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Effects of tyrosine kinase inhibitors on spermatogenesis and pituitary gonadal axis in males with chronic myeloid leukemia

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Abstract

Objective: The introduction of several classes of targeted therapeutics for the treatment of chronic myelogenous leukemia (CML) raises the question of whether male fertility is affected and the degree of this affection, if any, among the different generations of tyrosine kinase inhibitors (TKIs). Additionally, when two drugs are equally effective, the drug with less toxic effect on fertility is favourable. Our aims were to evaluate semen parameters and pituitary gonadal function before and four months after starting TKIs namely, dasatinib, nilotinib, and imatinib in patients with CML. *Design:* Prospective study. *Setting, patients and interventions:* We studied the effect of TKIs' first generation (imatinib) and second generation (dasatinib and nilotinib) on semen parameters and endocrine functions in 20 eugonadal male patients with CML, aged between 35 to 51 years. They were receiving imatinib (400 mg) once daily, dasatinib (100 mg) once daily or nilotinib (300 mg) twice daily as upfront therapy. We assessed the serum gonadotropins (LH and FSH) and testosterone (T) secretion and sperm parameters before and after four months of using these TKIs. *Results:* Four months after starting TKIs, serum testosterone, LH and FSH concentrations decreased significantly. The total sperm count (SC), total and rapid progressive sperm motility, and % sperms with normal morphology decreased significantly versus pre-treatment. After 4 months of therapy, dasatinib had comparatively the least deleterious effects on SC, ejaculate volume (SV), sperm motility and % of sperms with normal morphology (%NM) compared to imatinib and nilotinib. Significant correlations were found between serum T concentrations and semen parameters before and after TKIs therapy including SC ($r = 0.658$ and $r = 0.73$ respectively, $p < 0.001$), rapid progressive motility ($r = 0.675$ and $r = 0.758$, respectively; $p < 0.001$), and the % NM ($r = 0.752$ and $r = 0.834$, respectively; $p < 0.001$). After TKIs therapy, LH were correlated significantly with T concentrations, SV and SC ($r = 0.434$, 0.439 and 0.376, respectively; < p: 0.01). *Conclusions:* Our study showed that CML patients treated with TKIs have significant decrease of sperm parameters and decreased serum concentrations of serum T, LH and FSH. These potentially toxic effects on spermatogenesis are less prominent in patients treated with dasatinib compared to imatinib and nilotinib. The mechanisms and pathways for these effects need further human and/or experimental studies.

Keywords: chronic myelogenous leukemia (CML); tyrosine kinase inhibitors (TKIs); spermatogenesis; pituitary-gonadal axis

Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasms of the hematopoietic stem cells characterized by a triphasic clinical presentation, and the presence of a reciprocal translocation between chromosomes 9 and 22 or the Philadelphia chromosome (Ph+). The juxtaposition of the breakpoint cluster region (BCR) on chromosome 22 with the Abelson gene (ABL) on chromosome 9 leads to a dys-regulation of the ABL intrinsic non receptor tyrosine kinase activity. By controlling downstream pathways involved in cell proliferation, adhesion, and survival, the hybrid oncogene BCR-ABL is considered as a major driver of leukemogenesis in CML. The therapeutic approach for CML has undergone a revolutionary change during the last 2 decades with the introduction of the tyrosine kinase inhibitors. The use of these agents has changed overall survival as well as the

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quality of life of CML patients. Since the introduction of imatinib, newer agents have been developed and their role as first line treatment was examined [1, 2].

Dasatinib and nilotinib were first approved for patients resistant or intolerant of prior imatinib therapy, reactive against most BCR-ABL mutations with the exception of T315I, and phase III randomized trials later confirmed that dasatinib and nilotinib were superior to imatinib, inducing faster and higher rates of complete cytogenetic responses (CCyRs) and molecular responses. Therefore, both drugs were granted approval by the US Food and Drug Administration to be used in patients with newly diagnosed CML in chronic phase (CML-CP) [2, 3].

