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MULTIVITAMIN AND MICRONUTRIENT TREATMENT IMPROVES SEMEN PARAMETERS OF AZOOSPERMIC PATIENTS WITH MATURATION ARREST

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Abstract : The study was undertaken to evaluate the efficacy of multivitamin and micronutrient supplementation in azoospermic patients with maturation arrest. A total of 35 azoospermic patients showing maturation arrest on testicular biopsy were recruited in this study. The patients were divided into two groups. Untreated group (n=11) without any treatment and treated group (n=24) who received multivitamins, micronutrients and co-enzyme Q_{10}. The sperm concentration, motility and morphology were evaluated at monthly interval. The results showed reduction in liquefaction time and relative viscosity of the semen in the treated group. Further, in treated group there was appearance of spermatozoa (4.0 million/ml) exhibiting progressive motility (7%) and normal morphology (6%), even in the first follow up visit. The sperm count, motility and normal morphology increased significantly on subsequent visits. Within 3 months (3 visits) 2 pregnancies were reported. These observations indicate that multivitamin and micronutrient supplementation improve the qualitative and quantitative parameters of seminogram in patients with azoospermia of maturation arrest.

Key words : co-enzyme Q_{10} spermatozoa oxidative stress azoospermia maturation arrest

INTRODUCTION

Less than 1% of general population and 10 to 15% of infertile men suffer from azoospermia (1). Azoospermia is a condition associated with absence of spermatozoa in the semen (2) and can result from the absence of spermatogonial cells (germinal cell aplasia), arrest of spermatogonial cell division either at meiosis or mitosis (maturation arrest) or obstruction to the ductal system which transports the spermatozoa (obstructive). The azoospermia due to maturation arrest can result from number of conditions such as exposure to toxic chemicals, varicocele, testicular

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torsion, hormonal deficiency etc (3). The environmental toxicants are reported to cause oxidative stress in the testis with decreased spermatogenesis (3). In patients with varicocele a decreased anti-oxidant capacity of the semen was observed (4) and varicocelectomy improved the fertility status of these patients (5). In case of testicular torsion anti-oxidants provided beneficial effect (3). Hormonal deficiency was postulated to contribute or result from testicular oxidative stress (6, 7). Thus oxidative stress is a common factor in all the conditions of azoospermia due to maturation arrest (8–11). Since oxidative stress is implicated in azoospermia due to maturation arrest, it is hypothesized that anti-oxidants will be beneficial in the treatment of this condition. Vitamin A, vitamin C and vitamin E are natural anti-oxidants present in our diet. Further, minerals such as Zn, Cu, Fe, Mg, Mn etc are co- factor for mitochondrial enzymes. Therefore, this study was undertaken to evaluate the efficacy of multivitamins and micronutrients as anti-oxidants in azoospermic patients and compared with those who did not receive any treatment.

MATERIALS AND METHODS

Patient selection

Study was conducted at Male Infertility Clinic in Sir Sunderlal Hospital and Male Infertility and Reproductive Physiology Unit in the Department of Physiology, of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The study was approved by the Institutional ethical committee for conducting research on human beings. A total of 35 patients exhibiting azoospermia on 3 consecutive semen analyses (as per the W.H.O. protocol) and showing maturation arrest on testicular biopsy were recruited in this study (2). These patients were divided into untreated and treated groups. Untreated group (n=11), did not receive any treatment and treated group (n=24) received multivitamins, micronutrients and co-enzyme Q_{10}. All the patients (treated/ untreated) were advised for monthly follow up visit.

Collection of semen sample

Informed written consent was obtained from all the patients involved in this study. The semen samples were collected in a wide mouthed sterile container by masturbation after an abstinence of 3–4 days.

Semen analysis

The semen samples after collection were kept at room temperature. The liquefaction was ascertained at every 5 min till the time of liquefaction. Then the volume was measured and the pH was determined. The relative viscosity was measured as a ratio of the time taken by a known volume of semen to flow through a 20 gauge needle with that of equal volume of distilled water on that day.

