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INTERRELATIONSHIP OF CIRCULATING BIOCHEMICAL VARIABLES OF MEDICAL IMPORTANCE AND THEIR ROLE IN THE PATHOGENESIS OF PROSTATE CANCER

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ABSRACT

Vitamin-D is best known for its role in the regulation of calcium and phosphorus levels. Now a days with the development of advanced methodology other hormonal impacts, such as anti-proliferative activity of Vitamin-D in various classes of cancers is revealed. Vitamin-D modulates several functions including cell's growth, apoptosis and differentiation. Objective of present study was to evaluate the role of Vit-D, MDA and 8OHdG in the pathogenesis and progression of prostate cancer. Among 100 individuals 50 were taken as healthy controls and 50 with diagnosed prostate cancer were taken as subjects. Levels of Malondialdehyde (MDA) were measured through spectrophotometer, whereas levels of Vitamin-D and 8-Hydroxy deoxyguanosine (8-OHdG) were measured through commercially available ELISA kits. Current study elucidates significantly increased levels of MDA (p=0.019) and 8-OHdG (p=0.022) in the case of prostate cancer patients (2.19±0.0912 nmol/ml and 1.06±0.011 pg/ml) respectively which are the end-products of lipid peroxidation and DNA damage. As the lipid peroxidation increases it results in the increase oxidative stress within the body. Moreover levels of Vitamin-D (p=0.017) were significantly decreased in subjects (17.29±3.99 nmol/ml) as compared to the healthy individuals (31.06±4.26 nmol/ml). According to the current finding it can be concluded

that higher levels of ROS and decreased levels of Vitamin-D may lead to the production as well as progression of prostate cancer. Addition of antioxidant and maintenance of physiological levels of Vitamin-D in the body may be future of prostate cancer prevention as well as treatment.

Keywords: Vitamin-D, Prostate cancer, MDA, 8-OHdG

INTRODUCTION:

Despite Vitamin-D being best referred as a controller of the calcium and phosphorus, there is an expanding enthusiasm to explore additional hormonal impacts of Vitamin-D in different parts of the body. Vitamin-D modulates several functions including cell's growth, apoptosis and differentiation but not only limited to it. In skin cells, 7-dehydrocholesterol undergoes photolyzation by UV light to deliver preVitamin-D_3; converted to Vitamin-D_3 later by thermal isomerization, this whole process being self-maintained and controlled. It is noted that this conversion is reduced to less in the senile population [1]. Vitamin-D is fundamentally combined with Vitamin-D binding protein (DBP) after exiting the skin via circulation. Its activation in the body is started by hydroxylations catalyzed by cytochrome p450, first hydroxylation occurring in the liver aided by the mitochondrial sterol 25-hydroxylase at the position C-25, yields 25-hydroxyVitamin-D_3; the main Vitamin-D in circulation [2]. Second hydroxylation, happening in the kidney at position C-1 with the help of enzyme named 1α-hydroxylase, fabricates the most dynamic type of Vitamin-D to be specific 1α,25-dihydroxyVitamin-D_3. 1α, $25(OH)_2D_3$ and $25OHD_3$ is inactivated by 24-hydroxylase enzyme that is present in mitochondria. Hydroxylization of $25OHD_3$ or 1α, $25(OH)_2D_3$ beginning at C23 or C24 positions yields a more hydrophilic item for removal via the kidney. In kidney, 1α, $25(OH)_2D_3$ induces 24-hydroxylase while restraining 1α- hydroxylase expression [3]. Vitamin-D implicates its impact on gene expression by the receptors known as Vitamin-D receptors (VDRs), although it provides its focal part in pleiotropic activity of Vitamin-D [4]. 1α, $25(OH)_2D_3$ being the most active type of Vitamin-D_3 is involved in the regulation of calcium but now it has been recognized that this active form also has anti-proliferative activity in normal as well as oncogenic cells, so less calcemic analogues are suggested in treatment of potential cancer. The anti-proliferative function of 1α,$25(OH)_2D_3$ is because of the VDR mediated pathway that is present in the nucleus. This aids in controlling the target gene expression, eventually causing cell

apoptosis and differentiation after the cell cycle is curbed in the G1/S phase [5].

