Screening of Oxidative Stress and Prostate Cancer Biomarkers among Rural and Urban Elderly People in Erbil Governorate-Kurdistan Region

Peshraw S. Hamadamin¹*, Abbas B.Q. Salihî¹, Kamaran Abdoulrahman², Fikry A. Qadir¹, Rande Khasro¹, Jwana Najdat¹, Nishtiman Hamad² and Hawraz Najmaddin²

¹Department of Biology, College of Science, University of Salahaddin-Erbil, Erbil, Kurdistan Region, Iraq
²Department of Chemistry, College of Science, University of Salahaddin-Erbil, Erbil, Kurdistan Region, Iraq

1. INTRODUCTION

Prostate cancer (PCa) is an adenocarcinoma or glandular carcinoma, it is starts when the semen secreting epithelial cell mutate and become a cancer cells which result in deregulate of prostate growth (Galani, 2015). PCa is the most common form of non-skin malignancy in men and it is the second leading cause of cancer death after lung cancer among American males (Lara et al., 2004). Global Variation in the distribution of PCa incidence have been reported among different countries around the world (Scher and Chung, 1994). In developed countries such as Australia, United States and the Scandinavian countries, PCa is prevalent with about a 25-fold difference between high-incidence and low-incidence countries (Fleshner and Klotz, 1998). Furthermore, rural residents have higher cancer mortality than urban resident (Singh, G.K., et al. 2012). The reason for these differences is return to that rural residents may face challenges in accessing medical care and necessary support services because of extended and sometimes difficult travel and a limited number of health care facilities (Sabesan and Piliouras, 2009) Moreover, rural residents in general tend to be older, poorer, less educated, less likely to have insurance, and more likely to encounter transportation challenges, exacerbating health disparities (Hendryx et al., 2010).

Prostate cancer is usually screened by digital rectal examination and prostate-specific antigen (PSA) assay (Liong et al., 2012). Increasing the incidence of PCa and the early detection of low-risk tumors that may not clinically progress during lifetime is strongly related to the widespread use of PSA screening.

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*Corresponding Author:
Peshraw S. Hamadamin
Email: peshraw.hamadamin@su.edu.krd

ABSTRACT

The present study was designed to evaluate prostate-specific antigen (PSA) and oxidative stress (OS) markers in rural and urban old aged men in Erbil governorate, Iraqi Kurdistan region. The level of Prostate Specific Antigen (PSA), Malondialdehde (MDA), total antioxidant activity (TAC) and nitric oxide (NO) were determined among men aged over 40 years living in different rural regions and center of Erbil city. The results indicated that TAC in urban males was significantly higher than rural people, while the level of NO in rural males was significantly higher than urban males. On the other hand, there were no statistical significant differences between serum PSA and MDA levels of rural and urban residences. In conclusion, these findings indicated obvious differences between urban and rural people regarding the OS markers excluding prostate tumor marker.
and extended prostate biopsy protocols (Ferro et al., 2013). The broad prevalent of total PSA testing in blood has revolutionized PCa screening and resulting in a decrease of PCa metastasis and death (Shariat et al., 2011), because patients with PCa have high levels of serum PSA, this is return to enhanced production of PSA together with architectural distortions in the gland that increases PSA access to the circulation (Liong et al., 2012).

Although the causes of the high incidence of prostate cancer are poorly understood, epidemiological, experimental and clinical studies, suggest that oxidative stress (OS) plays a major role in explaining prostate cancer development and progression (Freitas et al., 2012). Cells are facing OS either by inadequacy of cell antioxidant system, or through excessive production of reactive oxygen species (ROS). Raising the ROS production in the cell is highly contributed to tissue injury, DNA damage, neoplastic transformation and aberrant growth and proliferation (Lambeth, 2007). The incidence of PCa and aberrant changes in ROS become more common with aging (Khandrika et al., 2009) due to the modulation of androgens, inflammation, vitamin D, tumor suppressor protein and antioxidants (Minelli et al., 2009). Because of the lack of information about the distribution of PCa between rural and urban people in Kurdistan region, this study was designed to determine the differences of PCa incidence between males of old rural and urban residents in Erbil governorate through measuring the level of serum PSA and to elucidate the role of OS in the progression of PCa.