A drop of undiluted semen sample (vortexed) was placed on a glass slide to see the presence of spermatozoa microscopically (400x). If no spermatozoa, then the sample was centrifuged (3000g for 15 min) and the pellet was examined for spermatozoa. Only those samples showing spermatozoa microscopically were evaluated by Sperm Quality Analyzer (SQA-IICP from MES, Israel) for sperm concentration, motility and morphology at room temperature.

Hormone estimation

After the diagnosis of azoospermia, the plasma concentrations of LH, FSH and testosterone were estimated using the standard radioimmunoassay technique.

Testicular fine needle aspiration cytology

Under aseptic conditions the testicular fine needle aspiration cytology was performed. Smear was prepared with the aspirate and was stained by Papanicolaou stain. Precaution was taken to minimize the tissue injury at the time of aspiration.

Treatment protocol

The azoospermic patients were divided into 2 groups. The untreated group (n=11) did not receive any treatment. The patients in treated group (n=24) were prescribed a preparation available commercially containing a combination of multivitamins and micronutrients thrice daily/orally, co-enzyme Q_{10} (100 mg, once daily/orally) and in addition they were advised to consume 2 lemons per day. The composition of multivitamin and micronutrient preparation was as follows: Vitamin A, 5000 IU; Vitamin B_1, 15 mg; B_2, 5 mg; B_6, 2 mg; B_{12}, 5 μg; Nicotinamide, 45 mg; d-panthenol, 5 mg; Folic acid, 1 mg; Vitamin C, 75 mg; Vitamin D_3, 400 IU; Vitamin E, 15 mg; Potassium iodide, 0.025 mg; Calcium dibasic phosphate, 70 mg; Magnesium oxide, 0.15 mg; Ferrous fumerate, 50 mg; Copper sulphate, 0.1 mg; Manganese sulphate, 0.01 mg; and Zinc sulphate, 50 mg. The patients were advised for follow up at monthly interval.

Analysis of data

The data in each category were pooled to compute the mean and standard deviation. The statistical differences were evaluated by using X^2 test (2X2 table) for qualitative analysis, one- way ANOVA and Student-Newman-Keuls test for multiple comparisons for quantitative analysis. A $P<0.05$ was considered significant.

RESULTS

During 10 months, 35 azoospermic patients visited the Male Infertility and Reproductive Physiology Unit and 11 of them did not receive any treatment and 24 received treatment comprising of multivitamins and micronutrients. The mean age of the patients (in years) of untreated group was 34.8±3.2 (n=11), treated group was 36.4±2.6 (n=24) and of the patients who visited for follow up regularly was 34.2±2.8 (n=9). Patients who did not receive any treatment failed to turn up after 1st follow up visit, inspite of our instructions. The testicular FNAC of all the patients recruited in this study showed maturation arrest mostly at spermatid level as compared to normal spermatogenesis (Fig. 1). The LH, FSH and testosterone were within the normal range (Table I).

Multivitamin and micronutrient therapy improved seminogram parameters

In both treated and untreated groups no spermatozoa was seen in the semen on the initial visit. In untreated group, the seminogram picture remained unchanged on subsequent visit also. They did not turnout for the next follow up visit. In treated group, positive changes in semen were observed

TABLE I : Endocrine profile of azoospermic patients in treated and untreated groups.

Hormone levels	*Normal range (from Rao and Deshpande[16])*	*Untreated (n=11)*	*Treated (n=24)*	*Patients visiting subsequently (n=9)*
LH (mIU/ml)	1–15	4.2±0.8	4.4±0.9	4.6±1.0
FSH(mIU/ml)	1–15	7.9±1.5	7.7±2.1	8.0±1.6
Total Testosterone (ng/dl)	300–1100	340±51.3	385±85.4	386.3±89.4

The values are mean ± SD from "n" number of patients in each category.