Cancer remained a condition reported highest occurrence of death worldwide out of which second most common type is prostate cancer among the American males. In 1989, approximately 28,500 men died because of prostate cancer among 103,000 diagnosed cases of prostate cancer [6]. VDRs are found in prostate epithelium as well as in oncogenic cells of prostate and clinical evidence shows its role in inhibiting the growth of epithelium found in prostate and cancer-causing cells [7,8]. Epidemiological studies also reinforce the hypothesis that an important role against prostatic cancer is played by the Vitamin-D [9]. There are three important epidemiological risk factor for prostate cancer; age, race, and geography. In advance age prostate cancer is very common due to less exposer of ultraviolet light and synthesis of Vitamin-D declines with age. Second important factor is race. The occurrence rate of prostate cancer in American black is twice as compared to the Whites. In black the high risk of prostate cancer is due to the higher level of melanin that prevents the conversion of previtamin in skin. Certain geographical areas i.e. U.S has more mortality rate due to prostate cancer that is strongly associated with decreased exposer of ultraviolet light. Conversely, high levels of $25OHD_3$ might possibly lead to prostate malignancy [10]. Previously anti-proliferative mechanism of Vitamin-D_3 was not well known but with the development of microassay technology its investigation brought to light the presence of many cells and genes being reactive due to Vitamin-D. Lymph Node Carcinoma of the Prostate (LNCaP) being overly sensitive to Vitamin-D has aided in screening for Vitamin-D target genes. The LNCaP cell growth is almost repressed by 10 nM of $1\alpha,25(OH)_2D_3$ and Vitamin-D3 stops the LNCaP cells in G1 phase [11]. In prostate malignant cell cycle is arrested mainly due to the cycline dependent kinase inhibitor P21/WAF1 and P27 pathways. $1\alpha,25(OH)2D_3$ up regulates the P21/WAF1 in both mRNA and protein, and hence the resulting cell arrests in G0/G1 phase [12]. As far as P27 is concerned $1\alpha,25(OH)_2D_3$ causes increased expression by decreasing CDK2 protein where CDK2 degrades the P27. As a result cells do not progress from G1 to S phase [13]. Studies conducted on prostate cancer indicate that decreased levels of Vitamin-D may lead to prostate cancer and in contrast it is also noted that increased levels of Vitamin-D beyond the physiological limits also increases the risk of cancer [10]. The present study focused on the relationship of

Vitamin-D, oxidative markers MDA and 8-OHdG in the pathogenesis of prostate cancer.

MATERIALS AND METHODS:

For the current study about 50 patients of prostate cancer and 50 age and sex matched controls were selected. All of the work was carried out by approval of Ethical committee of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore. They were screened for the possible oxidative stress and Vitamin-D deficiency. An informed consent was obtained from the patients before including them into the study. 5ml of blood was taken from cubital vein and stored at -70^{o}C after centrifugation for further analysis.

INCLUSION CRITERIA

Patients with clinically diagnosed cases of prostate cancer were included in the present study.

EXCLUSION CRITERIA

Patients suffering from diabetes, hypertension, myocardial infarction or any other hepatic, pulmonary pancreatic or renal diseases were excluded from the present study. 5.0 ml of venous blood was drawn from healthy individuals (controls) and cancer patients undergoing anticancer therapy.

BIOCHEMICAL ANALYSIS

The samples were processed and analyzed for the estimation of Vitamin-D and 8-OHdG by ELISA kit method and MDA by spectrophotometer method.

