Plasma and semen ascorbic levels in spermatogenesis

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Summary

Background: **Conflicting reports on the mechanism of action of ascorbic acid level in male reproductive system exist and very little is known about the ascorbic acid status in Nigerian males with weak fertility.**

Method: **Ascorbate that accumulates preferentially in the testis, the lipid and lipoprotein levels were determined in the plasma of Nigerian males. Twenty-seven (27) male with inadequate spermatogenesis (36 ±1.0) years, with mean value of 15.6 ± 6.90 million/cm³ sperm count and fourteen (14) controls (34 ± 0.6) years, with mean value of 108.0 ± 25.42 million/cm³ sperm count were selected for this study. The anthropometric indices were also determined.**

Results: **There were highly significant decreases in sperm cell count, percentage motility and percentage vitality (p<0.001) in each case, while percentage morphologically abnormal sperm cells was significantly elevated (p<0.001) compared with the control values. There were significant decreases in the seminal and plasma ascorbic acid concentrations (p<0.001) in the males who had inadequate spermatogenesis compared with the control values.**

The plasma total cholesterol (TC) and body mass index (BMI) were not significantly different from the corresponding control values, but the plasma low density lipoprotein (LDLC) (p<0.001) and triglyceride (TG)(p<0.01) concentrations were significantly increased in all the patients. While the plasma high density lipoprotein cholesterol (HDLC)(p<0.001) was significantly decreased compared with the controls. The plasma lipid and lipoprotein levels did not demonstrate any definite pattern with the sperm characteristics.

Conclusion: **The decreased semen ascorbate level may play a significant role in the reduced sperm characteristics in these patients.**

Key words: ***Semen and plasma ascorbic acid, Lipids, Lipoproteins, Infertility.***

Résumé

Introduction: Il y a des rapports qui s'opposent sur le mécanisme des actions de niveau ascorbique d'acide dans l'appareil reproducteur et on ne sait pas grand chose au sujet de statut d'acide ascorbique chez les Nigerians du sexe masculin atteint du faiblesse de la fertilité

Méthode: L'ascorbate qui s'accumule préférentielement dans le testicule, les niveaux des lipides et des lipoprotéines ont été évaluées dans le plasma de Nigerians du sexe masculin. Vingt sept (27) du sexe masculin atteints de la spermatogenèse insuffisante (36±1,0)ans, avec une valeurmoyenne de 15,6± 6,90-cm³ compte de sperm et quatorze (14) controles (34± 0,6) ans, avec une valeur moyenne de 108,0±25,42 million:cm³ compte de sperm ont été choisi pour cette étude. On avait également décide l'indices antropometriques.

Résultats: On avait noté des diminution fortement remarquable dans le compte de la cellule de sperme, le pourcentage de la mortalité et le pourcentage de la vitalité (P<0,001) dans chaque cas, tandis que le pourcentage morphologique des cellules du sperme abnormal était remarquablement élevé (P<0,001) par rapport aux valuers de contrôles. Il y avait des diminutions remarquable dans le titre séminal et le plasma d'acide ascorbique (P<0,001) chez les sexes masculins qui avaient la spermatogène insouffisante par rapport aux valeurs de contrôle. Le plasma cholestérole total (CT) et Indice de Masse de corps (IMC) n'était pas remarquablement different de valeurs de contrôle correspondantes, mais le plasma de la densité lipoprotéine basse (LDLC) (P<0,001) et triglyceride (TG) (P<0,01) concentrations étaient remarquablement élevées chez tous les patients. Tandis que le plasma cholestérol lipoproteine d'une densité très élevée (DECL) (P<0,001) était remarquablement base par rapport aux contrôles. Le lipide plasma et niveaux lipoprotéines n'avaient pas indiqué aucun modèle défini avec les traits de sperme.

Introduction

Adequate ascorbic acid level is required for both normal spermatogenesis and improved sperm cell quality in part, owing to its antioxidant properties[1].

The excessive generation of reactive oxygen species (ROS) by abnormal spermatozoa and sometimes by contaminating leukocytes has been implicated as one of the aetiologies of male infertility[1]. Evidence from studies has shown that ROS can induce damage to sperm cells[1,2]. Sperm cells are particularly sensitive to ROS induced damage of polyunsaturated fatty acids in the membrane[3, 4].

