Original Article

Utility Of Antioxidants and Nutrients In Sperm Motility And Altered Sperm DNA Fragmentation Levels: A Study Protocol

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Abstract

Background: Numerous studies have manifested that sperm deoxyribonucleic acid fragmentation levels can be lowered by use of antioxidants and some studies have shown the consequences of oral antioxidants on sperm chromatin probity. Supplementation of antioxidants in the body is likely to increase the motility and concentration of sperm, and sperm DNA fragmentation. Specific antioxidants such as CoenzymQ10 , Vit-E,C , L-Carnitine are supplemented into the body to decrease oxidative stress, reactive oxygen species further sperm DNA disintegration levels. Supplementation of folate helps in improving the sperm concentration. Further, supplementation of zinc and selenium plays essential role in increasing sperm motility. Objectives: To identify the deficiency of the antioxidants and nutrients in infertile male partner. To study the consequences of antioxidants on motility, morphology and count of sperms. To evaluate sperm DNA disintegration levels before and after performing SCD test. Methodology: This non-invasive methodology includes recording of treatment history and the indications. Counselling of the participants for research will be done. Further protocol include supplementation of antioxidants like CoenzymQ10 , Vit-C, E to study their effects on Sperm kinetics , concentration of sperm and Sperm DNA disintegration levels.

Keywords: Antioxidants like vitamin-C, vitamin-E , CoenzymQ10 , Zinc , Selenium, Omega- 3 -fatty acids, Reactive oxygen species, Sperm DNA fragmentation levels, Sperm motility.

Introduction

Infertility itself is a troublesome word meaning not able to conceive after having intercourse for 1 year.(1) Modern science is introducing new techniques and ART is one of them. ART refers to Assisted Reproductive Technology in which embryologist forms the embryo by fusion of both the gametes outside the female body. Worldwide infertility cases occur mostly due to abnormalities in male.

Semen contains some special antioxidants like vitamin- C, E, Coenzyme Q10, L-Carnitine Omega-3- fatty acids which perform crucial function to reduce the catabolic stress and reactive oxygen species (ROS) in the body and reduce the sperm DNA breakage levels in body. (2)

ROS basically includes free radicals and peroxides.(3) Changes in dietary pattern in people of this era is leading to obesity causing Hypogonadism among males that deteriorates sperm count in the body. Carnitine when supplemented in the body has been found to increase sperm motility by causing changes in fatty acid metabolism in the body.(4)

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S Supplementation of antioxidants like vitamin-E has improved implantation incidence by reducing oxidative stress and lipid peroxidation potential.(5) Supplementation of zinc and selenium in the body has improved sperm motility.(6) Folate supplementation in the body is found to increase sperm count and concentration.

So, lack of these antioxidants has adverse affects on body as it leads to increase in levels of ROS. In turn ROS levels further damage the amount of DNA present in the body which is associated with mitochondrial membrane.(7) ROS levels in the body not only increase due to lack of antioxidants but due to certain environmental factors like high temperature, electromagnetic waves, consumption of alcohol, obesity and poor nutrition, High ROS levels also effect the structure of DNA in sperm causing its fragmentation. Sperm DNA disintegration then destroys structure of deoxyribonucleic acid preventing it's fertilization with embryo causing infertility. Sperm DNA breakdown levels can be evaluated by certain methods :

1.) Sperm chromatin structure analysis (SCSA)

2.) Terminal transferase mediated dUTP end labelling (TUNEL)

3.) Single cell gel electrophoresis (SCGE)

4.) Sperm chromatin dispersion test (SCD).(8)

While now a days the most preferably used method to test the sperm DNA fragmentation levels is SCSA.

Studies among men in some fertile couples have shown that by consuming Mediterranean diet which includes higher intake of fruits, vegetables, seafood, whole grains have resulted in higher levels of testosterone, increased sperm motility and concentration.(9) Altered diet like consuming more of saturated fats and trans fatty acids leads to oxidative stress in the body that decreases sperm motility and count further leading to infertility.(10) Therefore, here I am discussing the importance of antioxidants and nutrients for how they help in improving fertilization rates.