DETERMINATION OF MDA

Patients were screened for the determination of increased lipid peroxidation due to stress by determining the amount of MDA with the help of spectrophotometer. Lipid peroxidation (MDA) in blood was estimated calorimetrically by measuring Thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa [14]. To measure MDA, 0.2ml of sample, 0.2ml of 8.1% Sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA was added. After centrifugation at 3000 rpm for 10 min the upper organic layer was taken and its OD was read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as milimoles of Thiobarbituric acid reactive substances (TBARS)/g of blood using standard curve.

RESULTS

For the current study total of fifty (50) patients of prostate cancer and fifty (50) age and sex matched controls were added in the study and were analyzed for their antioxidant status, Vitamin-D and lipid peroxidation. All of the statistical analysis

was done with the help of SPSS version 16. It depicted that in the case of prostate cancer the patients had increased lipid peroxidation which was estimated in terms of Malondialdehyde (MDA) as it is believed to be the end-product of the lipid peroxidation. Furthermore, increased lipid peroxidation is signified by the increased levels of MDA. Figure (A) showed that MDA levels were increased significantly in patients of prostate cancer (2.19±0.247 nmol/ml, p=0.019) when compared with the healthy group of population (0.94±0.153 nmol/ml). Increased level of MDA displays aggravation of a condition known as oxidative stress and decreased levels of antioxidants in the body. Similarly Figure (B) describes elevated levels of 8-OHdG in the diseased group (1.06±0.11 pg/ml, p=0.022) as compared to controls (0.018±0.0017 pg/ml). When MDA is increased significantly it plays vital role in the formation of DNA adducts and its damage which is on the later stages is estimated in terms of (8-OHdG). On the other hand when the patients were estimated for the level of Vitamin-D in their body they have reflected towards new sight. As known major source of Vitamin-D is through sunlight (UV-B)-induced photo-biosynthesis in the skin and it is found to play its major role in skin and bones, however significant amount of Vitamin-D can be involved in the pathogenesis of prostate cancer. In case of current study a similar trend was seen which states decline in the level of Vitamin-D in diseased group as shown in Figure C in subjects Vitamin-D was recorded as (17.29±3.99 nmol/L, p=0.017) while in controls it remained (31.06±4.26 nmol/L). Reduction in the level of Vitamin-D signifies toward the new roots which may be involved in the pathogenesis of the disease and its aggravation. There may be several reasons involved including (socio-demographic distribution etc.).

Table 1: Levels Of Extrapolative Variables Of Medical Importance And Their Interplay In The Development Of Prostate Cancer

VARIABLES	CONTROL (n=50)	SUBJECT (n=50)	*P*- VALUE (0.05)
MDA (nmol/ml)	0.94±0.003	2.19±0.0912	0.019
8-OHdG (pg/ml)	0.018±0.0017	1.06±0.011	0.022
Vit-D(nmol/L)	31.06±4.26	17.29±3.99	0.017

