ORIGINAL ARTICLE

Assessment of Pituitary Gonadal Axis and Sperm Parameters in Anemic Eugonadal Males Before and After Correction of Iron Deficiency Anemia

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Abstract

Iron deficiency anemia (IDA) is one of the most common nutritional anemia worldwide. Anemia imposes a significant hypoxic environment in different organs and tissues including the testes. This study evaluated the effect of treatment of IDA on the pituitary gonadal axis (Serum FSH, LH, Testosterone) and sperm parameters in adult eugonadal males.

Methodology: A hospital based interventional, analytic study was conducted at a tertiary care center among 25 eugonadal males (fully sexually developed, fertile) with newly diagnosed and untreated IDA, admitted in medicine wards and not suffering from any inflammatory disorders (excluded by C-reactive protein) after exclusion of patients having other forms of anemia/ hemoglobinopathies/ any malignancy/having MCV >80 fL, aplastic anemia and primary hypogonadism. Sexual maturation was assessed according to maturity stages 5. Investigations were performed before and 6 weeks after treatment of IDA with intravenous iron sucrose included CBC, peripheral blood smear, serum ferritin, serum iron, TIBC, serum FSH, serum LH, serum Testosterone and semen analysis (Semen volume, Sperm count, Sperm motility and Sperm morphology).

Results: The change in mean Hb level before (5.66 ± 1.97gm/dl) and after treatment (11.96 ± 0.87 gm/dl) was statistically significant. (P<0.001) Patients who had subnormal and normal serum level of FSH, LH, Testosterone and sperm parameters before treatment were divided into group A and group B respectively. Serum levels of FSH, LH and testosterone along with sperm parameters significantly improved after correction of anemia (p<0.01). The mean change in these parameters was significantly higher in patients having subnormal value of these parameters before treatment (Group A) than in patients having normal pre-treatment level (Group B) (p<0.01). The level of anemia (hemoglobin) had significant positive correlation with serum FSH, serum LH, serum testosterone levels and sperm parameters (semen volume, sperm count, sperm morphology, RPM and sperm motility) (p<0.001).

Conclusion: IDA had significant negative association with the pituitary gonadal axis (Serum FSH, LH, Testosterone) and sperm parameters in adult eugonadal males. The serum levels of FSH, LH and testosterone along with sperm parameters significantly improved after correction of anemia, especially in patients having subnormal value of these parameters.

Introduction

Iron deficiency anemia (IDA) is one of the most common nutritional anemia worldwide.[1] Anemia imposes a significant hypoxic environment in different organs and tissues including the testes. Spermatogenesis in the seminiferous tubules of the testes occurs under a high proliferation rate, which demands considerable oxygen consumption.[2,3] Hence spermatogenesis is affected considerably by hypoxia.[2,3]

Morphological studies in animals reveal that hypoxia causes damage to germinal epithelium, folding of the basal membrane, degeneration, sloughing of spermatogenic cells in lumen of seminiferous tubules and lipid droplet deposition in sertoli cells and spermatogonia degeneration with chromatin margination.[4] Studies indicate that hypoxia reduces the fertility of male rats, rhesus monkeys and men by decreasing sperm count and sperm motility in semen.[5-8]

Up to best of our knowledge, there is no Indian literature available which has assessed pituitary gonadal axis and sperm parameters in anemic eugonadal males before and after correction of anemia. Hence, we undertook the present study to evaluate the effect of treatment of IDA on the pituitary gonadal axis (Serum FSH, LH, Testosterone) and sperm parameters in adult eugonadal males.