Recently however, the role of ascorbic acid in the maintenance of sperm cell lipid membrane has generated increasing interest. Available studies have shown that ascorbic acid may protect gametes from damage by free radical during production[5].The exact relationship between

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Table 1 Age, weight, height, BMI, and semen characteristics in all patients and controls

	(Non-biochemical Parameters) (Mean ± SEM)			
	Patients (n=27)	**Controls (n=14)**	**t-value**	**p- valu**
Age(years)	35.80 ± 0.97	34.78 ± 0.61	0.5760	ns
Weight(kg)	70.64 ± 2.4	60 .07± 2.21	1.4984	ns
Height (m)	1.73 ± 0.07	1.67 ± 0.81	2.5747	0.01
BMI(kg/m²)	23.53 ± 3.26	23.35 ± 0.74	0.1684	ns
Seminal volume(cm³)	3.29 ± 0.28	2.9 ± 0.34	0.8128	ns
count(million/cm³)	15.57 ± 2.92	108.00 ± 25.42	4.7417	0.001
Motility (%)	18.24 ± 2.92	62.86 ± 2.44	9.9906	0.001
% age abnormal morphology	80.65 ± 1.46	37.86 ± 6.35	8.0834	0.001
Vitality (%)	27.48 ± 2.93	58.93 ± 2.63	6.8434	0.001

BMI= Body mass index abn= abnormality

plasma/seminal ascorbic acid concentration, motility and morphologically normal sperm cells is not certain. This study was therefore designed to determine the levels of plasma/seminal fluid ascorbic acid concentrations as well as lipid profiles in relation to sperm characteristics in weakly fertile Nigerian males.

Materials and methods

Subject selection

Twenty-seven (27) male patients with mean age (35 ±1.0) years who were attending the Surgical Out Patient Clinic and with a follow up laboratory investigations in the Surgical Research Laboratory were selected for this study. Fourteen (14) male healthy volunteers with mean age (34± 0.6) were included as controls. Informed consent was obtained from the patients.

Health questionnaire was administered to each patient. The ethical committee of the College of Medicine University of Ibadan and University College Hospital Ibadan Nigeria approved the study.

Anthropometric measurements: The height and weight of all subjects were measured using standard procedures. The body mass index (BMI) was calculated using W/kg/ HT^2(m)[6] W= weight in kilogram, H= height in m^2

Sample collection

Blood samples were collected from the antecubital fossa with pyrogen free syringe and sterile needle with minimal stasis from both patient and control subjects into ethylene diamine tetra acetic acid (EDTA) bottles after an overnight fast. These were immediately wrapped in black carbon paper and placed in ice bag to prevent oxidation. After centrifugation each, separated plasma sample was divided into two aliquots. One aliquot was immediately analyzed for ascorbic acid while the second aliquot was stored at – 20°C until analyzed for lipid and lipoprotein profiles.

Semen collection

Semen samples were obtained from all subjects by masturbation after 3-5 days of abstinence into plain bottles. These samples were also assayed for ascorbic acid and total cholesterol (TC). The motility, vitality, morphology, viscosity, volume and sperm count were assessed using the method of WHO[7]

Table 2 Plasma total cholesterol (TC) triglyceride(TG) high density lipoprotein cholesterol (LDLC) and low density lipoprotein cholesterol (LDLC) in pateints and controls

Patients (n=27)	**Controls (n=14)**	**t-value**	**p-value**
Ascorbic acid (mg/dl)	0.38 ± 0.03	1.16 ± 0.08	10.60	0.001
T.C (mg/dl)	185.82 ± 6.90	174.36 ± 8.29	1.01	ns
T.G (mg/dl)	92.04 ± 4.61	71.36 ± 5.30	2.77	0.01
HDL.C (mg/dl)	58.85 ± 5.02	86.35 ± 7.37	3.77	0.001
LDL.C (mg/dl)	108.63 ± 7.39	70.00 ± 4.80	3.55	0.001

Values are in mean ± SEM
SEM= Standard error of mean

Table 3 Ascorbic acid and total cholesterol levels in the seminal fluid of patients and controls. (Mean ± S.E.M)

	Patients (n=27)	Controls (n=14)	t-value	p- value
Ascorbic acid (mg/dl)	2.08 ± 0.09	6.19 ± 0.73	7.0759	0.001
T. C(mg/dl)	68.20 ± 6.42	60.93 ± 6.81	0.7072	ns

T.C = Total cholesterol
S.E.M=Standard error of mean
ns= Not significant

Methods

The method of Kyaw[8] was used for the assay of plasma/semen ascorbic acid levels. Enzymatic colorimetric methods were employed for the estimation of plasma total and HDL cholesterol and triglyceride as well as semen TC [9],[10] The LDL cholesterol was calculated using the formula of Friedwald et al [11]. Semen counts; volume, % motility, % vitality and % morphological abnormality were obtained using WHO recommended methods[7] Quality control samples were included in all assays. Results were only accepted if they fell within acceptable limit.