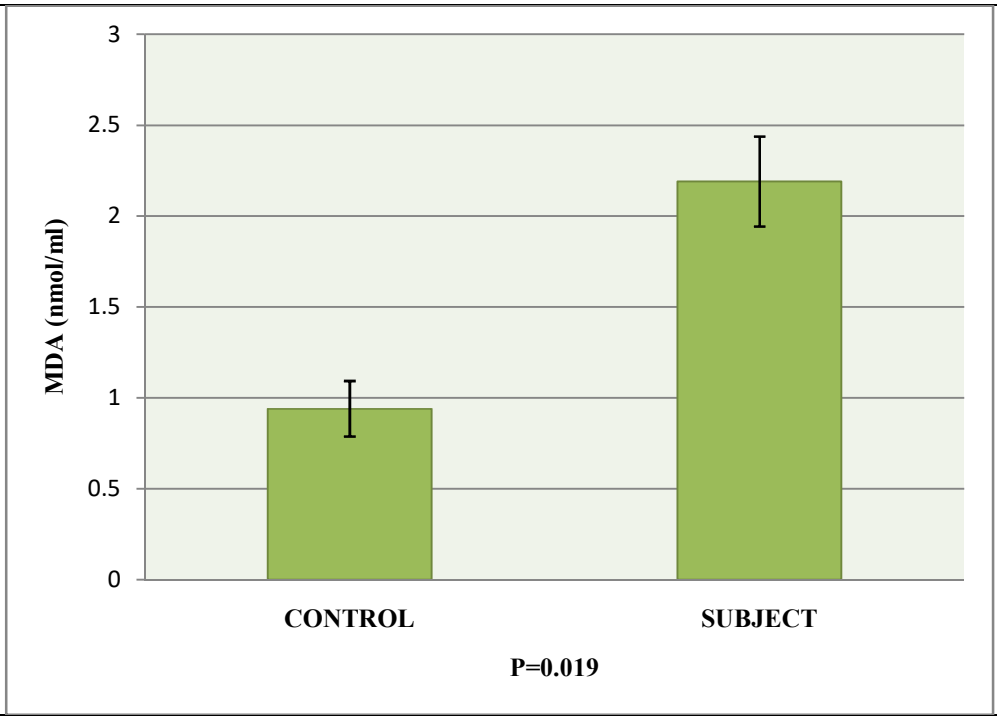

FIGURE:A

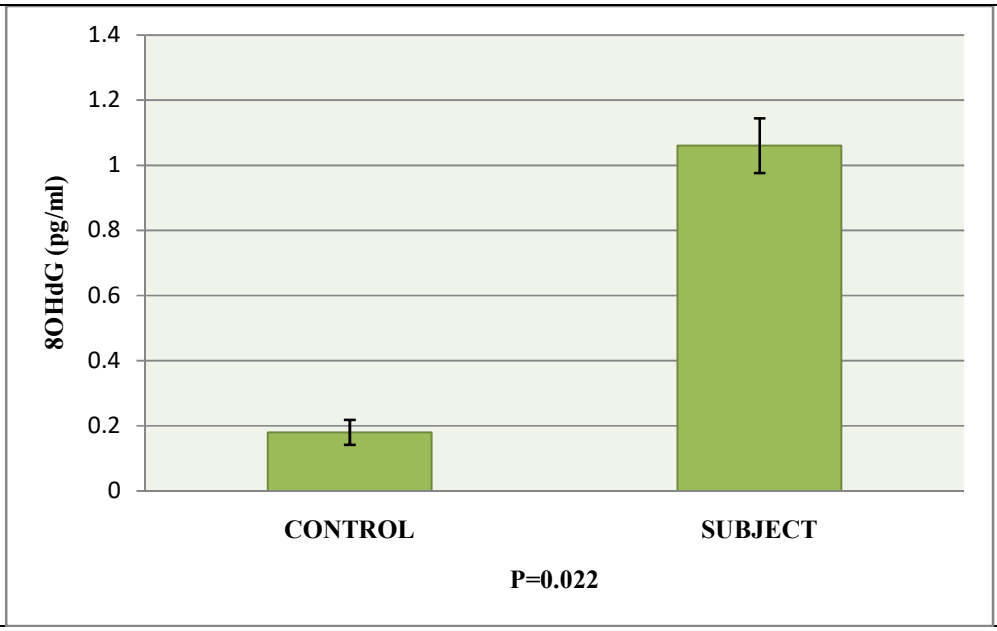

FIGURE:B

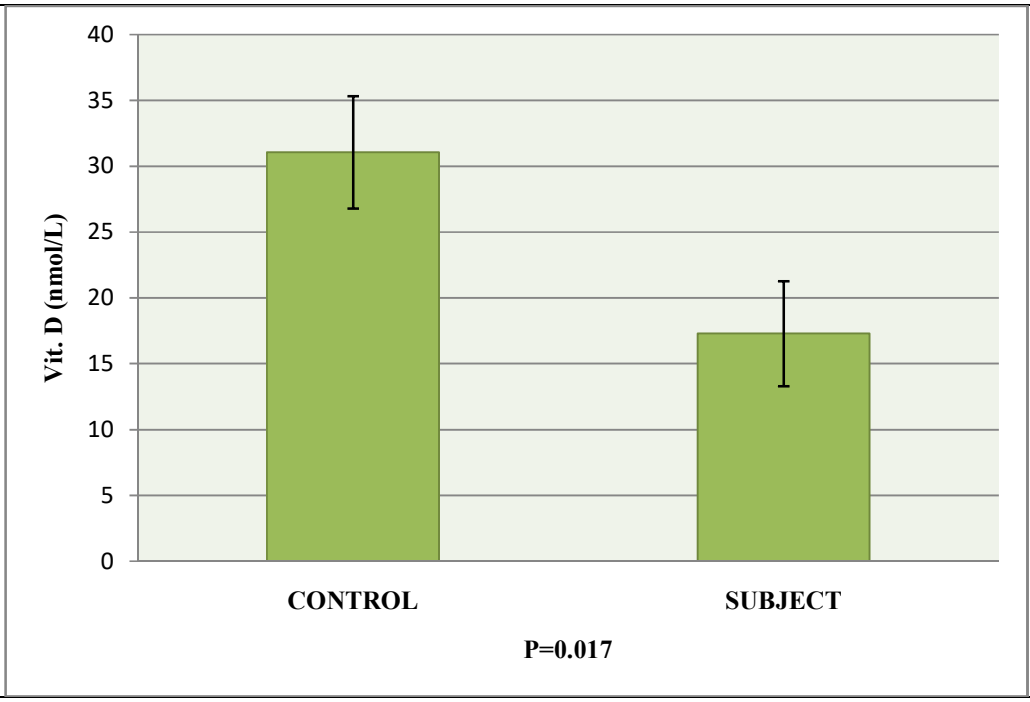

FIGURE:C

DISCUSSION

Progression of disease is related with number of factors including physiological and biochemical variables. Disease may be directly associated with the increased oxidative stress of free radical production and increased levels of NADPH Oxidase that plays a key role in the production of Reactive Nitrogen Species (RNS). Exact cause for the occurrence of disease is still unknown but several set of studies along with the current research work enabled to demonstrate some of the causative factors that may be involved in the aggravation of the pathological condition. Disease can either related with the increased lipid peroxidation under the influence of oxidative stress or may be the reason for the production of stress markers such as MDA. High levels of MDA might be involve in the formation of DNA adducts and leads to the DNA Damage which is estimated in terms of 8-OHdG.

Numerous clinical trials have suggested that increased oxidative stress (OS) is related to PCa and antioxidants can be employ to protect men from PCa [15,16]. Several studies conducted on both *In-Vivo* and *In-Vitro* to elucidate the mechanism of initiation and progression of PCa in relation to OS [17]. Oxidative free radicals are produced in biological system through multiple reasons such as modulation of androgens, inflammation, deficiency of

Vitamin-D, mutation in tumor suppressor genes (p53) and advanced age also involved in the production of OS which may instigate PCa [18]. It has been suggested that androgens promote production of ROS in PCa cells through signal transduction of multimeric redox-sensitive transcriptional factors, enzymes and genetic modifications. Increased levels of ROS in prostate epithelium due to androgens play an important role in development, progression and recurrence of PCa [19]. The aforesaid fact supported the study that castration or estrogen therapy involved in the regression of PCa specifically for androgen sensitive PCa [20]. How prostate cancer becomes castrate resistant is unclear, but some studies showed selective advantage of androgen ablation to androgen-independent cells [21]. Prognosis of castration-resistance prostate cancer (CRPC) is poor as compare with the castration-sensitive prostate cancer. Treatment option is limited to symptomatic relief of bone metastases most commonly in CRPC [14,22]. Hence, it may be postulated that antioxidant combine with other conventional therapies may restrain the progression of PCa [23].

