local and special factors of those population.
In regard to response of central nervous system to the repeated stress, it is that found changes occurred with progress of age through perform study on experimental animals (rats), the findings of this study indicated increase stress with advanced age resulting in up regulation of responses by serotogenic neurons to stress related with age (Yamaguchi et al., 2016) so, the presented results can be explained there are some problems affected hypothalamic-pituitary thyroid/adrenal axis at a level thyroid or
adrenal cortex. From physiological point view, that the thyroid hormones have abilities to produce ROS through their functions involve increasing the basal metabolic rate and accelerating respiratory chain rate (Villanueva et al., 2013) and enhancing the production of ROS that confers the prove increase oxidative stress.

Cortisol

Levels of cortisol indicated a significant lowering (p<0.05) in older men. Study of Wong and Shobo (2017) found that older men have high risk for development of hypothalamic–pituitary–adrenal dysfunction than of younger men. Among hormones, Cortisol represents the common hormone that has fluctuations and diurnal variations during the day, the cortisol peak appear after 20 to 30 minutes from beginning of wakefulness and then return to lower limits in the remaining time of the day (Fries et al., 2009).

The axis of HPA and thyroid become less effective when individuals reach the advanced age (Chahal and Drake, 2007). Previously, it is well established that younger men is more reactive to stressful conditions than of older men because younger men have little cognitive behaviors in a comparison with older men to deal with stress states (Neupert et al., 2007).

Data of the present study are inconsistent with study of Rehman and Masson (2001) that established dysregulation of negative feedback mechanism to stress conditions including HPA axis and they confirmed that adrenocorticotropic and cortisol hormones can remain with normal lower limits in younger individuals than older men and who suggested that a high level of activation and impairment of adequate responses is less active in older men.Larsson et al. (2009) showed different results and confirmed increase secretion of cortisol with progress of age. Indeed, the increase of HPA axis reactivity in response to stressful conditions in older ages continue for long time in contrast to younger ages (Bazhanova et al., 2000).

Alemany (2012) in his study confirmed the facts that sexual hormones (androgens and estrogens) inhibit inflammatory processes and tissue responses to actions of glucocorticoids, with age, there is a down regulation of androgen and estrogen lead to increase factors that can mediate by increasing levels of glucocorticoids. Previous research that has carried out on lab animals to explain how the advanced age can affect adrenal cortex, it is found that zona fasciculata and reticularis become hypertrophy in aged animals with the size and number of their intracellular organelles, these finding are attributed to increase the levels of adrenocorticotrophic hormone and cells of adrenal cortex unable to uptake and utilization of cholesterol molecules, and concluded there is a drop in the levels of glucocorticoids in older animals (Rebuffat et al., 1992).

Most if not all, older human undergo from many neurodegenerative diseases at different levels and these diseases can be masked, a higher levels of glucocorticoids, in particular, cortisol are found significantly associated with hippocampal abnormalities and impairment of memory. such alterations in aged men can lead to prolonged secretion of cortisol and disturbances of circadian rhythm (Lupien et al., 1998). The present data appear conflicted with results of Van et al. (1996) that established HPA axis affect with minor changes that related with aging and levels of glucocorticoids remain with normal values or moderately increased.

It is well documented in experimental studies that injection of animals with cortisol did not suppress or activate antioxidant systems and these studies concluded that high levels of cortisol can produce negative physiological events and not affect oxidant–antioxidant systems (Gauvin et al., 2017).
Testosterone

It is not surprising that ageing affects androgen hormone (Testosterone), the drop of serum testosterone in older men is called late hypogonadism (Perheentupa and Huhtaniemi, 2009). The previous study indicated decrease sexual activity with ageing and accompanied with drop of total and free testosterone levels (Oconnor et al., 2011; Orwoll, 2016).

