DOI : 10.35124/bca.2019.19.1.507

Biochem. Cell. Arch. Vol. 19, No. 1, pp. 507-511, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

STUDY FOR TESTOSTERONE AND OXIDATIVE STRESS IN MALES WITH BRONCHIAL ASTHMA

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(Accepted 25 February 2019)

ABSTRACT : Bronchial asthma, a chronic inflammatory condition, often triggered by allergy and oxidative stress, initiate lipid peroxidation and enhanced release of arachidonic acid from cell membrane.The effects of sex hormones on asthma symptoms and progression are complex and seem to be particularly associated with the fluctuation dynamics of the hormonal levels. Therefore, we have conducted this study. 50 clinically diagnosed bronchial asthma cases for Testosterone and oxidative stress levels were compared with 50 healthy controls. The results showed a significant decrease in serum Testosterone levels in all patients in comparison with control group (P≤0.05). Whereas, serum levels of the (MDA) and (CP) were significantly increased in all patients in comparison with control group (P≤0.05). No significant difference in concentration of serum TF in all patients in comparison with control group (P≤0.05). Bronchial asthma decreases the levels of both testosterone in males. Lipid peroxidation associates with bronchial asthma. Disorder the antioxidant system in patients with bronchial asthma, according to the levels of ceruloplasmin and transferrin.

Key words : Bronchial asthma, testosterone, lipid peroxidation, ceruloplasmin, transferrin.

INTRODUCTION

Asthma might be seen as a diffuse obstructive lung malady with hyper-reactivity of the airways to a range of stimuli and a high level of reversibility of the obstructive process, which may follow either spontaneously or as a because of treatment (William and Robert, 2001). When people talk about bronchial asthma, they are certainly talking around asthma, a common chronic inflammatory disease of the airways that causes the airway of the lungs to swell and narrow leading to coughing, shortness of breath, wheezing, chest tightness and excess mucus production (Joshua and Ruth, 2011). Asthma is a complex condition including biochemical, autonomic, immunologic, infectious, endocrine and psychological factors in variable degrees in different persons; it can be averted by staying away from causes, for example, irritants and allergens (Conboy-Ellis, 2006). Most persons can develop a severe exacerbation of asthma from various triggering agents. Bronchial asthma triggers may include smoking and second hand smoke; infections for exampleflu, colds, or pneumonia; allergens such as food, mold, mites, pollen, dust and pet dander; exercise; air pollution and toxins; weather, mainly great changes in temperature; drugs (for example aspirin and beta-blockers); food additives; emotional tension and anxiety; singing, crying, or laughing; perfumes and fragrances and acid reflux. Both virus and bacterial infections of the upper respiratory tract infection can worsen asthma (Kanazawa *et al*, 1991).

Epidemiological studies of both incidence and prevalence have reported a male predominance of asthma before puberty and a female predominance after puberty (Zannolli and Morgese, 1997). During the fifth or sixth decade, asthma seems to again become slightly more prevalent in men than women (Zannolli and Morgese, 1997). This time frame overlaps with a statistical decline of testosterone levels in aging men. The prevalence of hypogonadism was found to be 38.7% in men aged e"45 years presenting at primary care offices (Mulligan *et al*, 2006).

The reversal of the male/female prevalence of asthma and other atopic conditions at puberty strongly suggests a role for sex hormones. Although, the immunomodulatory properties of sex steroids have been known for many years, this information has seldom been applied to asthma and allergy, although there is evidence of their contribution to their underlying mechanisms (Canguven and Albayrak, 2011).

Atopy, production of specific IgE antibody, is the major risk factor for asthma and non-atopic individuals have a very low risk of developing asthma. IgE has the unique function of mediating immediate hypersensitivity reactions; it binds to specific receptors on basophils and mast cells and triggers the release of mediators. The IgE levels in atopic male subjects were significantly higher than in atopic female subjects after the age of 35 years (Barbee *et al*, 1987). In support of this observation, orchiectomy promotes and testosterone inhibits, production of antigen-specific IgE in males (Yamatomo *et al*, 2001) and dehydroepiandrosterone, the precursor of sex steroids and a weak androgen itself, lowers total IgE levels in males (Sudo *et al*, 2001).

Bronchial asthma is a chronic, inflammatory condition often triggered by allergy and oxidative stress. Oxidative stress, developed from imbalance between oxidants and antioxidants, may contribute to origin and development of several diseases including bronchial asthma. Lungs have the highest exposure to atmospheric oxygen and vulnerable to oxidative damage by oxidants and pollutants. The large endothelial surface makes it the major target site for circulating oxidants xenobiotics. Oxidative damage play one of the essential roles in development and persistent of bronchial asthma (Gutteridge and Halliwell, 2000).

Reactive oxygen species (ROS) can adversely affect airway cells and initiate lipid peroxidation, protein oxidation, DNA modification resulting in enhanced release of arachidonic acid from cell membranes, contraction of airway smooth muscle, increasing vascular permeability, increasing airway reactivity and airway secretion, synthesis and release of chemo attractants (Comhair and Xu, 2005). Cholesterol is the second most abundant lipid component of pulmonary surfactant, composing of 10-25% of total surfactant lipid. At least 80% of lung cholesterol appears to be derived from plasma lipoproteins (Turley *et al*, 1981).