Methodology

This hospital based interventional, analytic study was conducted at a tertiary care center in Rajasthan, during one calendar year, after obtaining due permission from Research Review board/ Institutional Ethics committee and informed written consent of the study participants. Twenty five eugonadal males (fully sexually developed, fertile) with newly diagnosed and untreated iron deficiency anemia (Hb<10g/dl), admitted in medicine wards and not

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Table 1: Study parameters in participants before and after treatment of anemia and mean change in parameters after treatment of iron deficiency anemia

	Group A (n=6)			Group B (n=19)			Mean change after treatment		
	Before treatment	After treatment	P	Before treatment	After treatment	P	Group A	Group B	P
S. FSH (mIU/ml)	0.65 ± 0.014	1.65 ± 0.02	<.001*	2.61 ± 0.20	3.14 ± 0.20	<.001*	1.005±0.002	0.53±0.025	<0.01!
S.LH (mIU/ml)	0.54 ± 0.05	1.36 ± 0.08	<.001*	2.52 ± 0.18	3.0 ± 0.17	<.001*	0.82 ± 0.03	0.48 ± 0.04	<0.01!
S. Testosterone (ng/dl)	62.14±2.68	159.14±4.36	<.001*	238.17±20.30	296.33±17.12	<.001*	97.0 ± 1.84	58.16 ± 6.0	<0.01!
Semen volume(ml)	1.08 ± 0.124	3.01 ± 0.159	<.001*	1.927±0.319	3.55±0.257	<.001*	1.93 ± 0.08	1.63±0.08	<0.01!
Total sperm count (million/ ml)	34.63 ± 3.29	91.62±5.82	<.001*	50.12 ± 7.90	91.88 ± 8.84	<.001*	56.99 ± 5.91	41.76 ± 5.13	<0.01!
Normal Sperm Morphology (%)	20.25 ± 1.92	52.25± 1.92	<.001*	43.04 ± 6.67	56.09± 4.05	<.001*	32.0± 3.54	13.09±3.62	<0.01!
RPM (%)	15.66 ±0.75	51.40 ± 0.4	<.001*	27.70 ±1.31	56.7 ± 1.12	<.001*	35.80 ± 0.37	29.00 ±0.46	<0.01!
Total Sperm Motility (%)	25.66±1.33	75.83±0.30	<.001*	51.21±1.52	83.84±1.23	<.001*	50.17±1.07	33.16±0.82	<0.01!
Hemoglobin (gm/dl)	3.27 ± 0.96	10.9 ±0.27	<.001*	6.42 ± 1.56	12.28 ± 0.69	<.001*	7.63 ± 0.69	5.86 ± 0.87	<0.01!

S. FSH= Follicle stimulating hormone, S.LH= Leutinizing hormone, RPM= Rapid progressive sperm motility, * = Highly Significant, ! = Significant

suffering from any inflammatory disorders (excluded by C-reactive protein) were included in this study. Patients having other forms of anemia/ hemoglobinopathies/ any malignancy/ having MCV >80 fL and aplastic anemia were excluded from this study.

All cases of primary hypogonadism were excluded from the study. Sexual maturation was assessed according to maturity stages 5.[9] All patients were married and had children (≥1).

Investigations were performed before and 6 weeks after treatment of IDA with intravenous iron sucrose[10] included CBC, peripheral blood smear, serum ferritin, serum iron, TIBC, serum FSH, serum LH, and serum Testosterone in a fasting venous sample and semen analysis (Semen volume, Sperm count, Sperm motility and Sperm morphology). Semen collection was done by masturbation after minimum 3 days of abstinence and was analyzed as per WHO criteria.[11]

Blood samples were drawn from patients in EDTA vials for CBC including peripheral blood smear; and in plain vials for serum ferritin, serum iron and TIBC. Patients having microcytic hypochromic anemia, serum ferritin below 20 ng/ml and transferrin saturation < 20% were selected. Patient's clinical history, findings of physical examination and other relevant data, including lab test results, were recorded in structured forms. CBC was done on Sysmex XT 4000i automated analyzer. Serum ferritin was measured on IMMULITE 2000 Systems analyzer using a solid-phase, two-site chemiluminescent immunometric assay. Serum iron was measured using colorimetric assay. TIBC was measured using saturation – precipitation method. Transferrin saturation (TSAT) was calculated as TSAT = (serum iron/TIBC) x100 and expressed as percentage. Hormonal analysis was done by chemiluminescent immunometric assay in our institutional lab. Semen analysis was done by single pathologist who was blinded to the study and clinical state of the patients. All lab workers were blinded to the study and clinical state of the patients.