Background:

In the study conducted by B.Eskenagi , in April 2005 which implemented to know whether intake of diet and supplements of specific antioxidants have good effect on sperm count and motility. This study was conducted on 97 men and their food intake and supplements were derived from a questionnaire . Intake of antioxidants and micronutrients were distributed into three categories as low, moderate and high intake. Analysis of covariance was used to examine the connectionamong antioxidants and semen parameters. The study demonstrated that 92 to 100% of men took supplements in high intake groups,48-64% were considered in the moderate group category and 5-38% were considered in low intake category . The men with high ingestion levels of antioxidant composition have higher concenteration of sperm than men with lesser intake.(5)

There was a study conducted in December 2016 by Sedigah Ahmadi, ReihaneBashiri ,AkramGhadiri, Anari and AzadehNadjarzadel. The analysis was aimed to review the consequences of oral antioxidant substituents on improving motility and morphology of sperm and the fertility rate .Ordinary antioxidants in semen are Vit-E ,VIT-C ,glutathione etc. Evidence shows that lower the antioxidant capacity, lower is the fertility rate and quality of sperm function is seen to be reduced. Also, studies that show the effect of antioxidants combined with drugs have shown some positive effects on fertility were also referred. The study was conducted on total 60 men with asthenoteratozoospermia by providing Coenzyme Q10 therapy for 6 months . After 6 months CQ10 increased the sperm motility in those individuals .It was concluded that,CQ10 does not specifically increases live parturition or gestation rates but there is a remarkable enhancement in semen variables including sperm motility ,concentration and also quality of sperm function was to be improved.(7-14)

The study conducted by Ahmad Majzoob, Ashok Aggarwal, Sandro C .Estcries in Jan 27,2017 was implemented to study the effects of antioxidants on quality of sperms ,their morphology ,count and sperm DNA breakage . DNA of sperm is found to be associated with protamine in the body. This helps sperm DNA to remain in a binding position that protects them from damages which may occur during transferring in the female cervix. Sperm Deoxyribonucleic acid fragmentation occurs due to certain factors like remodeling, chromatin packing, high amount of ROS and due to lesser amount of antioxidants. 3 studies were being discussed in this article mainly to implement that administration of antioxidants leads to reduced levels of sperm DNA fragmentation.

1st study was conducted by 'Greco er al' in which he took 64 patients who were known to have unexplained fertility and increased levels of sperm DNA fragmentation He divided them into 2 groups treatment group and placebo group . He provided both the groups with antioxidant combinants (1g Vit-c and 1g Vit -E) for 2 months. Not much changes were seen in semen parameters but sperm DNA levels were decreased

Another study was conducted by Kodama in which he randomized 14 infertile man with Vit-C(200mg), Vit-E (200mg),glutathione to check their effects on sperm DNA oxidation. After 2 months ,decreasedamount of sperm damage with significant rise in sperm count was seen.

Abade conducted a analysis to study the effects on sperm DNA fragmentation by supplementation of antioxidants .he conducted this study through Sperm Chromatin Dispersion (SCD) .Combined antioxidants were given to 20 infertile man for 3 months. Sperm concentration , motility were found to be increased and sperm DNA fragmentation levels were reduced.(15-17)

Ermano Grecco, Marcello Jacobelli, published a study in 02 JAN ,2O13 to review the effects of antioxidant administration

on sperm DNA breakage. It's main aim is to check whether sperm DNA disintegration levels were reduced by oral administration of antioxidants .Antioxidants in combined form (VIT-C and VIT-E) 1g each were given daily for 2 months were given to 64 infertile man divided into 2 groups which were treatment and control groups . These men were infertile due to unknown causes and they had increased levels of sperm DNA fragmentation. so, after administration of antioxidants sperm DNA fragmentation levels were estimated by end deoxyribonucleotidyl transferase nick end labelling method before and after the treatment. Changes in semen parameters very less in both the groups after treatment but sperm DNA fragmentation amounts were reduced in the group of treatment than in control group . so, hereby the author says that sperm DNA levels can be altered effectively by oral administration of antioxidants.(18)

OzlemTunc, Jeremy Thompson et al published an article on Jan 01, 2009 to study the improvement in quality of sperm DNA by administration of combined antioxidants like {zinc , selenium} 50 infertile men were administrated to a combination of antioxidants for 3 months to check sperm DNA integrity we perform sperm DNA fragmentation test by d(Utp) nick end labelling Sperm concentration , sperm motility, morphology of the sperm along with hormones (FSH,LH, Testosterone) were also monitored . Results came out as a significant rise in sperm DNA integrity levels ,reduced ROS levels but no such changes were observed in semen parameters like concentration and motility of sperm.(19)

Objectives:

Aim: To study the effect on sperm motility and sperm DNA fragmentation by supplementation of antioxidants and nutrients.

Hypothesis:

To study the effect on sperm motility and sperm DNA fragmentation by supplementation of antioxidants and nutrients.

Methods:

Study design:

Observational Study

Methodology:

This study will be performed in Wardha test tube baby centre in tertiary care hospital. Pertinent facts on the census and past therapeutics as well as the indications will be recorded. Counselling of all participants for research work will be done. Written as well as verbal consent will be taken from the participants. The practice protocol is as follows: To check patient history like he should not be having any prior ailments like Diabetes mellitus, hypertension, hormonal imbalance, problems of urogenital tract which may further affect sperm motility and disturb it's count.