Design of study

"This study conducted at AL-Hussein Teaching Hospital in Thi-Qar," especially, in respiratory counselling," Biochemistry Laboratory in the College of Science (University of Thi-Qar)" at the period between 1/10/2017 to 1/5/2018.

The study included (100) subjects, (50) controls and (50) patients of males. It is notable that the smokers were excluded.

The controls and patients were divided into two groups:

1- Control group: included (50) supposed healthy subjects of males aged (15-50 years).

2- Patient group: included (50) patients with bronchial asthma of males aged (15-50 years).

Collection of blood samples

"About 6mL of blood samples from bronchial asthma patients and controls were taken and allowed to clot at room temperature in disposable tubes centrifuge to separate it in the centrifuge at 3000 rotor per minute (rpm) for 10 min, Serum samples were separated and stored at (-20ºC) for later measurement of biochemical parameters, unless used immediately."

METHODS

Determination of serum testosterone

"Serum testosterone concentrations are determined by enzyme-linked through enzyme linked fluorescence test using the Mini-VIDAS Automatic immunofluorescence assay system (bioMerieux, Marcy letoile, Lyon, France)".

Determination of Serum Malondialdehyde (MDA)

"The level of serum malondialdehyde was determined spectrophotometrically according to the method of (Muslih *et al*, 2002). In brief; to 150 ìl serum sample the following was added: 1ml of 17.5% trichloroacetic acid TCA provided by BDH, England and 1ml of 0.66% TBA provided by BDH, England, mixed well by vortex, incubated in boiling water for 15 minutes and then allowed to cool. One ml of 70% TCA was added and the mixture allowed to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, the supernatant was taken out for scanning spectrophotometrically at (532nm)." The concentration of MDA calculated as follow:

$$MDA\left(\frac{\mu mol}{L}\right)=\frac{absorbance\ at\ 532}{L\times\varepsilon}\times D\times 10^{6}$$

L : light path (1cm)

ε : Extinction coefficient $1.56\times10^{5}\ M^{1}.cm^{-1} = 6.7$

D : Dilution factor = 1 ml Vol. used in ref./0.15

Determination of Serum Ceruloplasmin (Cp)

"Serum Cp concentration was measured according to the method of Menden *et al* (1997) 50 mg of PPD provided by BDH, England was dissolved in 5 mL (4 mL of distilled water (D.W) and 1 mL of glacial acetic acid). 8.15gm sodium acetate hydrate provided by BDH, England was dissolved in 30 mL of D.W then added to first solution, mixed and completed the volume to 50 mL with distill water. 100 mg of sodium azide provided by Reidledehain, Germany was dissolved in 500 mL of D.W then was kept cold in a refrigerator prior to use. One mL

of the first solution was pipetted into glass test tubes, incubated at 37°C for 1 min then 0.1 mL of serum was added, incubated at 37°C for 15 min, then tubes were removed and placed in iced-bath for 30 min five mL of cold sodium azide solution was added, mixed under 25°C in water bath. The absorbance of test and blank was read in spectrophotometer at 525 nm."The concentration of Cp was calculated as:

$$Cp\left(\frac{mg}{L}\right)=\frac{T-B}{\varepsilon}\times 10$$

ε = The extinction coefficient of Cp equal to 0.68

Determination of Serum Transferrin (Tf)

"Serum Tf concentration was measured by colorimetric method (Burtis *et al*, 1999). In which an excess of iron is added to the serum to saturate the Tf. The unbound iron is precipitated with basic magnesium carbonate. After centrifugation the iron in the supernatant is determined. The concentration of iron remaining is assayed and the result expressed as total iron binding capacity (TIBC). The serum Tf concentration was calculated from the following equation: (Serum Tf (g/L) = TIBC (μmol/L)/ 25.1) (Gambino *et al*, 1997)."

Statistical analysis

"The statistical analysis was done by using Microsoft Excel 2010. The results were expressed as mean ± standard deviation (mean ± SD) with LSD test. One way analysis of variance (ANOVA) was used to compare parameters in different studied groups. P-values (P≤0.05) were considered statistically significant."

RESULTS AND DISCUSSION

Serum Testosterone Concentration

Table 1 shows a significant decrease in concentration of serum testosterone in all patients in comparison with control group (P≤0.05).

Testosterone as well as its metabolites keep up maintain the physiological balance of autoimmunity and protective immunity by safeguarding the number of regulatory cells. Testosterone is an immunosuppressant and is probably going to be defensive against immunological and inflammatory processes that trigger asthma. We hypothesized that the testosterone or particular androgen receptor modulators would have beneficial effects on asthma and could diminish the danger of asthmatic attacks (Canguven and Albayrak, 2011).

In men, asthma bleakness remains generally stable from pubescence to the age-related drop in serum testosterone levels, which increases the risk of asthmatic impairment (Zannolli and Morgese, 1997).

