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# Study of Association among Vitamin D, Testosterone and Semen Quality in Fertile and Iraqi Infertile Men

**MUSTAFA T. ALBALDAWY[1] AND ALAAULDEEN S. ALSALAMI[2]**
*[1]MSc. Student, Department Of Biology, Faculty of science, Kufa University*
*[2]Assist professor Dr., Department Of Biology, Faculty of science, Kufa University*

**Abstract**

**Objective:** The objective of the study was to determination the biochemical changes and it's relationships in each of three infertile groups (Teratozoospermia, infertile normozoospermia compare with fertile normozoospermia).

**Methods:** Samples were collected at the Fertility Center Laboratories in Sadr Medical City, Najaf ,Iraq. Seminal fluid analysis were carried out on the samples, and samples that taken were 37 of Teratozoospermia, 34 of unexplained infertility samples and 17 of control group samples. A serum was then taken for biochemical tests, where vitamin D, vitamin D receptor (VDR), Testosterone, parathyroid hormone (PTH),Protamin enzyame 1( PRM1) by ELISA method and Calcium, Zinc by spectrophotometer test was measured.

**Results:** The result showed a significant decrease ($p<0.05$) of VD, Ca+2 and Zn+2 levels in both Teratozoospermia and infertile normospermia compared with control group, While significant increased ($p<0.05$) of PTH and PRM1 levels in both Teratozoospermia and infertile normospermia compared with control group. And the results showed non-significant ($p<0.05$) in both VDR and testosterone hormone.

**Conclusion**: A significant decrease and increase in the levels of each of above biochemical markers may be causes problem in infertile patients .

**Keywords: Infertility, VD, PTH, PRM1, $Ca^{+2}$, $Zn^{+2}$**

## INTRODUCTION

Infertility is a typical illness of the reproductive system, incapacity to have healthy birth following one year of effectively endeavors of unprotected free intercourses [1]. Around 15% of the couples on the planet confront failure in the primary involvement in pregnancy. These issues in these couples can be explained as infertility [2]. Male fertility depended on coordinated between hormonal and neural mechanism or amongst male reproductive system and these mechanisms. So any hindering, for at least one of these mechanisms will lead to infertility [3]. The imbalance of hormones have a critical significant among the reasons of infertility; hence, the examination of this flaw is essential in numerous pathologic cases that mirror the functional status of the endocrine glands [2], and because infertility in the world is a typical medical and social issue since the fifties of the most recent century and is around 15% of couples are suffer from infertility and around 40% of these percent are infertile by male factors which causes male infertility [4]. Semen examination is the most well-known essential test, considered unaltered, inexpensive, quick and rout test, that used as bedrock to determine the male infertility, but other reasons of infertility can't discovered by this test. So in view of different examinations that adopted as more precise screening test however not replaced to test of the seminal plasma [5]. In some cases, the sperms that have typical shape and normal count, despite this sperms can't fertilize the ova, because of biochemical disorder, this implies the typical values of seminal plasma test don't give ensure fertilization. Also, some defects in semen characteristics can know well the reasons for infertility for prevention from difficulties and treatment it all the more precisely [6]. On the other hand, most researchers show the significance of hormonal examination, other than screening of semen, the role functions of hormones by means of complex activities that is fundamental for spermatogenesis [7]. The factors of Male infertility are various, for example, varicocele that found in around 2-22% of infertile men that result from lessening level of Testosterone in serum [8]. In addition to hormonal defects, hereditary factors, environmental factors, coital factors and idiopathic factors that constitute around 25% of male infertility [9]. Some researchers classified reasons for infertility into four categories: male factors, female factors, congregated factors and idiopathic factors [10]. Additional factors that influence on male fertility involve weight of the body (body mass index), smoking and work [11]. Large quantities of biomarker proteins, a large number of essential protein and specific proteins in tissue that have been found in the seminal plasma that represent precise indicator for pathologic status related with reproductive system [12]. Vitamin D plays an important role in metabolism of calcium and phosphorus ions. Its primary activities involve absorption of intestinal calcium and reabsorption of renal calcium, and additionally an immediate impact on chondrocyte and osteoblast differentiation and resulting in bone formation [13]. The Accumulating evidence from human studies proposes that vitamin D, is side from its regulatory effects on musculoskeletal health, and is included in reproductive role in both sexes. The basis of the exchange between vitamin D and reproduction lays on the existence of both vitamin D receptors (VDR) and 1a-hydroxylase (CYP27B1) enzyme in the reproductive organs [14]. The Epidemiological studies supports a positive relationship among the concentration of serum 25-hydroxy-vitamin D [25(OH) D] and motility of the sperm in both fertile and infertile male [15]. Protamines (PRMs) includes the biggest amount of nucleoproteins in develop sperm of human. These proteins are translated in steps 1-4 of spermatids. While synthesis of the relating proteins begins, with temporal delay, in step 4 spermatids [16]. Different studies reported that abnormal expressions of protamine gene in sperm of fertile men. Additionally, relationship of the changed PRM1/PRM2 ratio has been appeared with low count in sperms, reduced in motility of sperm and morphology, diminishes the fertilization capacity and increased sperm chromatin defect [17], [18]. Altered P1/P2 ratios in the sperm have additionally been accounted for to be one of the critical reasons for male infertility [19], [17], [20]. The purpose for this changed ratio might be an interrupted post-translation modification or mutation in the PRM/TNP genes [17], [20]. This study deals with some hormones (Testosterone hormone and Parathyroid hormone) and some Vitamins (Vitamin D and Vitamin D receptor (VDR)), and with some trace elements (Calcium and Zinc), and also with nuclear protein (protamine) . Each of these, have an important role in the spermatogenesis and detection part of problems that related with the male infertility, along with the seminal plasma parameters.