The improved survival rates among patients with hematological malignancies, such as leukemia and lymphoma, are shifting areas of focus towards understanding and preventing treatment-induced complications. Of these, infertility is one of the most devastating consequences for patients with reproductive potential. CML generally occurs in the 4th decade of life, when sexual and reproductive functions are important biological functions in men that can be adversely affected by the disease or its therapy. Many side effects have been reported using old CML chemotherapy drugs in relation to sexual functions. CML itself seems to have adverse effect on fertility before any form of treatment [4, 5]. With the improved survivals offered by the TKIs has come the necessity to address issues relating to quality of life and one such area is that of fertility and parenting. The degree of treatment-induced gonadal dysfunction depends on age and gender-related differences, the type and dosage of therapy used. Animal data suggest that imatinib at standard dosages is unlikely to impair fertility in either adult males or females but human data remain limited [4-7].

Children born to men who are actively taking imatinib at the time of conception appear healthy and current advice is not to discontinue treatment. In contrast the data relating to children born to women exposed to imatinib during pregnancy are less encouraging. Although numbers are small, there has been a disturbing cluster of rare congenital malformations such that imatinib cannot be safely recommended, particularly during the period of organogenesis [4-9].

This study was intended to observe the effect(s) of imatinib, dasatinib and nilotinib on the pituitary- gonadal axis and sperm parameters in patients with CML.

Materials and methods

We studied the effect of Tyrosine Kinase Inhibitors (TKIs') first generation (imatinib) and second generation (dasatinib and nilotinib) on semen parameters, endocrine functions in 20 eugonadal male patients with chronic phase CML, and aged from 35 to 51 years. They were receiving imatinib, dasatinib or nilotinib as upfront therapy.

Anthropometric measurements included: Weight, height, body mass index (BMI). All patients were fully sexually developed. Sexual maturation was assessed according to Tanner et al. maturity stages [10], and testicular volume was assessed by Prader orchidometer. All of them were married, and had children.

The following investigations were performed before and after 4 months of treatment with TKIs for the first time and no prior treatment with Hydroxyurea or Interferon therapy: i) Semen analysis (total sperm count, morphology, and motility) at least after 3 days of abstinence. ii) Measurement of serum levels of FSH, LH, and T in a fasting venous sample at 8 AM.

Conventional semen analysis was carried out using manual procedures and light microscopy, in the central hospital laboratory, according to the last WHO guidelines [11]. The semen analyser was blinded to patients' names and diagnosis. Two semen samples were taken from each patient 4 days apart, and the average of the two readings was calculated for different semen parameters.

The following nomenclature was used to define the semen quality: i) Normozoospermia: A total number of spermatozoa, and a percentage of progressively motile and morphologically normal spermatozoa, equal to or above the lower WHO reference limit [11]. ii) Oligospermia: A total sperm concentration per ejaculate below the lower reference limit (5^{th} percentile: 33 million) [11].

The normal serum levels of T, LH, and FSH in our central lab for subjects aged 30-45 years are: total testosterone = 21.4 ± 5.9 nmol/L, LH = 4.5 ± 1.2 IU/L, FSH = 3.2 ± 0.9 IU/L). All hormone levels were measured by radioimmunoassay (Diagnostic Products Corporation, USA). Serum hormone levels were measured in one assay after completion of the study to avoid critical between-assay variability. The Ethical Committee of Hamad Medical Center (HMC) approved the study, and informed consent was obtained from all the patients.

The results are presented as mean ± standard deviation (SD), and the paired t-test was applied to analyse the data when normally distributed, and Wilcoxon test was applied when the data were not normally distributed. Linear regression equation was used to study the relations between different variables. ($p: < 0.05$ was chosen as the limit of significance.)

Results

We investigated the effects of TKIs on pituitary gonadal axis and spermiogram in 20 adult males with chronic phase CML who had spontaneous pubertal development (Tanner's stage 5) with normal secondary sex characteristics, testicular volume (17.7 ± 4.5 ml) and spontaneous spermatogenesis.

Four months after starting TKIs there were significant decreases in serum T, LH, FSH concentrations. The total sperm count (SC), total and rapid progressive sperm

motility, and % sperms with normal morphology (%NM) decreased significantly versus before treatment (Table 1). After 4 months of TKIs therapy, it was shown that dasatinib had significantly less deleterious effect on sperm count (SC), volume (SV), all sperm motilities and % NM compared to imatinib and nilotinib (Table 1).

Table 1 Hormonal and sperm parameters before versus after TKIs therapy for 4 months (mean+/-SD).