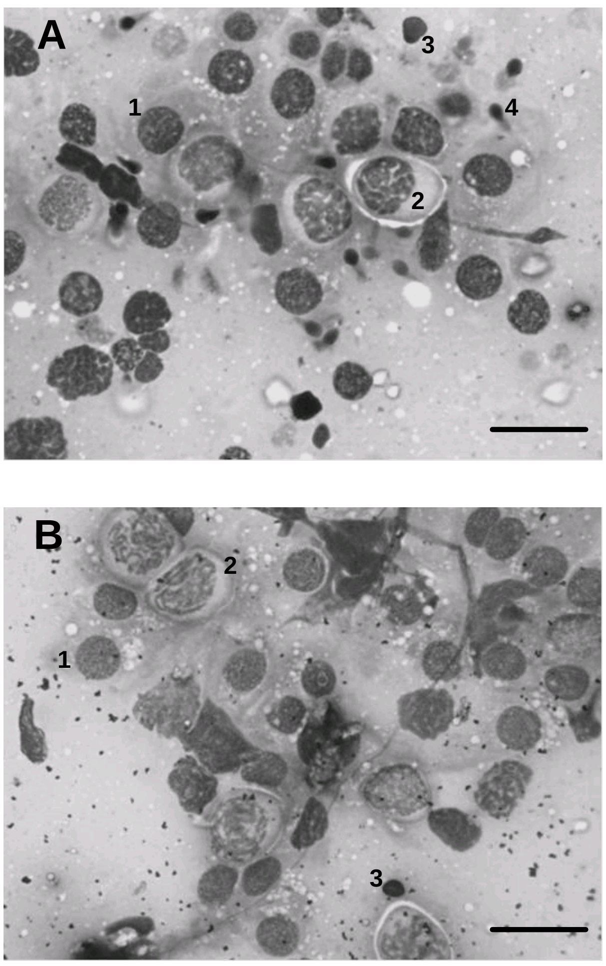

Fig. 1 : Photomicrographs of fine needle aspiration cytology (FNAC) slides in persons with normal spermatogenesis (A) and in patients with maturation arrest (B). Representative cells at different stages of spermatogenesis are indicated by numericals in both A and B. 1- Spermatogonia; 2-Spermatocyte; 3-Spermatid and 4-Spermatozoa. In (B) note the absence of spermatozoa. Horizontal bar= 25 μ.

on the very next visit (Tables II and III; Fig. 2). The pooled data of the treated group revealed decrease in liquefaction