Patient with male infertility having sperm count less than 5million/ml registered for semen analysis was excluded from study because the SCD test needs a concentration of 5–10million per ml of sperms. Sample collection from 40 male patients at WTTBC observed with damaged sperm DNA integrity/ infertility. The washed semen sample was diluted in phosphate buffer solution (pH 6.88)to a concentration of 5–10 × 106/ml. Put the Agarose Eppendorf in the hot water bath for 5 mins at 90–100°C to melt the agarose. Eppendorf with melted agarose is shifted to hot water bath maintained at 37°C for five minutes. Washed 25 μL of semen sample is mixed well with agarose. 14 μL fractional of the sperm suspension placed from the agarose on the treated glass slide side and covered with coverslip, without formation of air bubbles. Place slide in horizontal position through the entire process. Place the slide on cold surface (4 degree celcius) for around five minutes. Remove the coverslip gently and immerse it in acid denaturation solution provided in kit. Incubate for 7 minutes horizontally. Place the slide in incubator tray containing 10 mL of tempered lysis solution provided in the kit . Incubate at room temperature for 25 minutes. Transfer the slide to another incubation tray containing abundant distilled water to wash out the extra lysis solution and incubate for five minutes. Place the slide into a tray containing 70% ethanol for 2 minutes followed by 90% ethanol for 2 minutes, andfinally add 100% ethanol for 2 minutes. Keep the slide to dry at room temperature and stain it with Wright stain solution (Sigma-Aldrich, Tokyo, Japan). SDFI evaluated from test is calculated by particular method: SDFI (%) = 100 × (number of sperm with disintegrated DNA)/(count of sperm). Sperm with large or medium halos were considered to be without sperm DNA breakage. Sperm with small or no halos wereconsidered to besperm with DNA disintegration. Degraded sperm was also considered to be with sperm DNA breakage .

Statistical analysis: Results are reported showing 4 statistical categories of fertility potential:

- ≤ 15% DFI = excellent to good sperm DNA integrity
- 15 to < 25% DFI = good to fair sperm DNA integrity
- 25 to < 50% DFI = fair to poor sperm DNA integrity
- ≥ 50% DFI = very poor sperm DNA integrity.

Setting:

Location : Wardha Test Tube Baby Centre, Sawangi, Wardha.

Relevant dates, including periods of recruitment : 2021 – 2022

Participants:

INCLUSION CRITERIA

• Infertile couple attending Wardha Test Tube Baby Centre, AVBRH

EXCLUSION CRITERIA

• Patient with psychological illness

. Patient who is not willing to give consent form.

. Patient that might be having certain disorders like Diabetes mellitus, heart diseases, reproductive disorders which may become a hinderance in our study.

Study size:

Sample size :

50 infertile couple

$$N = \frac{\chi 2 * N * p(1-p)}{C2(N-1)+\chi 2 p(1-p)}$$

Total population = N=100 during18 months

χ2= Value of Chisquare for 1 degrees at some desired probability level. This is 3.84 at 5% level of significance

P = 50% proportion

Q = 100 – p

= 50

C= Confidence interval of the one choice (95% CI)

= 0.05

N= 3.84*100*0.5*0.5

(0.05)2 *15+3.84*(0.5*0.5)

= 50

Expected Outcome:

50 patients were taken into consideration and then semen analysis of each patient was done . Patients with sperm count less than 5million/ml were administrated with antioxidants such as Vitamin-C, Vitamin-E , CoenzymQ10. Motility and concentration of sperms and sperm DNA disintegration levels were observed to be increased.

Discussion:

The major motive of this study is to check the effect of antioxidant supplementation on motility and concentration of sperm and sperm DNA disintegration levels. Intake of nutrients in the diet helps to bring up the motility and concentration of the sperm to a certain extent but with additional supplementation of specific antioxidants like CoenzymQ10 ,Vit – E, C quality of sperm , it's motility , concentration highly improves. Antioxidants help to reduce reactive oxidative species thereby increasing the oxidative capacity which further helps to reduce oxidative DNA damage.

