American Journal of Immunology

Immunomodulatory Treatment of Infertility and Subfertility in Men with Elevated Antisperm Antibodies

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ABSTRACT

Etiologycaly, possible causes of subfertility or infertility in men are poorly defined, but one important category may be elevated level of antisperm antibodies (ASA) caused by infective and/or auto-immune diseases of male reproductive system. In this study, blood sera from 45 infertile men of different age with ASA positive ELISA test were examined for the serum level of ASA before and after treatments with 1α,25-dihydroxy-Vitamin-D3 and Dexamethasone. We observed 23 infertile men treated with Vitamin-D3/Dexamethasone during 30 days, 12 infertile men treated with Dexamethasone only during 30 days and 10 infertile men without any treatment. All selected patients showed poor parameters of semen analysis and high level of ASA serum concentration (>75 U/ml). Serum concentration of ASA in non-treated group (312.6 U/ml), Dexamethasone only treated group (288.0) and Vitamin-D3/Dexamethasone treated group (123.6 U/ml) are significantly different. In addition, serum level of ASA in Vitamin-D3+Dexamethasone treated group was significant less as compared to the level before the treatment ($P<0.01$). Immunomodulatory and suppressive effects of Dexamethasone and Vitamin D3 regarding antibody production, co-stimulatory molecules expression, immune cells communications and profile of cytokine network could explain these results. Since the approach is relatively new, this is the first study of Vitamin D3 and Dexamethasone treatment concerning the suppression of ASA production.

Key Words: *Antisperm antibody, ASA, Dexamethasone, Vitamin D3, Infertility.*

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INTRODUCTION

Autoimmunity to sperm can occur because sperm cell molecules are first expressed during sexual maturation, long after the perinatal period when immunological self-tolerance is induced and finished [1,2]. Protection against autoimmunity is provided by the blood-testis barrier composed predominantly of Sertoli cells isolating the tubular content from the vasculature, as well as limited lymphatic drainage of the testis [3]. Several other immunoregulatory mechanisms also play a significant role in prevention of antisperm immunity such as immunosuppressive prostaglandins of seminal plasma, as well as both systemic nonspecific and specific factors (immunoregulatory cells, cytokines, absence of co-stimulatory molecules expression etc.) [4]. When the blood-testis barrier is disrupted by disease and/or injuries humans can be autoimmunized by previously sequestered sperm and testicular molecules [5,6]. Generally, ASA formation can be induced primarily during infectious and noninfectious inflammations, or by obstruction of testicular efferent passageways [1,7]. The ASA was also induced after accidental

and/or surgical injury of testicles, exposure to very low temperature or cryptorchism [5,6]. Subsequently, infertility can result from antibodies directly binding the sperm, or from lack of spermatogenesis due to autoimmune orchitis. A similar phenomenon occurs in vasectomized laboratory rodents and humans, so many men choose to be vasectomized as a form of safe birth control [5]. A high percentage of these individuals develop granulomas of epididymis and testicular degeneration associated with the formation of ASA [5,8]. These data support well established belief that presence of ASA reacting with molecules on the spermatozoa can be considered as typical and specific immunological infertility [8].

ASA can impair fertilization in many different ways. They can interfere with sperm motility by immobilizing or agglutinating the sperm, or interfere with sperm-cervical mucus interaction and disturb transport of the spermatozoa [9]. At the level of uterine or oviduct fluids a similar phenomenon can occur, such as interference of the penetration into the oocyte, and perhaps zygote development by impairing early cleavage, or even damaging the implantation process [10]. Whether antisperm antibodies are involved in pregnancy loss is still debatable as no conclusive evidence is available in the present literature, so that this subject needs further research [9,10].

There are many evidences and experiences that corticosteroids and Vitamin D3 can affect immune response in humans on different levels, including IgG responses to self molecules [11,12], so we hypothesize that treatment with Vitamin-D3 + Dexamethasone can be useful and safely treatment of infertile ASA positive man.

MATERIALS AND METHODS

A total of 45 infertile men with serum level of ASA higher than 75 U/ml (as recommended by the ASA Kit manufacturer) and poor parameters of semen analysis comprised the study groups. Average age of all patients was 35.3±6.9* (*Values are mean ± SD). As a possible etiologic factor of ASA presence, we found cryptorchism in 11.43% (unilateral and bilateral), orchitis in 11.43%, varicocele in 25.71%, accidental trauma in 2.86%, surgical intervention in 2.86%, epididymitis in 11.43% and unknown etiology in 34.29% of patients. Basic parameters of semen analysis and serum level of ASA studied at the time of starting therapy and after 45 days are summarized in table 1.