Statistical analysis

Microsoft Excel® and SPSS® 17 for Windows® were used for data storage and analysis. Continuous variables were expressed as mean ± standard deviation. Student's t test was used to determine statistical difference between variables. Correlation analysis was done by using Pearson correlation coefficient and linear regression was performed. Statistical significance was set at P value ≤ 0.05.

Results

Out of 25 eugonadal anemic male patients with IDA, 14 patients (56%) were below 25 years. and 11 (44%) patients were more than 25 years (age range 23-45 years). All patients had iron deficiency with Hb <10 gm/dl at the time of recruitment. The change in mean Hb level before (5.66 ± 1.97gm/dl) and after treatment (11.96 ± 0.87 gm/dl) was statistically significant. (P<0.001)

Patients who had subnormal and normal serum level of FSH, LH, Testosterone and sperm parameters before treatment were divided into group A and group B respectively.

Serum level of FSH, LH and Testosterone (Table 1)

The serum FSH level in group A were significantly high after correction of anemia (1.65 ± 0.02mIU/ml) compared to pre-treatment level (0.65 ± 0.014mIU/ml) (p <0.001). Similarly in group B, after treatment Serum FSH level were significantly higher (3.14 ± 0.20mIU/ml) compared to pre-treatment level (2.61 ± 0.2mIU/ml) (p <0.001). The mean change in Serum FSH level was significantly of larger magnitude in group A than group B (1.005 ± 0.002, 0.53 ± 0.025 respectively) (p <0.01).

The serum LH level in group A were significantly high after correction of anemia (1.36 ± 0.08mIU/ml) compared to pre-treatment level (0.54 ± 0.05 mIU/ml) (p <0.001). Similarly in group B, after treatment Serum LH level were significantly higher (3.00 ± 0.17mIU/ml) compared to pre-treatment level (2.52 ± 0.18 mIU/ml) (p <0.001). The mean change in Serum LH level was significantly of larger magnitude in group A than group B (0.82 ± 0.03, 0.48 ± 0.04 respectively) (p <0.01).

The serum testosterone level in group A were significantly high after correction of anemia (159.14 ± 4.36 ng/dl) compared to pre-treatment level (62.14 ± 2.68 ng/dl) (p <0.001). Similarly in group B, after treatment Serum testosterone level were significantly higher (296.33 ± 17.12 ng/dl) compared to pre-treatment level (238.17 ± 20.30 ng/dl) (p <0.001). The mean change in Serum testosterone level was significantly of larger magnitude in group A than group B (97.00 ±1.84, 58.16 ± 6.00 respectively) (p <0.01).

Sperm parameters (Table 1)

The semen volume in group A was significantly high after correction of anemia (3.01 ± 0.159 ml) compared to pre-treatment level (1.08±0.124ml) (p <0.001). Similarly in group B, after treatment semen volume was significantly higher (3.55±0.257ml) compared to pre-treatment level (3.01±0.159ml) (p <0.001). The mean change in semen volume was significantly of larger magnitude

Table 2: Correlation of hemoglobin and study parameters in participants, after treatment for iron deficiency anemia

Correlation	r-value	P value
Hb and serum FSH	+0.975	<.01
Hb and serum LH	+0.985	<.01
Hb and serum testosterone	+0.978	<.001
Hb and semen volume	+0.976	<.001
Hb and sperm count	+0.852	<.001
Hb and normal sperm morphology	+0.891	<.01
Hb and RPM	+0.901	<.001
Hb and total sperm motility	+0.981	<.001

P <.01-significant; P<.001-highly significant

in group A than group B (1.93±0.08, 1.63±0.08 respectively) (p <0.01).