## Materials And Methods

Semen and serum specimens were collected from teratospermic, infertile normospermic patients in addition to control group (Normozoospermia) that attended to fertility center. The average age of infertile patients was (33± 1.24) years, the samples were collected are 215 and sample which tested are 88 samples, the sample which obtained from control group (fertile) was 17samples (Normozoospermia), and 37 samples from teratospermic and 34 samples from infertile normospermic patients. A biochemical test was performed on (88) samples. The following hormones (Testosterone and PTH) and (Vitamin D, VDR) and PRM1 had been measured by immunological method (Enzyme-Linked-Imuno-Sorbent- Assay) by using ELISA reader (Huma Germany origin), while $Ca^{+2}$ and $Zn^{+2}$ had been measured by using spectrophotometer. All specimens and reagents must be allowed to come to room temperature before use. All reagents must be mixed softly without foaming. Once the procedure has started, all steps must be completed without interruption, and biochemical tests were conducted in the laboratories of Biology Department/ faculty of Sciences/ University of Kufa. The ELISA kits used in this study was (25 (OH) Vitamin D (VD220B), PTH (PT311T), Testosterone (3725-300), CALBIOTECH company USA in Origin) and (Human VDR (Vitamin D Receptor) (E-EL-H2043), Human PRM1 (protamine 1) (E-EL-H5684) Elabscience company china in Origin), while $Ca^{+2}$ and $Zn^{+2}$ kits was (Calcium (BT294QY) UK in Origin and Zinc (IFUFCC56) Germany in Origin.

## Results

The result showed a significant decrease ($^*p<0.05$) of VD level in both Teratozoospermia (26.49 ± 3.73) and infertile Normospermia (26.67 ± 2.87) compared with control group (51.80 ± 5.23), While there was non-significant difference ($p>0.05$) between Teratozoospermia and infertile normospermia as in Figure 1. Also, significant decrease ($^*p<0.05$) of $Ca^{+2}$ level in both Teratozoospermia (2.040 ± 0.050) and infertile Normospermia (1.988 ± 0.050) in compared with control group (2.265 ± 0.032), While there was non-significant difference ($p>0.05$) between Teratozoospermia and infertile normospermia as in Figure2. The result showed a significant decrease ($^*p<0.05$) of $Zn^{+2}$ level in both Teratozoospermia (2.094 ± 0.040) and infertile Normospermia (2.047 ± 0.030) in compared with control group (2.265 ± 0.032), While there was non-significant difference ($p>0.05$) between Teratozoospermia and infertile normospermia as in Figure3. While significant increase ($^*p<0.05$) of PTH level in both Teratozoospermia (121.5 ± 15.30) and infertile Normospermia (120.3 ± 14.88) in compared with control group (59.13 ± 7.85), While there was non-significant difference ($p>0.05$) between Teratozoospermia and infertile normospermia as in Figure 4. Also the result showed a significant increase ($^*p<0.05$) of PRM1 level in both Teratozoospermia (416.3 ± 21.48) and infertile Normospermia (443.9 ± 34.94) in compared with control group (298.9 ± 11.31), While there was non- significant difference ($p>0.05$) between Teratozoospermia and infertile normospermia as in Figure 5. The result showed non-significant ($p<0.05$) of both Testosterone and VDR in both Teratozoospermia and infertile Normospermia in compared with control group.