The fundamental testosterone level changes are likely because of the stress, hypoxia and corticosteroid treatment. The possibility of a direct suppressive action of endo- and exoallergens on the testes are discussed (Mueva and Maleeva, 1988).

Serum Malondialdehyde concentration

Table 2 demonstrate a significant increase in concentrations of serum MDA in all patients in comparison with control group (P≤0.05), showing in thereby the relation of serum MDA level with that of the underlying inflammatory process in bronchial asthma. These imply that patients during asthmatic attack are wide-open to a great degree of lipid peroxidation (LPO). This result is consistent with the observations of others (Sayyah, 2011). MDA is a pointer of lipid peroxidation (LPO), it has a solid correspondence with atopic asthma, proposing that oxidative stress occurs simultaneously on lipid peroxidation. Oxidative stress can have different effects on the function of the airway, including contraction of airway smooth muscle, induction of airway hyper responsiveness, hyper secretion of mucus, vascular exudation and epithelial shedding. Moreover, reactive oxygen species can induce the production of cytokine and chemokine of during the initiation of oxidative stress-sensitive transcription on nuclear impact in bronchial epithelial cells includes the consumption of oxygen and consecutive release of reactive oxygen species into adjacent cells (Nada *et al*, 2010). During the respiratory burst, the inflammatory cells have released a high concentration of $O2\bullet^{-}$, OH•, HOCl and H_2O_2 that may leak into adjacent cells may resulting in increased amounts of free radicals in airway tissues. Moreover, the inflammatory cell of asthmatics has an increased capacity to produce free radicals, which may further contribute to high concentrations of reactive nitrogen species. One marker of airway inflammation in the asthmatics is the excess generation of reactive nitrogen species (RNS) (Umit *et al*, 2011). Cytokines may excite increased production of nitric oxide radical (NO•) which reacts with $O2\bullet^{-}$ to form peroxynitrite ($ONOO^{-}$), a cytotoxic species that has many destructive effects, including lipid peroxidation (LPO). Therefore, the additional amounts of reactive oxygen species and reactive nitrogen species that are produced by asthmatics may overcome the host antioxidant defenses and cause oxidative stress (Stefano *et al*, 2010).

Antioxidant system

Serum Ceruloplasmin concentrations

Table 3 shows a significant increase in concentrations

Table 1: Serum Testosterone levels in control and patients groups.

Groups	No.	Testosterone (ng/mL) Mean±SD
Control	50	4.57±1.15[a]
Patient	50	3.82±0.91[b]
L.S.D		0.03

Note: Each value represents mean ± S.D values with non-identical superscript(a, b or c etc.)were considered significantly differences (P≤ 0.05).
- No: Number of subjects. **- SD:** Standard deviation.
- LSD: Least Significant Difference.

Table 2 : Serum MDA levels in control and patients groups.

Groups	No.	MDA(µmol/L) Mean±SD
Control	50	2.47±0.41[b]
Patient	50	3.31±0.63[a]
L.S.D		0.17

Table 3 : Serum CP levels in control and patients groups.

Groups	No.	CP (g/L) Mean±SD
Control	50	2.02±0.27[b]
Patient	50	3.26±0.75[a]
L.S.D		0.18

Table 4 : Serum Tf levels in control and patients groups.

Groups	No.	Tf (g/L) Mean±SD
Control	50	3.49±0.85[a]
Patient	50	3.82±0.99[a]
L.S.D		0.43

of serum Cpin allpatients in comparison with control group (P≤0.05).

Increased ceruloplasmin levels were demonstrated in asthma patients (Vural and Uzun, 2000). Levels of certain proteins in plasma increase during the acute inflammatory state or according to certain types of tissue damage. These proteins are named acute phase proteins or reactants. They are synthesized by the liver in response to the mentioned cases. Following stimulus through infection or injury, the macrophages liberate interleukin-1 and monocline, which induce the liver to secrete a lot of acute stage proteins. These reactants have been shown to serve different effective roles during tissue rehabilitation of inflammation or injury in various mechanisms of host immune defense. Cp is recognized to keep considerable peroxidase activity and capable of suppression superoxide radicals. It is responsible for restricting the damage caused by these radicals (Ramesh and Ravindra, 2012).

Serum Transferrin concentrations

Table 4 shows no significant difference in concentration of serum Tfin all patients in comparison with control group (P≤0.05).

It has been shown the presence of inflammatory cells for example eosinophil, lymphocytes, mast cells and macrophages in different specimens of bronchial asthma patients. Secrete several sorts of cytokines of these inflammatory cells (Barnes, 2008). As a result, allergic inflammation and bronchial asthma include wide cytokine network. This cytokine and their complicated relationship have an important role in immune and inflammation. The acute stage reaction is a general response to inflammation through interleukins, released from this website of inflammation or injury (Alexandrakis *et al*, 2000).

CONCLUSION

Bronchial asthma decreases the levels of both testosterone in males. Lipid peroxidation associates with bronchial asthma. Disorder the antioxidant system in patients with bronchial asthma, according to the levels of (ceruloplasmin and transferrin).

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