	Imatinib		*Dasatinib*		*Niolotinib*		*All*	
	Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
Age (yr)	43.57	43.95	46.14	46.49	46.17	46.52	45.25	45.60
SD	8.94	8.90	8.76	8.74	5.00	5.20	7.59	7.61
LH (U/L)	7.71	5.00*	5.29	5.14	4.83	3.50*	6.00	4.60*
SD	1.38	1.15	1.11	0.90	1.17	0.55	1.75	1.14
FSH (U/L)	7.14	4.14*	5.71	5.71	5.17	3.50*	6.05	4.50*
SD	1.07	0.69	0.95	0.95	0.75	0.55	1.23	1.19
Testosterone	18.34	13.17*	15.64	14.80*	15.05	12.33*	16.41	13.49*
SD	1.33	1.12	1.69	1.69	1.63	1.13	2.08	1.65
Sperm count (M/ml)	220.6	88.00*	271.57	256.43	202.17	123.67*	232.90	157.65*
SD	54.89	15.80	46.40	44.47	36.18	24.11	53.67	81.26
Ejaculate vol (ml)	2.86	2.29*	3.00	2.64*	2.42	2.17*	2.78	2.38*
SD	0.38	0.27	0.71	0.56	0.49	0.41	0.57	0.46
Total PM (M/ml)	135.0	44.57*	126.43	118.43	130.17	42.67*	130.5	69.85*
SD	47.22	6.68	32.67	32.65	13.01	8.41	33.15	41.32
RPM (M/ml)	88.14	31.29*	80.29	72.57*	70.17	25.83*	80.00	44.10*
SD	38.94	9.39	25.62	18.94	14.39	7.28	28.20	24.89
SPM (M/ml)	46.86	13.29*	46.14	45.86*	60.00	16.83*	50.55	25.75*
SD	10.82	5.09	14.65	20.89	8.69	1.72	12.85	19.44
NPM (M/ml)	43.57	20.29*	83.86	71.14*	36.50	35.50	55.55	42.65*
SD	31.17	7.16	26.60	20.93	25.89	10.73	34.20	26.16
Immotile (M/ml)	42.00	23.14*	61.29	65.71	35.50	45.50*	46.80	44.75
SD	12.06	7.24	29.76	19.90	19.09	17.39	23.40	23.56
Normal morphology %	57.57	41.57*	54.43	52.43	54.17	30.17*	46.80	41.95*
SD	3.21	4.61	3.69	2.23	4.62	7.19	23.40	10.31

Abbreviations: *= $P < 0.05$; Total PM= total sperm motility; RPM = rapid sperm motility; SPM = slow sperm motility; NPM = normal sperm motility.

Significant correlations were found between serum T concentrations and semen parameters before and after TKIs therapy including SC ($r = 0.658$ and $r = 0.73$; respectively, $p < 0.001$), rapid progressive motility ($r = 0.675$ and $r = 0.758$, respectively; $p < 0.001$), and the % NM ($r = 0.752$ and $r = 0.834$, respectively; $p < 0.001$).

After TKIs therapy, serum gonadotropins and testosterone concentrations were significantly correlated to some seminal parameters (motility and morphology). (Figures 1-4) LH concentrations were correlated significantly with T concentrations ($r = 0.52$, $p = 0.01$) (Figure 5).

Discussion

Tyrosine kinase inhibitors revolutionized the treatment of CML with increase in survival and significant improvement in quality of life thereby resulting in an increased number of patients willing to father or mother a child. Although animal data suggested that TKIs are unlikely to impair fertility in males and females, human data are lacking. This prospective study compared the effects of TKIs imatinib, nilotinib and dasatinib on patients with chronic phase CML previously untreated on hypothalamic- pituitary gonadal axis and sperm parameters [4-7].

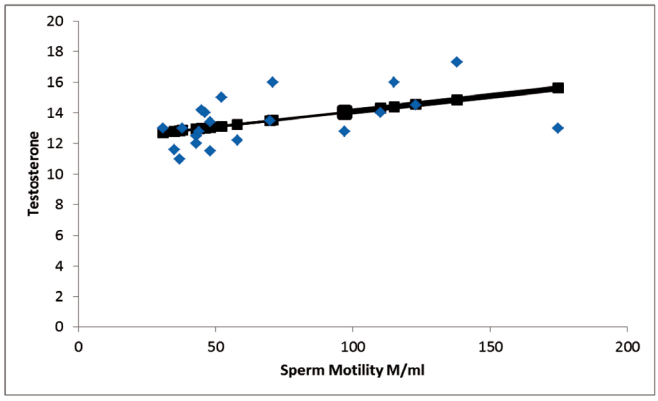

Figure 1 Correlations between testosterone (nmol/L) concentration and sperm motility after TKIs (r = 0.75, P < 0.01).