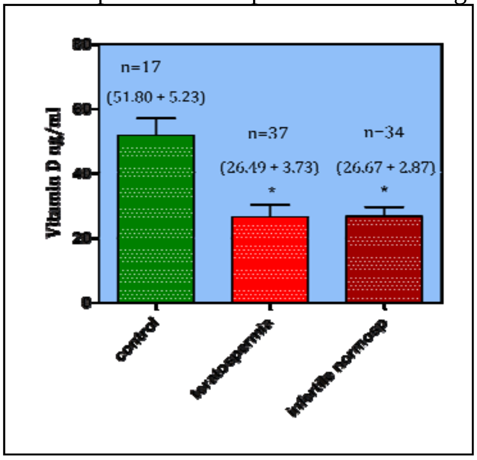


Figure 1: the comparison of Vitamin D concentration between Teratozoospermia, infertile Normospermia with fertile men serum (control).

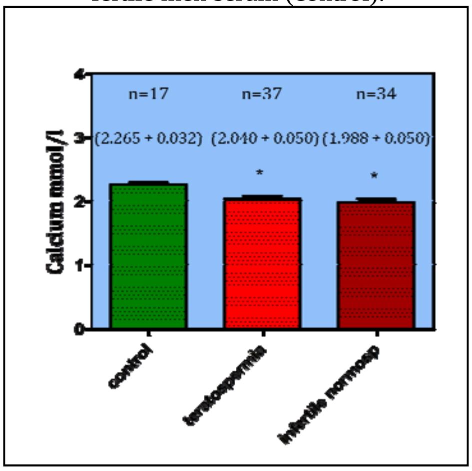


Figure 2: the comparison of $Ca^{+2}$ concentrations between Teratozoospermia, infertile Normospermia with fertile men serum (control).

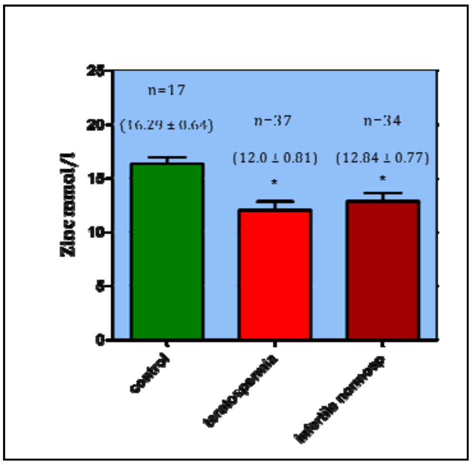


Figure3: the comparison of Zn+2 concentrations between Teratozoospermia, infertile Normospermia with fertile men serum (control).

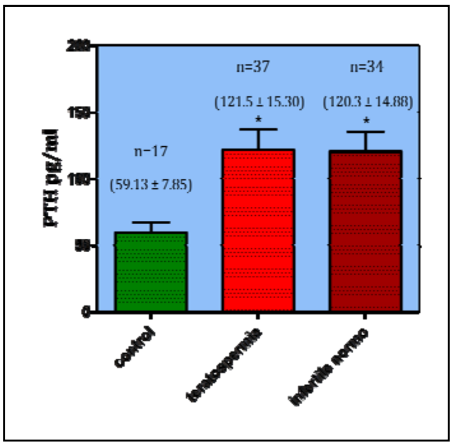


Figure 4: the comparison of PTH concentration between Teratozoospermia, infertile Normospermia with fertile men serum (control).

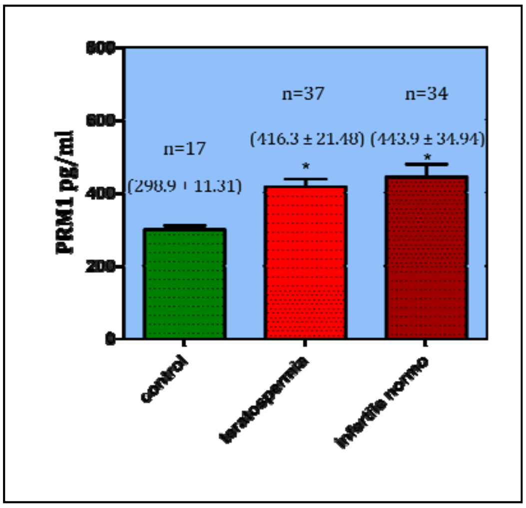


Figure 5: the comparison of PRM1 concentration between Teratozoospermia, infertile Normospermia with fertile men serum (control). * This mean significant difference (p<0.05).