2. MATERIALS AND METHODS

2.1 Study Subjects and Data

This study was conducted on 80 healthy men; 40 from villages of Mergasor, Soran and Harir provinces and 40 from center of Erbil city of ages 40-60 years old, who had no evidence for any prostatic diseases, none of them were smokers, alcoholic, or under medications like antihypertensive or had been already been diagnosed with PCa.

The current study was carried out in Department of Biology, College of Science University of Salahaddin-Erbil from November, 2015 to March, 2016. The specimen collection from both groups was carried out using venous blood. Serum was separated by centrifugation for at least 15 min at 2500 rpm within one hour of collection. The separated samples were either used immediately for the study of total PSA, MDA, NO and TAC or kept until further analysis.

2.2. Estimation of Total Prostate Specific Antigen

Total PSA was measured by electrochemiluminescent immunoassay (ECLISA) reagent kit and it was assembled into a ready-for-use unit on cobas e411 immunoassay analyzer. In brief, calibrator and quality control barcode were ascend, then barcode-labeled calibrator and quality control vials placed on sample disc, calibration and quality control were measured, calibration result was validated by quality control values. Serum samples were placed on sample disc then results were obtained after 14 hours. The analyzer automatically calculates the analytic concentration of each sample in ng/ml (Finne et al., 2002).

2.3. Determination of Serum Malondialdehyde

Malondialdehyde level was estimated according to method of Kartha and Krishnamurthy (D'souza et al., 2012). MDA was determined spectrophotometrically with a thiobarbituric acid (TBA) solution. In brief, 1ml of trichloroacetic acid (TCA) 17.5 % with 1ml of 0.66% TBA were added to 150μl serum
sample, mixed well by vortex, incubated in boiling water for 15 minutes, then allowed to cool at 25 °C. One ml of 70 % TCA was added and the mixture was left to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15minutes, then the supernatant was taken out for scanning spectrophotometrically at 532 nm.

2.4. Determination of Serum Total Antioxidant Capacity

Serum TAC was measured by quantitative colorimetric assay, using Total antioxidant Capacity - QuantiCromAntioxidant Assay Kit (BioAssay systems, USA; DTAC-100). Twenty five µl of standards and samples were pipetted into the respective wells of microtiter plate, then 100 µl reagent mixtures A and 50 µL of reagent mixture B respectively pipetted into the wells. The microtiter plate was incubated exactly for 20 min at 2–8 °C, 50 µL of the stop solution pipetted to each well and the absorbance of the solution in the wells were read at 450 nm (Hadzovic-Dzuvo et al., 2011).

2.5. Determination of Serum Nitric Oxide

Serum NO was determined by the Griess method. In brief, 0.5 ml of serum samples were deproteinized by adding 10 µl of sodium hydroxide and 300 µl of 0.15M Zinc sulfate, mixed well by vortex, incubated on ice for 15 min and centrifuged for 10 min at 3000 rpm. To reduce nitrate to nitrite, 0.5 ml of supernatant mixed with 0.5 of glysen and 2-3 gram of Copper coated Cadmium granules and shaked for 30 min. 0.5 ml of mixture was mixed with 0.5 ml of Griess reagents [250 µl of N-(1-naphthyl) ethylenediamine hydrochloride (0.1%) and 250 µL of 1 % Sulphanilic acid in 5 % phosphoric acid], then incubated for 15 min at 37 °C and absorbance was read at 543 nm using light spectrophotometer. Concentration of NO in serum samples was determined from the linear standard curve established by 0–250 µM sodium nitrate (Asl et al., 2008, Sun et al., 2003).

2.6. Statistical Analysis

Data obtained were presented as mean±SE. The differences for all variables studied were tested by unpaired t-test. All statistical tests were two-tailed and a (P≤0.05) was considered statistically significant. All the graph, calculation and statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, San Diego, California, USA).

3. RESULTS AND DISCUSSION

Recent studies showed a relative PCa mortality reduction of at least 20% by PSA-based population screening (Schroder et al., 2009). The present study is attempted to report to disparities of PCa markers PSA as a screening test for early diagnosis of PCa, MDA as OS and TAC as antioxidant capacity between rural and urban residents were studied.