Total sperm count in group A was significantly high after correction of anemia (91.625±5.829million/ml) compared to pre-treatment level (34.63±3.29million/ml) (p <0.001). Similarly in group B, after treatment sperm count was significantly higher (91.88±8.84) compared to pre-treatment level (50.12±7.90million/ml) (p <0.001). The mean change in sperm count was significantly of larger magnitude in group A than group B (56.99±5.91, 41.76±5.13 respectively) (p <0.01).

Normal sperm morphology in group A was significantly higher after correction of anemia (52.25±1.92 % of total sperm) compared to pre-treatment level (20.25±1.92 % of total sperm) (p <0.001). Similarly in group B, after treatment sperm with normal morphology were significantly higher (56.09±4.05 % of total sperm) compared to pre-treatment level (43.04±6.67 % of total sperm) (p <0.001). The mean change in sperm with normal morphology were significantly of larger magnitude in group A than group B (32.00±3.54, 13.09±3.62 respectively) (p <0.01).

In group A, sperm with rapid progressive motility (RPM) were significantly higher after treatment of anemia (51.40 ± 0.40 %) compared to pre-treatment level (15.66 ± 0.75 %) (p <0.001). Similarly in group B, sperm with rapid progressive motility (RPM) were significantly higher after treatment of anemia (56.70 ± 1.12 %) compared to pre-treatment level (27.70 ± 1.31 %) (p<0.001). The mean change in sperm with RPM were significantly of larger magnitude in group A than group B (35.80±0.37, 29.00±0.46 respectively) (p <0.01).

Total sperm motility increased significantly in group A after treatment (75.83±0.30%) compared to pre-treatment status (25.66±1.33%) (p <0.001). Similarly in group B, total sperm motility increased significantly after treatment (83.84±1.23 %) compared to pre-treatment status (51.21±1.52 %) (p <0.001). The mean change in sperm motility were significantly of larger magnitude in group A than group B (50.17±1.07, 33.16±0.82 respectively) (p <0.01).

Correlation of anemia (Hb) and study parameters (Table 2)

Hemoglobin level had significant positive correlation with serum FSH, serum LH, serum testosterone levels and sperm parameters (semen volume, sperm count, sperm morphology, RPM and sperm motility) (p<0.001).

Discussion

In this study, the effect of treatment of IDA on the pituitary gonadal axis (Serum FSH, LH, Testosterone) and sperm parameters in adult eugonadal males was evaluated. It was found that serum levels of FSH, LH and testosterone along with sperm parameters significantly improved after correction of anemia. The mean change in these parameters was significantly higher in patients having subnormal value of these parameters before treatment (Group A) than in patients having normal pre-treatment level (Group B). The level of anemia (hemoglobin) correlated well with all these parameters.

One possible explanation for improvement in study parameters after anemia correction is hypoxic effect of anemia which results in impairment in hormonal and sperm parameters.[5-8] Various studies are described in literature supporting hypoxia as a cause of impaired hormonal and sperm parameters.[5-8]

When effect of sickle cell disease on gonadotropin-thyrotropin releasing hormones (GnRH-TRH), LH, FSH and TSH was studied, it was found that blood transfusion (anemia correction) elevates level of LH, FSH and TSH without affecting Prolactin level. Correction of anemia improves hypoxia and subsequently elevates gonadotropin hormones.[12] In sickle cell anemic patients, abnormalities of semen volume, sperm morphology and sperm motility were found.[13-14] Correction of anemia was also found to be associated with gonadotropin hormones levels and sperm parameters in sickle cell disease.[15]

Similarly in thalassemia major patients, improvement in hormone levels (serum FSH, LH, Testosterone) and semen parameters (sperm count and sperm morphology) were significantly associated with increase in haemoglobin after packed red cell transfusion.[16]