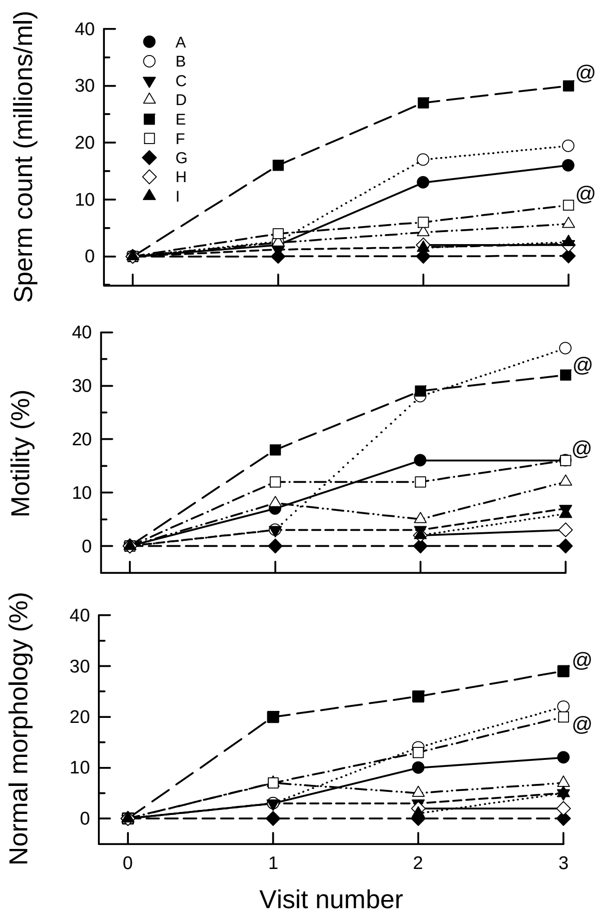

Fig. 2 : Seminal parameters of patients during 3 visits after advocating anti-oxidant treatment. Alphabets in the legend indicate the data of 9 patients in subsequent visits. Symbol (@) indicates the patients who reported pregnancy.

time and relative viscosity while no change in pH (Table II, values in square brackets). The observations of 9 patients who came for follow up when paired also provided similar results (Fig. 2). Thus, there was improvement in the physical properties of the semen in treated group (Table II).

In treated group, there was appearance of spermatozoa (4.0 million/ml) showing 7% and 6% progressive motility and normal morphology, respectively, on first follow up visit (Table III). In subsequent visits, the sperm concentration, progressive motility and normal morphology increased incrementally ($P<0.05$, one way ANOVA; Table III). Nine patients who visited us for follow up demonstrated improvement in seminal parameters (Fig. 2). The qualitative changes in these patients were analyzed by X^2 test (2X2 table) and were significant ($P<0.05$). At the end of 3 months two pregnancies were reported and are indicated in Fig. 2.

TABLE II : Physical properties of semen of azoospermic patients.

Groups	*Visits in Untreated group*		*Visits in Treated group*			
	Initial	*1*	*Initial*	*1*	*2*	*3*
Volume (ml)	2.2±1.3 (n=11)	2.3±1.1 (n=8)	1.7±0.7 (n=24)	2.0±0.4 (n=19)	2.3±0.3 (n=16)	2.4±0.2 (n=13)
			[1.7±0.6] (n=9)	[2.1±0.3] (n=6)	[2.1±0.3] (n=9)	[2.4±0.2]*# (n=9)
pH	8.0±0.5	7.8±0.5	7.6±0.7 [7.4±0.8]	7.6±0.5 [7.4±0.5]	7.6±0.5 [7.4±0.5]	7.6±0.7 [7.8±0.4]
Liquefaction time (min)	32.7±9.0	30.1±2.9	30.4±7.7 [31.2±10.0]	29.5±4.5 [28.3±2.5]	28.8±2.9 [28.3±2.5]	28.8±2.2 [28.9±2.2]
Relative viscosity to water	7.0±2.1	7.5±2.5	6.7±1.2 [6.8±1.5]	6.4±0.6 [6.2±0.4]	6.2±0.5 [6.2±0.4]	6.1±0.5 [6.1±0.3]

The values are mean±SD from "n" number of patients. The values in square brackets denote the data of patients who visited for follow-up. Number (n) indicated in treated group row 1 or 2 are applicable to all rows; respectively. Note the untreated patients did not turn up after 1st follow up visit.

*$P<0.05$, one way ANOVA; #$P<0.05$, Student- Newman- Keuls test for multiple comparisons as compared to initial visit. Other group comparisons are not significant.

TABLE III : Seminogram parameters of azoospermic patients

Groups	*Visits in Untreated group*		*Visits in Treated group*			
	Initial (n=11)	*1 (n=8)*	*Initial (n=24)*	*1 (n=6)*	*2 (n=9)*	*3 (n=9)*
Sperm count(millions/ml)	0.0±0.0	0.0±0.0	0.0±0.0	4.0±5.2*	8.0±9.1*#	9.7±10.1*#
Progressive motility (%)	0.0±0.0	0.0±0.0	0.0±0.0	7.3±6.2*#	12.1±11.2*#	16.0±12.3*#
Normal morphology (%)	0.0±0.0	0.0±0.0	0.0±0.0	6.1±6.6*#	9.0±7.9*#	12.8±9.8*#

The values are mean±SD from "n" number of patients reported at initial and subsequent visits. Note the untreated patients did not turn up after 1st follow up visit.