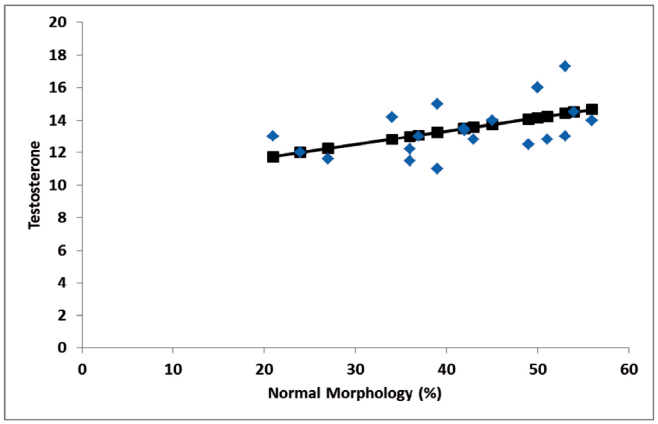

Figure 2 Correlations between testosterone concentration and normal morphology % after TKIs (r = 0.83, P < 0.01).

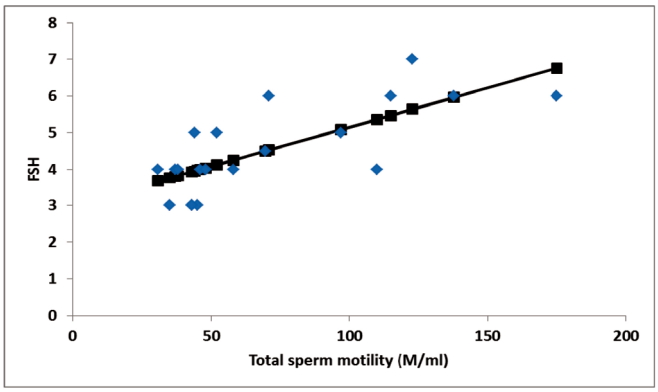

Figure 3 Correlation between FSH and sperm motility (r = 0.72, p < 001).

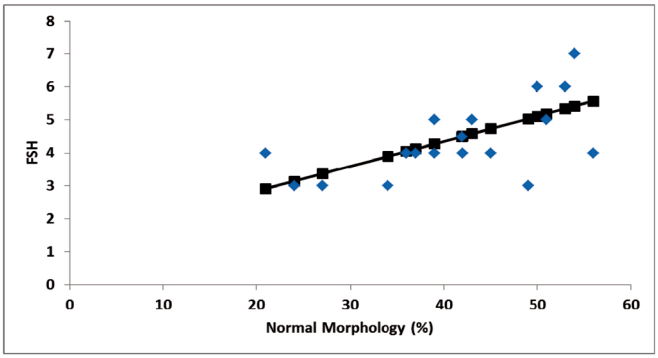

Figure 4 Correlation between FSH and sperm motility % (r = 0.65, p < 0.001).

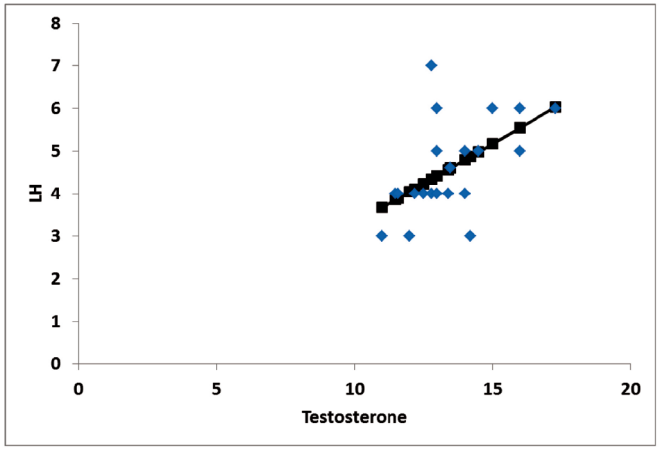

Figure 5 Correlation between LH and testosterone concentrations (r = 0.52, p = 0.01).

