American Journal of Immunology 1 (4): 130-134, 2005
ISSN 1553-619X

Immunomodulatory Treatment of Infertility in Men with Elevated Antisperm Antibodies

[1]Ivan Bubanovic, [2]Slobodan Kojic, [3]Stevo Najman and [4]Zlatibor Andjelkovic
[1]Department of Obstetrics and Gynecology, "Medica Centre", Nis, Serbia and Montenegro
[2]Department of Obstetrics and Gynecology, Medical Centre, Paracin, Serbia and Montenegro
[3]Institute of Biology, University Medical School, Nis, Serbia and Montenegro
[4]Institute of Histology, Medical Faculty, Pristina, Kosovska Mitrovica, Serbia and Montenegro

Abstract: Possible causes of subfertility or infertility in men are still poorly defined, but one important category may be elevated level of antisperm antibodies (ASA) caused by infective and/or autoimmune diseases, as well as injuries 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 (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. Before treatment all selected patients showed poor parameters of semen analysis and high level of ASA serum concentration (>75 U/ml). After treatment serum concentration of ASA in control group (321.8 U/ml) and Dexamethasone only treated group (287.7 U/ml) were significantly higher compared to Vitamin D3 + Dexamethasone treated group (120.2 U/ml). In addition, serum level of ASA in Vitamin D3 + Dexamethasone treated group was significant less as compared to the level before the treatment ($P<0.01$). The treatment by Vitamin D3 + Dexamethasone were accomplished by successful pregnancy in three cases (13%). These results can be explained by numerous immunomodulatory and immunosuppressive effects of Dexamethasone and Vitamin D3 regarding antibody production, co-stimulatory molecules expression, immune cells communications and development of specific cytokine network profile. Since the therapeutic 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, Dexamethasone, Vitamin D3, Infertility

INTRODUCTION

Autoimmunity to sperm can occur because sperm cell molecules are first expressed during sexual maturation, long after the prenatal 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, cryptorchism and exposure to very low temperature[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 source of 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

Corresponding Author: Dr. Ivan Bubanovic, Ob/Gyn Department, "Medica Center", Novosadska 1/c, 18000 Nis, Serbia and Montenegro

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 immunoglobulin responses to self molecules[11,12], so we hypothesize that treatment by 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 34.6±7.5* (*Values are mean ± SD). As a possible etiologic factor of ASA presence, we found surgical treated cryptorchism in 3 (6.67%) (unilateral or bilateral), other surgical treatments in 3 (6.67%), orchitis in 6 (13.33%), varicocele in 12 (26.67%), accidental trauma in 2 (4.44%), epididymitis in 4 (8.89%) and unknown etiology in 15 (33.33%) 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: The group of 23 men was 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). Further Dexamethasone treatment was followed by decreasing oral dose. The starting oral dose was 42 mcg/kg (on day 4 of the treatment), while finishing dose was 7 mcg/kg on day 30 of the treatment.

Men Treated with Dexamethasone Only: The group of 12 men was treated only with Dexamethasone that administrated during 30 days as well as in previous group.

Men without any Treatment (Control): The group of 10 men was 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 regarding demarcation of normal and abnormal semen analysis[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 using Immuno-Biological Laboratories (IBL) Sperm Antibody Enzyme Immunoassay Kit. The test is based on a non-competitive ELISA technique. The strips were incubated with patient diluted sera (1:50), 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. Elevated levels of ASA are indicated if ASA concentrations were higher than 75 U/ml in diluted sample patient sera as recommended by IBL.

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

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 of sperm count volume, spermatozoa concentration and percent of spermatozoa with normal morphology before and after treatment in 3 studied groups ($P>0.05$). Also, no significant differences in motility and viability of spermatozoa were found before and after treatment in Dexamethasone treated group and Control group ($P>0.05$). Interesting, the percent of mobile ($P=0.01$) and vital ($P<0.01$) spermatozoa in Vitamin D3 + Dexamethasone treated group was significantly higher after 45 days in relation to the percent before treatment.