Men Treated with Vitamin-D3 + Dexamethasone

Twenty-three men were treated with Vitamin-D3 + Dexamethasone. Vitamin D3 was administered orally (0.025 mcg/kg of body weight) during 30 days. Dexamethasone was administered during 30 days in dose-decreasing manner. On day one of the treatment, Dexamethasone was administered intramuscularly (im.) in one-day dose 110 mcg/kg. This was followed by one-day dose of 55 mcg/kg administered im. (days 2 and 3). The treatment with Dexamethasone was followed by decreasing oral dose, starting with 42 mcg/kg (on day 4 of the treatment) to 7 mcg/kg on day 30 of the treatment.

Men Treated with Dexamethasone Only

Twelve men treated only with Dexamethasone that administrated during 30 days as well as in previous group.

Men without any Treatment (Control)

Ten men were not treated and they will be included in treatment in one of further investigations.

Semen analysis

The semen analyses were performed according to the guidelines of the WHO. Normal values of descriptive semen parameters were also issued by WHO in 1992, that are generally used as reference [13]. Sperm count in all groups of patients was analyzed before the treatment and 15 days after treatment. Abstinence time before sperm sampling was 5 days.

Serum Antisperm Antibody ELISA Test

Serum concentration of ASA was performed on HUMAN ELISA READER instrument with *Immuno-Biological Laboratories* (IBL) Sperm Antibody Enzyme Immunoassay Kit. The test is based on a non-competitive ELISA technique. The strips were incubated with diluted sera (1:50) from patients, and after washing steps, were incubated again with peroxidase conjugated anti-human-Ig (IgA, IgG and IgM). Following the final wash and enzyme substrate addition, the developed color was determined using the HUMAN ELISA READER. Positive results are indicated by ASA concentrations > 75

U/ml in diluted sample of serum as recommended by IBL.

Statistical Analysis

All parameters of 3 study groups were analyzed. P value of less than 0.05 was considered to indicate statistical significance. Calculations were performed MS Excel® 2002 software by t-test.

RESULTS

Table 1 demonstrates basic parameters of semen analysis before and after treatment in all 3 groups of infertile men. No significant differences were found recorded in sperm count volume, sperm concentration and percent of spermatozoa with normal morphology before and after treatment in 3 studied groups (P>0.05). Likewise, no significant differences in motility and viability of spermatozoa were found before and after treatment in Dexamethasone and Control groups (P>0.05). Percent of mobile (P=0.021) and vital (P<0.01) spermatozoa in VD3D group is significantly higher after treatment in relation to the percent before treatment.

Table 1. Basic parameters of semen analysis and serum level of ASA before** and after*** treatments (45 days).

		Vitamin D3 + Dexamethasone n=23	Dexamethasone n=12	Control n=10
Volume (ml)	**	2.52±0.7	2.63±0.7	2.85±0.9
	***	2.61±0.7	2.69±0.5	2.95±0.4
Sperm concentration (10^6/ml)	**	17.0±9.4	16.0±11.4	17.4±9.0
	***	18.8±8.6	17.0±9.2	19.2±7.0
Mobile (%) (after 60 min.)	**	34.2±16.8	34.1±4.9	33.8±12.9
	***	45.2±14.9	35.3±6.6	34.5±8.3
Vital (%) (after 60 min.)	**	41.2±7.55	37.4±8.7	38.5±6.2
	***	51.6±9.7	39.1±9.5	36.0±8.2
Normal morphology (%)	**	61.0±15.2	64.2±11.3	62.7±6.0
	***	62.0±12.0	61.8±12.9	60.8±7.7
Serum level of ASA (U/ml)	**	311.2±77.5	321.9±87.2	317.2±80.0
	***	123.6±39.8	288.0±82.8	312.6±90.9

In control group, no significant changes were found in serum level of ASA regarding to start sample and after 45 days (P=0.54). Also, serum level of ASA in Dexamethasone treated group do not show significantly changes before and after the treatment (P=0.21), but the level of ASA in Vitamin D3 + Dexamethasone group was significant lower after treatment as compared to the level before the treatment (P<0.01).

DISCUSSION

Taking into consideration the function of blood-testis barrier and other micro-environmental immunomodulatory mechanisms that provide tolerance to sperm molecules, it is clearly that every breakdown of the barrier and the immunomodulatory mechanisms may lead to infertility with the autoimmune etiology. In most cases, the autoimmunity on testicular molecules resulting from trauma or infectious disease can generate ASA [1,5,6]. Mechanisms that can provide the autoimmunity and ASA production are micro-environmental acceleration of Th1 immunity, enhanced secretion of pro-inflammatory cytokines like IL-1, IFN-γ, TNF-α, reduced secretion of anti-inflammatory cytokines like IL-10 and TGF-β, up-regulation of MHC and

co-stimulatory molecules expression and down-regulation of immune cells apoptotic mechanism [1,4,5,6].