Hormones act on all phases of spermatogenesis. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) act directly on the testes to stimulate somatic cell function in support of spermatogenesis. Three phases of spermatogenesis that are regulated by gonadotropins in men are: i) The maturation of type A spermatogonia to type B spermatogonia, ii) meiosis, and iii) spermiation, both acutely and chronically. Both FSH and T actions are important for the progression of meiosis, perhaps by the regulation of the germ cell survival (inhibition of apoptosis) and also spermiation in both rodents and humans. Data show that FSH predominantly regulates spermatogonial development, while testosterone regulates the latter phase of spermiogenesis, while both FSH and testosterone seem to be equally important in supporting spermatocyte development. Testosterone is important for the conversion of step 7 round spermatids into step 8 spermatids by regulating the adhesion between Sertoli cells and round/elongating/elongated spermatids at the apical ectoplasmic specialization adherent junctions [12-14]. For humans, entire process of spermatogenesis takes 74 days. Including the transport on ductal system, it takes 3 months. For this reason we choose an interval period of 4 months before to reassess the pituitary gonadal axis of our patients.

In this study TKIs decreased LH, FSH and T secretion and T concentration was correlated significantly with sperm parameters before and after TKIs therapy. These findings can explain partially the decreased sperm parameter's during TKIs therapy. In vitro tyrosine kinase inhibitors have been shown to inhibit luteinizing hormone-releasing hormone (LHRH)-induced gonadotropin release from rat hemi pituitaries, as well as other pituitary hormones from pituitary cells [15, 16]. In addition TKIs have been shown to inhibit testosterone secretion by Leydig cells in animals [17, 18].

A comprehensive high quality semen analysis is an essential first line investigation for male fertility. Semen quality is conventionally determined according to the number, motility, and morphology of spermatozoa in an

ejaculate. Of all semen parameters, sperm morphology turns out to be the best predictor of a man's fertilizing ability. Besides, motility is an important fertility-relevant quality parameter [19, 20]. A link has been established between sperm morphological characteristics and infertility by many investigators. However, total number of sperm per ejaculate is another important measure for clinicians to provide advice to patient couples [19-21].

In this study, significant deterioration of sperm count, morphology, and motility occurred after 16 weeks of treatment with TKIs (Table1) and suggest a harmful effect of TKIs on spermatogenesis and potential fertility.

Consistent with our findings, selective protein tyrosine kinase inhibitors have been shown to inhibit sperm phosphor-tyrosine (PY)-containing proteins (located both at the tail and head of spermatozoa) and decreases sperm motility and motion parameters in vitro [22, 23]. Defective sperm flagellar motility and hyperactivated motility, production of volume changes that negatively affect motility of the sperm as well as defective capacitation of the sperms have been observed in animal studies [24-31]. In addition, three-day treatment of immature male rats (SD) with imatinib (150 mg/kg) on postnatal days 5-7 delayed the formation of germ-line stem cell pool, reduced proliferation of type A spermatogonia and induced germ cell apoptosis [32]. In human, tail protein PY-phosphorylation is related to sperm movement and especially to hyperactivated motility and its deficiency, during treatment with TKIs, may be associated with reduced sperm motility [33, 34].

It is reasonably reassuring that although the use of TKIs therapy significantly decreased sperm count and functions in our patients, it did not lead to severe oligospermia or asthenozoospermia (immotile spermatozoa) in any of the patients. However, it is prudent to follow-up sperm parameters in CML male patients on TKIs therapy. Sperm banking before the use of TKIs in those males can easily preserve healthy sperms that can be used for intrauterine insemination or in vitro fertilization. In addition, treatment of low testosterone is another treatment option for those with subnormal T level [35].

Conclusions

TKIs treatment of men with CML adversely affects sperm parameters coupled with decreased serum concentrations of T, LH, and FSH but is unlikely to completely knock out sperm production. The use of TKIs with the least adverse effect on spermatogenesis appears to be preferable on the long term treatment of male patients. Sperm banking before TKIs therapy can easily preserve healthy sperms for potential future fertility in those patients. The limitation of the study is the small number of CML patients enrolled in our study that may partially limit the significant findings of this pilot study. Larger studies, therefore, are needed to enforce these original results.

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