In mountain trekkers, the effect of chronic hypoxia due to high altitude (2000-5600 meters above sea level) resulted in alteration of human spermatogenic parameters (oligospermia) and these spermatogenic alterations restored 1-6 months after returning to sea level. Thus oxygen supply had some role in physiological mechanisms of spermatogenesis and male fertility.[17-18] High altitude might affect spermatogenesis and Leydig cell function, negatively but reversibly.[19] Hypobaric hypoxia (high altitude) also inhibits the spermatogenesis in rats and decreases primary spermatocytes and thus suppress spermatogenesis.[20] High altitude (hypobaric hypoxia) was also found to be associated with fall in LH, FSH and Testosterone levels in adult male and these hormones returned to normal level when the persons came to low land.[21]

One pilot study was in echo with our study as this pilot study evaluated semen parameters and hormone levels (serum FSH, LH, Testosterone) before and 12 weeks after iron therapy in adults with iron-deficiency anemia. They found that after correction of anemia, a significant increase of Hb was associated with an increase of Testosterone, FSH and LH. Semen volume, sperm count, sperm motility and sperm morphology improved significantly after anemia correction.[22] In current study, after correction of anemia, significant positive correlation of hemoglobin levels were found with serum FSH, LH, Testosterone levels and sperm parameters (sperm count, semen volume, sperm morphology, sperm motility). Similar findings were reported in previous study in IDA.[22]

Previous reports also had shown significant positive correlation of hemoglobin levels with serum FSH, LH, Testosterone levels and sperm parameters in sickle cell anemia and in

thalassemia.[15-16,23]

Physiologically, the testes are at risk of hypoxia as they have high metabolic requirements due to spermatogenesis and peculiar blood supply as approximately 50% of incoming arterial blood is siphoned off via arterio-venous anastomoses in the spermatic cord.[22] Blood vessels are located exclusively between the tubules, and oxygen reaches the lumen of the seminiferous tubules only by diffusion. So, seminiferous tubules operate in a state of relative hypoxia. Whenever oxygen delivery to testes became hampered as in anemia, it would negatively affect spermatogenesis. The deleterious effect of anemia on hormone levels (FSH, LH, testosterone) and sperm parameters with improvement after anemia treatment, therefore, consistent with this possible mechanism.[22]
Animal studies in male rats and rhesus monkeys proposed that hypoxia reduces the fertility by decreasing sperm count and sperm motility.[2,5-6,24] Hypoxic damage to germinal epithelium, folding of the basal membrane, degeneration, sloughing of spermatogenic cells in lumen of seminiferous tubules and lipid droplet deposition in sertoli cells and spermatogonia degeneration with chromatin margination also demonstrated.[2,5-6]

Hypoxia also increases interstitial spaces of testes, which extend the oxygen diffusion distance and would impair oxygen delivery to germ cell. The germ cell became more susceptible to damage, which is confirmed by degenerative germ cell in hypoxic rats under light and electron microscopy. The changes in testes point out to the "vulnerability" of spermatogenesis to hypoxia.[2-3]

So the anemia related testicular hypoxia negatively impact spermatogenesis which can be improved after anemia correction.

Limitations

The sample size of patients included in our study is smaller (n=25), which may partially limit the significant findings of this study. We included only IDA patients in our study. We did not include patients with megaloblastic/ dimorphic anemia as vitamin B_{12} and folate can impair spermatogenesis per se, apart from the hypoxic effect of anemia. Larger studies are required to enforce these results.

Conclusion

IDA had significant negative association with the pituitary gonadal axis (Serum FSH, LH, Testosterone) and sperm parameters in adult eugonadal males. The serum levels of FSH, LH and testosterone along with sperm parameters significantly improved after correction of anemia, especially in patients having subnormal value of these parameters.

So anemia has independent effect on pituitary-gonadal axis and spermatogenesis and should be kept in mind in evaluating patients with suboptimal sperm parameters.

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