In control group, no significant changes were found in serum level of ASA regarding to start sample and after 45 days ($P=0.44$). Also, serum level of ASA in Dexamethasone treated group do not show significantly changes before and after the treatment ($P=0.13$), but the level of ASA in Vitamin D3 + Dexamethasone group was significant lower after treatment as compared to the level before the treatment ($P<0.01$). Three cases from the group treated by Vitamin D3 + Dexamethasone were accomplished by successful pregnancy (13%).

Table 1. Basic parameters of semen analysis and serum level of ASA before† and after‡ treatment.

Parameters		Vitamin D3 + Dexamethasone n=23	Dexamethasone only n=12	Control n=10
Volume (ml)	†	2.9 ± 1.1	2.9 ± 0.9	3.18 ± 1.1
	‡	2.8 ± 0.9	3.0 ± 0.7	3.0 ± 0.8
Spermatozoa concentration (10^6/ml)	†	18.4 ± 10.3	17.5 ± 10.1	19.9 ± 9.5
	‡	18.9 ± 11.2	17.5 ± 10.1	20.4 ± 9.7
Mobile (%) (after 60 min.)	†	38.0 ± 17.2	38.6 ± 9.7	36.1 ± 12.0
	‡	45.2 ± 14.9	35.3 ± 6.6	34.5 ± 8.3
Vital (%) (after 60 min.)	†	41.7 ± 9.4	42.6 ± 12.4	42.8 ± 10.6
	‡	60.6 ± 10.9	43.6.1 ± 12.4	41.8 ± 10.6
Normal morphology (%)	†	59.1 ± 14.6	59.4 ± 13.1	63.2 ± 5.5
	‡	58.8 ± 15.7	60.5 ± 13.2	62.5 ± 7.1
Serum level of ASA (U/ml)	†	313.4 ± 77.8	322.2 ± 77.2	321.8 ± 76.7
	‡	120.2 ± 37.9	287.7 ± 72.6	316.1 ± 81.1

DISCUSSION

Taking into consideration the function of blood-testis barrier and other microenvironmental immunomodulatory mechanisms that provide tolerance to sperm molecules, it is clearly that every breakdown of the barrier and the protection 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 microenvironmental acceleration of Th1 immunity, enhanced secretion of proinflammatory cytokines like IL-1, IFN-γ, TNF-α, reduced secretion of anti-inflammatory cytokines such as IL-10 and TGF-β. Also, these mechanisms are associated with 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 all Th1-mediated autoimmune diseases[20]. In addition, Vitamin D3 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 Vitamin D3 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 Vitamin D3 + Dexamethasone treatment might be acceptable explanation for significant differences in serum level of ASA in Vitamin D3 + Dexamethasone group before and after the treatment. In addition, decreased level of ASA in Vitamin D3 + Dexamethasone 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 treatment by Vitamin D3 + Dexamethasone 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 mild gastro-intestinal disorders, increased body weight and slight edemas. In most cases the side effects did not need additional treatment, but some patients with gastro-intestinal disorders such as nausea and gastritis were successfully treated by ranitidine 150 mg twice daily orally, whereas edemas treated by furosemide 10 mg every 2-3 days orally.

CONCLUSION

In conclusion, infertile men with elevated level of ASA and poor basic parameters of semen analysis can be treated by Vitamin D3 + Dexamethasone combination with great chance for decreasing the level of ASA. Significantly increased motility and viability of the spermatozoa after Vitamin D3 + Dexamethasone treatment might be repercussion of low ASA activity. Due to ASA may play an important role in pathogenesis of male infertility, Vitamin D3 + Dexamethasone combination may be useful as pre-treatment in almost all procedures of assisted reproduction[22]. Further study is needed to identify the mechanisms of Vitamin D3 + Dexamethasone treatment regarding down-regulating the ASA level and to appoint the real benefits of this therapy.