A lot of biochemical changes are associated with decreased levels of testosterone in aged men, there is an increase of sex hormone binding proteins in blood circulation lead to decrease of free testosterone and the lowering levels of this hormone can impair negative feedback mechanism on hypothalamic – pituitary that exert by hormone self and lead to increase the gonadotropin (FSH and LH) levels (Lunenfeld et al., 2015). The drop of essential sexual hormone (testosterone) is correlated with many physiological changes in older individuals including hyperinsulinemia, increased triglycerids and low density lipoproteins, decreased high density lipoproteins, and neurophysiological changes (Bhasin et al., 2010). Recent study indicated that treatment of old men with testosterone leads to support and improve several sexual indicators including sexual activity, sexual desire, and erectile functions (Cunnigham et al., 2016).

Concerning the relation of testosterone with oxidative stress, previous researchers confirmed apposite and different outcomes; it is documented that testosterone can induce oxidative stress and observed decrease life span of red blood cells because they become less resistance to lyse generating by injection of testosterone to those experimental animals (Alvarez et al., 2007). In contrast, it is found that the drop of testosterone depress the expression of intratesticular antioxidants that in turn causes to suppress of spermatogenesis and extensive apoptosis of germ cells (Aitken and Roman, 2008). Also, recent further study confirmed that testosterone in healthy normal men can provide defense and neuroprotection to prevent incidence of neurodegenerative diseases, but in older men, there is increase of oxidative stress and low levels of testosterone, they become more susceptible to neurodegenerative diseases (Homes et al., 2016).

Antioxidants

The results of the present study showed clearly that antioxidant components undergo sharp decrease accompanied with a high level of lipid peroxidation products (MDA) in aged men. In recent years, many studies and efforts were focused to understand and explain the relation of oxidative stress and progress and incidence of ageing and chronic diseases (Verrugio et al., 2016). The present data are supported by Ide et al. (2002) who confirmed that men have higher levels of oxidative stress than of women.

In addition, recent study improve an understanding to explain how ROS have many deteriorations effects on many body organs and exert their harmful effects on mitochondria which become more subjected to much damage by ROS associated with lowering ATP production when human become aged (Abbas et al., 2017). Many of researches and studies are conducted to investigate the relationships between mitochondrial DNA (mtDNA) mutations and oxidative stress that take part in progress and development of ageing, mtDNA mutations are mostly originated from loss repairing mechanisms having nuclear DNA, according to these facts, there is no doubt that ageing is associated with accumulations of several mutations in mtDNA as a result of ROS (Pinto and Moraes, 2015).
Mitochondria and their components of respiratory chains represent the major pathway in generating reactive oxygen species as side products, respiratory enzymes become more exposed to these toxic compounds, moreover, reactive nitrogen species also are produced and synergized with ROS to increase injuries and damage of mitochondrial components causing several degenerative alterations that in turn lead to develop ageing (Murphy, 2009; Rju, 2014).

Although the toxic effects of ROS, they have some benefits and important to body functions such as killing of pathogens by immune system and intracellular signaling when they are presented at lower levels, but excess levels of ROS causes damage and deterioration to many organs and system rendering the body loss many vital functions and aging development (Jevas, 2016). mtDNA and nuclear DNA become more influenced by ROS, many modifications can occur in structures of DNA because of ROS and these modifications including break DNA strand, purine and pyrimidine changes. Also, many mutations hit the genomic potential such as point, translocation, and binding with other proteins (Girotti, 1985; Cui et al., 2012).

In conclusion, the results that obtained from this study indicated that there are many physiological disturbances occurring in hypothalamic adrenal and thyroid axis associated with fall levels of testosterone hormone. These hormonal dysfunction are also accompanied with down regulation of antioxidant systems and according to these data can be suggested the ageing is a physiological phenomenon resulted from accumulation of different abnormalities of homeostatic mechanisms responsible for adjustment of many body functions.

References


Zsanyi, A. (2001). On the true role of oxygen free radicals in living state. Age Ing