## DISCUSSION

In the current study, the level of VD has a significant decrease (*p<0.05) in serum in Teratospermia and infertile Normospermia compared with control group. This study agreed with Karras et al that found VD has a significant decrease (p<0.05) in infertility patients compared with healthy men. The study showed the lower gene and protein expression of CYP2R1 in patients, resulting in deficiency in vitamin D and lower in bone mass, in spite of normal testosterone concentrations [21]. Men with vitamin D deficiency (VD < 25 nM) had a lower ratio of motile, progressive motile and normal morphologically spermatozoa compared with healthy men [22]. In a Chinese study VD concentrations were independently associated with sperm motility and morphology, only in infertile men. Association between VD status and testosterone concentrations, exist conflicting data. Both positive and negative association between VD and total serum testosterone has been reported [23]. Furthermore, no difference in VD status was found in men with congenital hypogonadotropic hypogonadism in compared with healthy men. The reasons for these contradictions may be differences in age, metabolic parameters (BMI, insulin resistance), fertility status, or ethnicity between the participants, as well as androgen assessment methods [24]. In the present study, showed VDR level has no significant (p<0.05) in serum in infertility patients compared with control group. Our results disagreed with Martin Blomberg et al that found VDR has a significant increase (p<0.05) in infertility patients compared with healthy men, therefore, study suggest that sperm motility could be influenced through VDR-regulated active calcium transport in the male reproductive tract, which is a prerequisite for generating a 2- to 3-fold higher calcium concentration in the seminal fluid compared with serum [15], [25]. Loss of VDR-regulated calcium transporter, expressed in the epididymis, leads to impaired sperm motility and infertility due to impaired cellular calcium transport and subsequent changes of the epididymal fluid concentration [26]. The current study, the level of PTH has a significant increase (*p<0.05) in serum in Teratospermia and infertile Normospermia compared with control group. Foresta et al who agreed with our study, showed the level of PTH has a significant increase (p<0.05) in infertility patient`s serum compared with healthy men, in their study taking 98 patients with reduced in spermatogenesis showed that reduced in VD and higher PTH concentrations compared with healthy men. The study appeared the low gene and protein expression of CYP2R1 in these patients' men, result in deficiency in VD and low bone mass, despite normal testosterone concentrations [27]. Ogard et al also agreed with our results reported that a significant correlation has been found between PTH and VD classifications. Previous searches showed that higher Parathyroid hormone level was in those who had low vitamin D levels [28]. PTH play an important role to increase the concentration of calcium in the blood by acting on the PTH 1 receptor which is present at high levels in bone and kidney, and the PTH 2 receptor, which is present at high levels in the central nervous system, pancreas, testis, and placenta [29].

The results of our study showed a significant decrease (*p<0.05) in $Ca^{+2}$ level in infertile men compared with control group, therefore our study is agreed with Bassey et al that showed Ca+2 has a significant decrease (*p<0.05) in infertility patients compared with healthy men [30]. Also agreed with Wong et al that were worked in Netherland reported same results of low Ca+2 in the infertile men which accounts for low motility [31]. $Ca^{+2}$ are fundamental for reproductive functions in male include hyperactivation, acrosome reaction, spermatogenesis, and motility of sperm. VD regulates $Ca^{+2}$ levels through the Vitamin D receptor [32]. From result in the present study, the level of $Zn^{+2}$ has a significant deccrease (*p<0.05) in serum in Teratospermia and infertile Normospermia compared with control group is agreed with Jiang Zhao et al that showed zinc concentrations in infertile men were significantly lower than those in healthy men [33], and disagreed with Akinloye, O. that reported the zinc concentration in infertile men was significantly higher than normal men [34]. Results were appearing that zinc supplementation could significantly increase the sperm volume, sperm motility and percent of normal sperm morphology of infertile men. Also after supplementation of Zinc, the sperm quality was significantly increased in infertile men. Zinc concentration in human seminal plasma is higher than other tissues [35]. In the human reproductive system, Zn plays an essential role in spermatogenesis, from its formation and contribution to ultra-structural stabilization of chromatin compaction to change of mitochondria-dependent processes, like respiration of cell and apoptosis [36]. The results of current study showed a significant increase (*p<0.05) of PRM1 in infertile men serum compared with control group, therefore our results are agreed with Ni et al the study showed that the sperm protamine ratio on male fertility is significantly higher value of the PRM ratio in infertile men when compared with the healthy men [37]. Rogenhofer et al also agreed with our stud, this study estimate mRNA of protamine and protein ratio and found that infertile group exhibits a significantly increased PRM ratio in infertile patients compared with the controls group [38]. A number of former studies have reported a relationship between abnormal PRM1/PRM2 ratios and men infertility [19], [39] [17], [40], [41]. In spermatozoa of infertility men, deregulation of P2 occurs much more frequently than deregulation of P1, based on the cause that Protamine2 deregulation is responsible for the majority of cases including an aberrant protamine ratio [42].

## CONCLUSION

A significant decrease and increase in the levels of each of above biochemical markers may be causes problem in infertile patients and infertility patients present in Iraq in the current study have a linear shape association with vitamin D and not U shape.


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