Recent study also showed the Levels of MDA and 8-OHdG were significantly increased in the case of prostate cancer as compare to the control group. As shown in table-02 that increased levels of MDA directly effects on the DNA resulting in high levels of 8OHdG (MDA Vs. 8OHdG, r=0.568). To date, no randomized controlled trials have been led to decide the effect of OS on the d anger of creating PCa. Various investigations have endeavored to inspect the impact of exogenous cell reinforcements in anticipating tumor recur and decreasing the danger of creating malignancy. These investigations incorporate lung, breast, [24,25] colorectal, gastrointestinal, head and neck, leukemia, bladder cancer, and PCa [26] and discoveries have been conflicting. Further, there have been reported increased risks of antioxidant rather than their defensive impacts. The real restriction of these investigations is the absence of consideration of the redox balance amongst oxidation and antioxidants, and the twofold edged impact of exogenous cell reinforcements. Supplementation of exogenous antioxidants in the long-term without observing the redox balance can bring about gainful and in addition unsafe impacts relying upon the convergence of ROS and the expected adds up to keep up or re-build up redox homeostasis in every individual patient [27]. Studies propose that high measurements of exogenous cell

reinforcements could incomprehensibly go about as a genius oxidant by upsetting the redox balance. In this way, the harmony amongst oxidation and antioxidants agents is a basic issue to consider in an individual patient while evaluating the anticancer impact of cell reinforcements. As far as anyone is concerned, there have been no writing surveys looking at the relationship amongst OS and men with PCa. Besides, none of the previously mentioned examinations have analyzed the impact of the redox balance in men with PCa. Appropriate link in diet and cancer is also observed that how diet in higher calcium content is linked with increased risk for developing prostate cancer and how lower levels of Vitamin-D are involved in disease aggravation. Epidemiological studies also reinforce the hypothesis that an important role against prostatic cancer is played by the Vitamin-D [9]. Conversely, higher levels of $25OHD_3$ might possibly lead to prostate malignancy [10]. Previously anti-proliferative mechanism of Vitamin-D_3 was not well known but with the development of microassay technology its investigation brought to light the presence of many cells and genes being reactive to Vitamin-D. LNCaP being overly sensitive to Vitamin-D has aided in screening for Vitamin-D target genes. The LNCaP cell growth is almost repressed by 10 nM of $1\alpha,25(OH)_2D_3$ and Vitamin-D3 stops the LNCaP cells in G1 phase [11,28] In prostate malignant cell cycle is arrested mainly due to the cycline dependent kinase inhibitor P21/WAF1 and P27 pathways.$1\alpha,25(OH)2D_3$ up-regulates the P21/WAF1 in both mRNA and protein, and hence the resulting cell arrests in G0/G1 phase [12]. Since the impact of Vitamin-D on cell cycle is assisted by the retinoblastoma protein therefore when the retinoblastoma protein expresses vigorously it effect the Vitamin-D activity [5,22]. It is also proposed that insulin-like growth factor binding protein 3 essential for $1\alpha,25(OH)_2D_3$ induces cell cycle inhibition by enhancing the expression of P21/WAF in LNCaP cells [24]. In previous studies it was postulated that fatty acid synthetase is associated with the growth inhibition of LNCaP cells through Vitamin-D. Vitamin-D_3 exhibits different actions on growth of cells and gene expression in different prostatic cells by affecting different mechanisms [29,30]. Moreover, depletion of Vit-D leads to increased oxidative stress (r=-0.759** MDA Vs. Vit-D). Androgen is another factor that has a significant role in Vitamin-D dependant inhibition of cell growth [9]. It is seen that *In Vitro* studies, the outcome of Vitamin-D on