REFERENCES

1. Naz, R.,K., and R. C. Menge, 1994. Antisperm antibodies: origin, regulation and sperm reactivity in human infertility. Fertil Steril. 61: 1001-1013.
2. Koide, S.,S., L. Wang and M. Kamada, 2000. Antisperm antibodies associated with infertility: properties and encoding genes of target antigens. Proc Soc Exp Biol Med. 224: 123-132.
3. Setchell, B.,P., J.K. Voglamyer, and G.M. Waites, 1969. A blood-testis barrier restricting passage from blood rete testis fluid but not into lymph. J Physiol. 200: 73-85.
4. Sainio-Pollanen, S., T. Saari, O. Simell and P. Pollanen, 1996. CD28-CD80/CD86 interactions in testicular immunoregulation. J Reprod Immunol. 31: 145-163.
5. McDonald, S.,W., 2000. Cellular responses to vasectomy. Int Rev Cytol. 199: 295-339.
6. Sakamoto, Y., T. Matsumoto, Y. Mizunoe, M. Haraoka, M. Sakumoto and J. Kumazawa, 1995. Testicular injury induces cell-mediated autoimmune response to testis. J Urol. 153: 1316-1320.
7. Almagor, M., M. Dan-Goor, Y. Hovav and I. Kafka, 1998. Antisperm antibodies in men with psychogenic anejaculation. Arch Androl. 41: 1-4.
8. Shibahara, H., T. Tsunoda, A. Taneichi, Y. Hirano, A. Ohno, S. Takamizawa, C. Yamaguchi, H. Tsunoda and I. Sato, 2002. Diversity of antisperm antibodies bound to sperm surface in male immunological infertility. Am J Reprod Immunol. 47: 146-150.
9. Marshburn, P.,B. and W.H. Kutteh, 1994. The role of antisperm antibodies in infertility. Fertil Steril. 61: 799-811.
10. Lombardo, F., L. Gandini, F. Dondero and A. Lenzi, 2001. Immunology and immunopathology of the male genital tract – antisperm immunity in natural and assisted reproduction. Hum Reprod Update. 7: 450-456.
11. Slack, J., J.M. Risdahl, S.J. Valberg, M.J. Murphy, B.R. Schram and D.P. Lunn, 2000. Effects of dexamethasone on development of immunoglobulin G subclass responses following vaccination of horses. Am J Vet Res. 61: 1530-1533.
12. Lemire, J.,M., D.C. Archer, L. Beck and H.L. Spiegelberg, 1995. Immunosuppressive actions of 1,25 Dihydroxyvitamin D3: preferential inhibition of Th1 functions. J Nutr. 125: 1704-1708.
13. Mortimer D., 1994. Practical Laboratory Andrology. New York: Oxford University Press.
14. Wolff, H. and W.B. Schill, 1985. Antisperm antibodies in infertile and homosexual men: relationship to serologic and clinical findings. Fertil Steril. 44: 673-677.

15. Snyder, N.K., C.M. Kramer, R.K. Dooley and M.P. Holsapple, 1993. Characterization of protein phosphorylation by 2,3,7,8-tetrachlorodibenzo-p-dioxin in murine lymphocytes: indirect evidence for a role in the suppression of humoral immunity. Drug Chem Toxicol. 16: 135-163.
16. Curtis, G.,L., V.A. Kahl and W.L. Ryan, 1987. Serum sperm agglutinating antibody formation in vasectomized men treated with dexamethasone. Andrologia. 19: 597-601.
17. Shulman, J.F. and S. Shulman, 1982. Methylprednisolone treatment of immunologic infertility in male. Fertil Steril. 38: 591-599.
18. Muller, K. and K. Bendtzen, 1996. 1α,25-Dihydroxyvitamin D3 as a natural regulator of human immune functions. J Investig Dermatol Symp Proc. 1: 68-71.
19. Muller, K., C. Heilmann, L.K. Poulsen, T. Barington and K. Bendtzen, 1991. The role of monocytes and T cells in 1α,25-dihydroxyvitamin D3 mediated inhibition of B cell function in vitro. Immunopharmacology. 21: 121-128.
20. Bubanovic, I., 2004. 1α,25-dihydroxyvitamin D3 As New Immunotherapy In Treatment of Recurrent Spontaneous Abortion. Med Hypotheses. 63: 250-253.
21. Pichler, J., M. Gerstmayr, Z. Szepfalusi, R. Urbanek, M. Peterlik and M. Willheim, 2002. 1α,25$(OH)_2$D3 inhibits not only Th1 but also Th2 differentiation in human cord blood T cells. Pediatr Res. 52: 12-18.
22. Bubanovic, I., S. Najman and S. Kojic, 2004. New Immunomodulatory Treatment for Infertile Men with Antisperm Antibodies. Fertil Steril. 81(3):S7-31; p: 20.