*$P<0.05$, one way ANOVA; #$P<0.05$, Student- Newman- Keuls test for multiple comparisons as compared to initial visit. Other group comparisons are not significant.

DISCUSSION

This study was conducted to ascertain the usefulness of anti-oxidant therapy in patients presenting with azoospermia of maturation arrest. This was achieved by advocating a treatment protocol consisting of multivitamins and micronutrients. The present results reveal that the azoospermic patients with maturation arrest who received the treatment were benefitted. Further, the anti-oxidant treatment resulted in two pregnancies.

Oxidative stress affects the testicular function by disrupting germinal cell epithelial division as well as differentiation (12) and also induces germ cell apoptosis (8). The mechanisms underlying the apoptosis induction by oxidative stress are not clear. However, they are shown to be due to the involvement of cytokine-induced stresskinase and E-selectin expression in the testicular vascular endothelium (8–10). Induction of apoptosis leads to testicular neutrophil recruitment and increases the generation of intra-testicular reactive oxygen species (ROS). ROS in turn, cause peroxidative damage to cell membranes and also activate germ cell apoptosis (8–10). The rate of phagocytosis by Sertoli cells is also enhanced by increased germ cell apoptosis so as to clear the dying and damaged germ cells (13, 14). The toxic effects of ROS can be neutralized by anti-oxidants as they scavenge the free radicals. This in turn decreases the free radical-induced damage to germ cells to facilitate the maturation process. The beneficial effects of multivitamins and micronutrients as anti-oxidants in our study support this proposition.

Histological observations in these patients revealed the presence of cells up to the stage of spermatids but no spermatozoa, indicating maturation arrest. The normal time duration for spermiogenesis (process of differentiation of spermatids to spermatozoa) is 21 days (15). It is presumable that the free radical scavenging effect of the anti-oxidants used in the treatment decreased the oxidative stress and restored the spermiogenesis. Our observations support this as there was improvement in seminal parameters even in the first follow up visit (1st visit as in Fig. 2). The increase in sperm concentration on subsequent visits signify more and more spermatogonial cells escaped apoptosis and are able to complete their cell cycle.

In this study both enzymatic (co- enzyme Q_{10}) and non-enzymatic (multivitamins and micronutrients) anti-oxidants were prescribed to the patients. The enzymatic anti-oxidants decrease the formation of free radicals and the non enzymatic anti-oxidants neutralize the pre-formed free radicals. However, it will be interesting to isolate the contribution of enzymatic and non-enzymatic anti-oxidants in these subjects to identify the mechanisms.

The ROS produces toxic effects at 3 different levels. Firstly ROS activates apoptotic mechanism on gamete cells (8–10). Secondly suppress the cell division and differentiation directly (12). Thirdly, activates the phagocytic mechanism in Sertoli cells so that damaged and apoptotic cells are phagocytosed (13, 14). The anti-oxidants thus neutralize the oxidative damages at all the levels to transform azoospermia to oligospermia. Improvement of seminal parameters in the first visit indicates the overcoming of oxidative stress at spermiogenesis level. The improvements in subsequent visits probably involve spermatogonial cells.

In this study nearly 2/3rd of the patients did not report for the follow up which is beyond the control of any such investigation. The present observations are not merely by chance because untreated group did not show any improvement in the seminal profile on the visit after 1 month. Further, in the treated group the X^2 analysis revealed significant improvement in the sperm count, motility and morphology ($P<0.05$). We did not measure the oxidative stress parameters in semen. But even in the absence of these indicators the patients exhibited improvement, demonstrating the role of anti-oxidants in normal spermatogenesis.

In conclusion, anti-oxidants improve seminogram parameters qualitatively and quantitatively of azoospermic patients with maturation arrest. Further, anti-oxidant intervention may be useful in the treatment of these patients.

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