LNCaP (which is inhibitory in essence) has been proven to be androgen- dependant [8]. 1α-hydroxylase is found in both human prostate malignant cells and cultures of non-cancerous prostatic cells [30]. It was observed that 1α-hydroxylase activity is more in benign prostatic cells as compared to prostate cancer cells. In cultures, $25OHD_3$ (physiological concentration of100nM) encouraged the appearance of 1α-hydroxylase mRNA, but $1\alpha,25(OH)_2D_3$ did not show any effect on 1α-hydroxylase mRNA (Lou *et al*., 2004). Since the finding of 1α-hydroxylase activity in prostate leads to the autocrine role of $1\alpha,25(OH)_2D_3$ has been gaining significant recognition, In cells having 1α-hydroxylase activity $25OHD_3$ is shown to arrest the propagation of prostatic epithelium cells. As $25OHD_3$ is converted to $1\alpha,25(OH)_2D_3$ intra-cellularly, the side effect of hypercalcemia can be evaded, thus $25(OH)_2D_3$ can be used in the cure of prostate cancer [17]. Hence it can be concluded that as $25OHD_3$ is activated by the 1α-hydroxylase that might be key to the anti-proliferative activity of Vitamin-D. As in senescence, levels of 1α-hydroxylase decrease, which might be the cause of prostate cancer in senility. With the discovery of VDR in prostate cancer cells it was found that 1α-$25(OH)_2D_3$ increases the level of 24-hydroxylase in DU125 and PC3 and DU145 cells but it is not true in the case of LNCaP cells. Utilizing real time-polymerase chain reaction (RT-PCR) helped demonstrate that $1\alpha25(OH)_2D_3$ remarkably raises the 24-hydroxylase mRNA in LNCaP cells and also in stroma of human prostatic cells. Human prostatic carcinoma cells were observed to have 24-hydroxylase involvement, which was up-managed by $1\alpha25(OH)_2D_3$ [14]. It is also observed that the growth inhibition of prostatic cells by $1\alpha25(OH)_2D_3$ decreases with the increase of 24-hydroxylase. Hence it can be conclude that the levels of 24-hydroxylase with age increase and the anti-proliferative action of $1\alpha,25(OH)_2D_3$ decreases which may lead to prostate cancer in old age [19].

In humans the levels of vitamin metabolites in prostate and serum were measured which showed that the amount of $25OHD_3$ is equal or slightly higher in prostatic tissue whereas the levels of $1\alpha,25(OH)_2D_3$ and $25,24(OH)_2D_3$ are much higher in prostate. So it suggests noteworthy metabolism of $25OHD_3$ in the prostate [30]. Recently a new Vitamin-D endocrine system has been discovered. In light of this, the new system indicated that $25(OH)D_3$ was seems to an active hormone in controlling proliferation and gene regulation of prostate

stromal cells. It was also observed that in physiological concentrations, $1\alpha,25(OH)_2D_3$ was rendered inactive but $25(OH)D_3$ was the active hormone. The action of $25(OH)D_3$ is not due to the 1α-hydroxylase because inhibition of the 1a-hydroxylase leads to depletion of anti-proliferative activity of $25OHD_3$. Hence it can be concluded that $25OHD_3$ can effectively controls the proliferative activity and differentiating effects of prostate, and not the $1\alpha,25(OH)_2D_3$, which in turn is controlled by the levels of calcium and PTH, therefore; $1\alpha,25(OH)_2D_3$ is not linked with a decrease in the danger of prostate tumor [5]. Thus decreased levels of $25OHD_3$ may lead to prostate cancer and in contrast it is also noted that increased levels of $25OHD_3$ beyond the physiological limits also increases the risk of cancer. It is postulated that elevated levels of $25OHD_3$ may induce the production of 24-hydroxylase.

Table 2: Pearson S' Correlation Coeefficients Of Different Variables In Prostate Cancerpatients

VARIABLES	MDA (nmol/ml)	8-OHdG (pg/ml)	Vit-D(nmol/L)
MDA (nmol/ml)	1	0.568	-0.759**
8-OHdG (pg/ml)		1	-0.881***
Vit-D(nmol/L)			1

CONCLUSION

The current study concluded that as the levels of ROS and Vitamin-D are disrupted such as ROS is increased and Vitamin-D is decreased, they may contribute in the pathogenesis of prostate cancer. Therefore, by the supplementation of antioxidants and Vitamin-D the risks of prostate cancer may be decreased.

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