The results of the present study showed that there were no significant differences between the level of serum PSA in rural (0.85±0.19 ng/ml) and urban (1.2±0.15 ng/ml), as shown in figure 1. This data suggested that the incidence of PCa was very low and there was no difference in its occurrence between residents of rural and urban. This result is entirely not compatible with data reported during 1970-1990, which showed a large rural–urban cancer mortality inequality exists worldwide and rural residents are certainly at higher risk (Miller et al., 1987). Furthermore, Coory and Baade (2005) reported that in Australian regional and rural areas 110 extra deaths from PCa occurred each year and that this has been increasing over time. The reason for equality of rural-urban of PSA may be related to similarity of lifestyle and improvement of education level of rural areas as well increasing the facility for of rural people to get to medical accesses. Overall
access to health care is a strong cancer mortality predictor and this may have a prominent role in rural–urban inequality in PCa mortality. Due to geographical limitations and limited transport options, many rural residents have limited access to clinics and hospitals with the advanced technology necessary for early cancer detection (Pruthi et al., 2006).

Figure 1: Serum total PSA (ng/ml) level in rural and urban males of Erbil city. No significant difference was observed between these two groups of residences. Data are expressed as Mean±SE. Statistical analysis was performed using unpaired t-test.

Malondialdehyde is the final product of lipid peroxidation, so it is an indicator of OS and antioxidant status of cell (Gaweł et al., 2003). Researchers have proved that increased cell OS play important role in PCa initiation and progression by directly affecting on DNA or through regulation of enhancers, transcription factors and cell cycle regulators (Gupta-Elera et al., 2012). An increase in free radicals causes overproduction of MDA. Therefore, the level of MDA was measured to compare the differences between rural and urban aged male residents of Erbil city regarding OS. The results showed that there were no significant differences between rural (9.76 ± 1.28 mM/L) and urban (9.05±1.74 mM/L) males, as shown in figure 2. The similarity of serum MDA level of male rural and urban population was supported with the finding of (Reddy et al., 1993), which was achieved in India. However, according to another study which held in Mexico city, urban males had higher level of MDA compared to rural residents (Sánchez-Rodríguez et al., 2006).

Figure 2: The level MDA (µM/L) among rural and urban men of Erbil city. Data are expressed as Mean±SE. Statistical analysis was performed using unpaired t-test.

The antioxidants are the substances that reduce oxidation of substrates and constitute the body’s main protection materials against free radicals injury. Rajneesh et al. (2008) showed that PCa is positively associated with OS, since antioxidants reduce oxidation by OS action; it is inversely associated with PCa (Vance et al., 2016). According to previous study, high aggressive prostate cancer patients had lower plasma TAC compared to low and intermediate aggressive PCa cases (Vance et al., 2014). In the present study, the TAC in urban males (1.44±0.087µM/L) is significantly higher (P<0.001) than rural males (1.2±0.15µM/L) as shown in figure 3. This could be possibly due education levels of urban areas being higher than that of rural areas, this
education making the urban residents more knowledgeable of healthy lifestyle choices such as diet and exercise (Pitsavos et al., 2005).

Figure 3. Rural-urban differences in serum TAC (mmol/L) level in Erbil city. Urban males had significantly (P<0.001) higher concentration than urban males. Data are expressed as Mean±SE. Statistical analysis was performed using unpaired t-test. *** indicates significant difference at level P<0.001.

Nitric oxide (NO) is endogenous, free radical gas, which plays an important role in many physiological and pathological processes. However there is a great contravery and confusion about the role of NO in cancer biology (Choudhari et al., 2013). It is said that NO is double-edge sword which has both pro and antitumeral effects depend on its timing, location, and concentration (Weiming et al., 2002). In this study, NO level among rural males (51.87±2.57µM/L) was significantly higher (P<0.01) than urban males (39.07± 2.35µM/L), (Figure 4). The level of NO is highly sensitive to altitude, the levels of NO and NO-derived molecules increase at high altitude. Limited data suggest processes including hypoxic upregulation of NO synthase gene expression, hemoglobin-NO reactions and genetic variation (Beall et al., 2012).

Figure 4. NO concentration (µmol/L) of rural and urban males of Erbil city. Rural males had significantly (P<0.001) higher concentration than urban males. Data are expressed as Mean±SE. Statistical analysis was performed using unpaired t-test. *** indicates significant difference at level P<0.001.

4. CONCLUSIONS

These results showed that the level of TAC is higher in residences live inside Erbil city due to educational status and type of food intake, while serum NO is extremely increased in rural residences due to high altitude. On the other hand, serum PSA and MDA levels were in normal range, these results together imply the negative prediction of PCa in Erbil city.

Conflict of Interest

There is no conflict of interest.

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