In this study, we found that poor parameters of semen analysis, elevated level of ASA and infertility in men are linked with history of cryptorchism, orchitis, varicocele, epididymitis and accidental or surgical trauma of male genital tract. Only a minority of the patients has no clear etiologic factor for ASA and infertility, although we have in mind that ASA may form as a result of exposure of sperm molecules to the rectal mucosa, and they have been detected in the sera of a high percentage of homosexual men [14].

The immunosuppressive effects of dexamethasone are multiplex. For instance, the drug suppresses molecule presenting cells, dendritic cells, down-regulates co-stimulatory and MHC molecules expression, as well as Th1 cells and production of pro-inflammatory cytokines. Dexamethasone has strong suppressive effects on macrophages and T cells, so that the effects can indirectly inhibit antibody production by B cells and proliferation of B cells clones [15,16]. Nevertheless, in our study the drug has no significant effect on ASA level in patients treated with dexamethasone only. Curtis et al. [16] instigate similar findings that dexamethasone has no effects on serum level of sperm agglutinating antibody in vasectomized men [16]. However, Shulman et al. [17] found that methylprednisolone could increase incidence of pregnancy and live birth rates in infertile couples, albeit the ASA level was not significantly different before and after the therapy [17].

Vitamin D3 inhibits production of monocytes-derived cytokines such as IL-1α, IL-6, and TNF-α. The proliferation of T cells and their release of cytokines such as IL-2 and IFN-γ are also suppressed by Vitamin D3, partly because pre-transcriptional reduction of T cell-activating cytokines production, but also because of a direct effect on the T cells [18]. Although Vitamin D3 has no apparent effect on B lymphocytes, the T cell suppression may indirectly inhibits antibody production by B cells [19]. Vitamin D3 directly inhibits IFN-γ secretion by Th1 clones while it has little effect on IL-4 secretion by Th2 clones. These facts are important due to IFN-γ and IL-2 induce B cells to produce IgG2a while IL-4 and IL-10 induce the production of IgG1 and IgE by B cells [18,19]. These actions of the vitamin D3 suggest that it may have potential therapeutic quality in Th1-mediated autoimmune disease [20]. In addition, VD3 inhibits the ability of molecule presenting cells to induce T cell activation and might involve down regulation of co-stimulatory molecules. The inhibitory effect of VD3 on dendritic cell maturation was comparable to that induced by IL-10, a cytokine which inhibits molecule presenting cells at different levels, including inhibition of IL-12 secretion and MHC molecules expression [18,21].

Synergistically immunomodulatory effects of VD3D treatment might be acceptable explanation for significant differences in serum level of ASA in VD3D group before and after the treatment. In addition, decreased level of ASA in VD3D group probably contributes to a significantly higher percent of vital and mobile spermatozoa after the treatment.

There are several techniques of processing semen to select the antisperm antibody-free sperm, or to free up sperm already coated with antisperm antibodies. Collection of the sperm samples directly into a culture medium, followed by rapid washing of the sperm seem to increase the proportion of antibody-free ejaculate and to improve the fertilization rate for in vitro fertilization and intrauterine insemination. Current techniques are partly efficient, so we suppose that VD3D protocol can be usable as pre-treatment of infertile men in procedures, such as IUI, IVF and ICSI.

We found that most frequent side effects of the treatments were gastro-intestinal disorders, increased body weight and slight edemas. In most cases, the side effects were not treated, but in some patients with gastro-intestinal disorders such as nausea and gastritis were successfully treated by *ranitidine* 150 mg twice daily orally, whereas edemas where treated by *furosemide* 10 mg every 2-3 days orally.

In conclusion, infertile men with elevated level of ASA and poor basic parameters of semen analysis can be treated with VD3D protocol with great chance for decreasing the level of ASA. Consequently, significantly increased motility and viability of the spermatozoa after VD3D treatment might be repercussion of low ASA activity. ASA may play a mayor role in pathogenesis of male infertility, so that VD3D combination may be useful as pre-treatment in different procedures of assisted reproduction. Further study is needed to identify the mechanisms of VD3D treatment action(s) in down regulating the ASA level and to appoint the real benefits concerning VD3D protocol.

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