References

1. Majzoub A, Agarwal A. Systematic review of antioxidant types and doses in male infertility: Benefits on semen parameters, advanced sperm function, assisted reproduction and live-birth rate. Arab J Urol [Internet]. 2018;16(1):113–24. Available from: https://doi.org/10.1016/j.aju.2017.11.013
2. Arafa M, Agarwal A, Majzoub A, Selvam MKP, Baskaran S, Henkel R, et al. Efficacy of antioxidant supplementation on conventional and advanced sperm function tests in patients with idiopathic male infertility. Antioxidants. 2020;9(3).
3. Bansal AK, Bilaspuri GS. Impacts of oxidative stress and antioxidants on semen functions. Vet Med Int. 2011;2011.
4. Nassan FL, Chavarro JE, Tanrikut C. Diet and men's fertility: does diet affect sperm quality? Fertil Steril [Internet]. 2018;110(4):570–7. Available from: https://doi.org/10.1016/j.fertnstert.2018.05.025
5. Eskenazi B, Kidd SA, Marks AR, Sloter E, Block G, Wyrobek AJ. Antioxidant intake is associated with semen quality in healthy men. Hum Reprod. 2005;20(4):1006–12.
6. Salas-Huetos A, Rosique-Esteban N, Becerra-Tomás N, Vizmanos B, Bulló M, Salas-Salvadó J. The effect of nutrients and dietary supplements on sperm quality parameters: A systematic review andmeta-analysis of randomized clinical trials. Adv Nutr. 2018;9(6):833–48.
7. Sc PM, Bashirip R, Ghadiri-Anarip A, Nadjarzadehp PMDA. Evidence based review Antioxidant supplements and semen parameters: An evidence based review Sedigheh AhmadiP 1. Int J Reprod BioMed. 2016;14(12):729–36.
8. Qiu Y, Yang H, Li C, Xu C. Progress in research on sperm DNA fragmentation. Med Sci Monit. 2020;26:1–11.
9. Karayiannis D, Kontogianni MD, Mendorou C, Douka L, Mastrominas M, Yiannakouris N. Association between adherence to the Mediterranean diet and semen quality parameters in male partners of couples attempting fertility. Hum Reprod. 2017;32(1):215–22.
10. Skoracka K, Eder P, Łykowska-Szuber L, Dobrowolska A, Krela-Kaźmierczak I. Diet and Nutritional Factors in Male (In)fertility—Underestimated Factors. J Clin Med. 2020;9(5):1400.
11. Majzoub A, Agarwal A, Esteves SC. Antioxidants for elevated sperm DNA fragmentation: A mini review. Transl Androl Urol. 2017;6(21):S649–53.
12. Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. J Androl. 2005;26(3):349–53.
13. Tunc O, Thompson J, Tremellen K. Improvement in sperm DNA quality using an oral antioxidant therapy. Reprod Biomed Online [Internet]. 2009;18(6):761–8. Available from: http://dx.doi.org/10.1016/S1472-6483(10)60024-7
14. Ahire, Abhijit Murlidharrao, Shweta Parwe, and Milind Nisargandha. "A Comparative Evaluation of Efficacy of Mustadi Yapan Bastiand Baladi Yapan Basti in the Management of Oligozoospermia-Study Protocol." Journal of Pharmaceutical Research International, June 11, 2021, 208–16. https://doi.org/10.9734/jpri/2021/v33i31A31686.
15. Mohan, Manju, Sawarkar Punam, and Sawarkar Gaurav. "Contribution of Ayurveda in the Management of Ksheena Shukra Vikara with Special Reference to Asthenozoospermia: A Case Report." Journal of Pharmaceutical Research International, July 12, 2021, 140–49. https://doi.org/10.9734/jpri/2021/v33i36B31961.
16. Nisargandha, Milind Abhimanyu, and Shweta Dadarao Parwe. "Evaluation of Spermatogenic Action in the Management of Oligospermia." International Journal of Pharma and Bio Sciences 11, no. 2 (April 15, 2021): 218–23. https://doi.org/10.22376/ijpbs/lpr.2021.11.2.P218-223.
17. Nisargandha, Milind Abhimanyu, and Shweta Dadarao Parwe. "Evaluation of Spermatogenic Action in the Management of Oligospermia." International Journal of Pharma and Bio Sciences 11, no. 2 (April 15, 2021): 218–23.

https://doi.org/10.22376/ijpbs/lpr.2021.11.2.P218-223.
18. Tayawade, Akshay, and Akash More. “A Successful ART Treatment of Severe Asthenoteratozoospermia with Donor Sperms: A Case Study at Wardha Test Tube Baby Centre, India.” Journal of Pharmaceutical Research International, July 19, 2021, 64–69. https://doi.org/10.9734/jpri/2021/v33i37B32022.
19. Patel, Mimansha, Madhuri Nitin Gawande, Minal Shashikant Chaudhary, and Alka Harish Hande. “DNA Methylation Patterns in Saliva of Tobacco Users with High-Risk Oral Potentially Malignant Disorders.” Journal of Pharmaceutical Research International, June 22, 2021, 129–37. https://doi.org/10.9734/jpri/2021/v33i32B31754.
11, 63–71. https://doi.org/10.1055/s-0039-1700610