Statistical analysis

All results were subjected to statistical analysis using the EP- INFO statistical package analysis; Student t-test and Pearson correlation coefficient were employed. Differences were regarded as significant at $p< 0.05$.

Results

Tables 1-3 show the results obtained from this study.

Tables 1-2 show the physical and biochemical characteristics of patients and controls. The patients were taller than the corresponding controls. Significant decreases were obtained in the sperm count, percentage motility and percentage vitality ($p<0.001$), while the percentage morphologically abnormal sperm cells was elevated ($p<0.001$) in all the cases when compared with the corresponding control values. No significant differences were obtained in the seminal volume and BMI. Interestingly; the plasma ascorbic acid and HDLC concentrations were significantly decreased ($p<0.001$) in all the cases when compared with the corresponding controls, while the plasma LDLC and triglyceride were significantly higher ($p<0.001$, $p<0.01$) in all the cases respectively when compared with the corresponding control values. The total plasma cholesterol was however, not significantly different from the control value.

As shown in table 3, there was remarkable significant decreased in the seminal ascorbic acid level ($P<0.001$) in all the patients when compared with the corresponding control value No significant difference was observed in the seminal total cholesterol in all patients when compared with the corresponding control value.

The mean seminal total cholesterol that was slightly higher in the patients was however, not significant when compared with the control value.

Discussion

This study has demonstrated significantly reduced plasma and semen ascorbic acid concentrations in male patients with inadequate spermatogenesis. In a similar study, decreased plasma and seminal ascorbate were linked with decreased sperm motility and count number [2, 12]. This perhaps suggests that adequate concentration of ascorbic acid in semen may play an important role in maintaining the well being and functioning of sperm cells. The exact relationship between plasma/seminal ascorbic acid concentrations, the motility and morphological normal sperm cells is uncertain. However, available evidence has speculated that inadequate ascorbic acid concentration in the semen may have an adverse effect on sperm quality[12]; our results probably suggest that these patients lack adequate ascorbate level that may at least in part contribute to the manifested semen quality. An earlier report[5, 12] showed that the patients with inadequate spermatogenesis due to agglutination who took supplements of ascorbic acid had improved sperm motility after supplementation. However; the baseline plasma/seminal ascorbic acid data were not reported

The present study appears to have significantly extended the findings in these earlier reports in that it measured plasma and seminal ascorbic acid baseline. The increased plasma LDL cholesterol and triglyceride and decreased HDLC are striking. Oxidation of increased circulating LDL cholesterol may give rise to lipid peroxyl radicals that are deleterious and these could be injurious to the sperm cell membrane invariably leading to the death of the sperm cells[3]. When the antioxidant nutrients available to scavenge these injurious free radicals are inadequate in the body as demonstrated in this study in the patients with low plasma and seminal ascorbic acid concentrations, the life span of the sperm cells is likely to be reduced[12]. Although the exact mechanism by which ascorbic acid participate in or modulate spermatogenesis is not well defined, the result of this study may suggest that reduced ascorbic acid in the semen may at least in part be responsible for abnormal spermatogenesis in these patients. Increased plasma LDL cholesterol has been associated with non-specific sperm agglutination[13].

Excessive generation of ROS by abnormal spermatozoa because of the high content of LDLC and decreased

plasma and seminal ascorbic acid in these patients sperm cell membrane has earlier been identified as one of the few defined aetiologies for male infertility[3]. Ascorbic acid acts as the first line of defense, against oxidative free radical and lipid peroxidation reaction[1, 3]. Therefore the protective effect of ascorbic acid is likely mediated through its ability to scavenge the excessive free radical generated through LDLC oxidation [13]. The plasma total, HDL and LDL cholesterol concentrations did not show any definite pattern with the percentage motility, morphological abnormal cells and vitality.

The results of this study suggest that the seminal and plasma ascorbic acid levels may be related to the seminal quality as well as sperm count, motility, vitality and increased abnormal sperm cell morphology, decreased plasma and seminal ascorbic acid in the patients with abnormal spermatogenesis.

This report appears to suggest that to improve fertility in an individual with suboptimal sperm count; ascorbic acid supplementation may be helpful intervention .The studies also suggest that low ascorbate level is probably a major disorder in infertile males. A study to determine the optimal dose is on going.

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