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Sperm Preservation by Electroejaculation in Adolescent Cancer Patients

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Background. The increasing prevalence of cancer survivors who are infertile due to gonadal failure highlights the importance of fertility preservation prior to gonadotoxic treatments. Adolescent cancer patients may not be mature enough to produce sperm by masturbation, leading to the use of alternative methods for obtaining sperm for cryopreservation. The aim of the current study was to evaluate the safety and efficacy of electroejaculation (EEJ) for cryopreservation among adolescent cancer patients. ***Procedure.*** This retrospective cohort study included 45 adolescent (12–18 years old) cancer patients who underwent EEJ during 2002–2012 in an academic tertiary referral fertility center. Sperm cryopreservation, ejaculate parameters, and procedure complications were evaluated. ***Results.*** EEJ was performed without documented complications. Sperm was successfully obtained in 30 (66.7%) patients. Retrieval failures included ejaculates without sperm in 8 patients (17.8%) and no ejaculate in 7 patients (15.5%). Cryopreserved ejaculates were characterized by severe asthenospermia, normal sperm concentration, and low volume. Retrieved sperm group was further divided to 19 motile sperm ejaculates with significantly higher volume, sperm concentration, and total count compared to 10 non-motile sperm patients. ***Conclusions.*** EEJ is a safe and feasible procedure for cryopreservation in adolescent cancer patients who are unable to masturbate. The wide diversity of EEJ outcome and ejaculate parameters may represent a continuum of pubertal changes in that population. Pediatr Blood Cancer 2014;61:286–290.

Key words: adolescent cancer patients; cryopreservation; electroejaculation; gonadal failure

INTRODUCTION

Cancer disease among adolescents carries tremendous medical, psychological, and sociological challenges among patients, families, and care givers. Total international incidence of cancer in male adolescents (age 15–19 years) ranged from 85 to 228 cases per million person years in the studied series [1]. Cancer is diagnosed annually among 20,000 adolescents/young adults (age 15–24 years) across Europe with a 5-year survival estimation of 87% [2]. Similar to childhood malignancies, hematopoietic cancers are the most common with variable survival between that of Hodgkin lymphoma (93%) and acute lymphoid leukemia (50%).

Fortunately, treatment efficacy has improved significantly during the last decades and the overall number of survivors has increased substantially [3]. Therefore, the prevalence of adults who were previously treated by chemotherapy and/or radiotherapy has increased significantly, resulting with more secondary malignancies and other late sequelae that require medical treatment [2]. Infertility due to gonadal failure is one of the major consequences of cancer therapy [4]: an average of 11–30% of cured cancer patients remain sterile in the long-term [5,6] with higher infertility rates among males compared to females [5]. The risk for infertility is influenced by the age of the oncological patient, underling disease, type of therapeutic agent, cumulative doses and duration of treatment [7,8]. Gonadal failure in these patients is due to accelerated and premature depletion of germ cells in the gonads [9].

The increasing prevalence of infertile adults due to previous cancer treatments highlights the importance of fertility preservation prior to initiation of gonadotoxic treatments. The only established method to secure fertility in male cancer patients is semen cryopreservation (SCP) [10], which should be strongly considered in each cancer patient since recovery or spermatogenesis cannot be guaranteed for an individual patient [8]. In the past, SCP could only be used for intra-uterine insemination (IUI), but since freezing and thawing of the sperm have a deleterious effect on the cryopreserved sperm, only a few patients had compatible semen samples to use with that method [8,10]. The introduction of assisted reproduction technologies (ART) such as IVF (In Vitro Fertilization) and ICSI (Intra Cytoplasmic Sperm Injection) provided cured cancer patients with a reasonable opportunity to become fathers even with the poorest semen sample [11].

While semen banking is well accepted in adult male cancer patients, the same is not true for adolescents [8]. Although Bahadur et al. [12] demonstrated a success rate of 86.1% among post-pubertal males who produced semen for cryostorage, Muller et al. [13] demonstrated major psychological obstacles that resulted in only 44.5% SCP. It seems that adolescent patients may be shy or too immature to obtain sperm for cryopreservation by masturbation. Therefore, alternative methods should be offered such as vibrostimulation or electroejaculation (EEJ) [3,14].

EEJ is performed by transition of electrical stimulation from a rectal probe to the short post-synaptic fibers in the wall of ejaculatory organs [15]. Improvements in the stimulator and the techniques have established EEJ as a simple and promising method for sperm retrieval in unejaculatory men [16] such as those with spinal cord injury and psychogenic unejaculation [17] with sperm retrieval of more than 90% [16–18]. However, hormonal profiles of adolescents with a diagnosed neoplastic disease such as acute

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lymphoblastic leukemia (ALL), non-Hodgkin lymphoma (NHL), and Hodgkin lymphoma (HL) revealed gonadal function that may represent spermatogenesis dysfunction [19]. The current data regarding sperm retrieval by EEJ in adolescent cancer patients is limited. Relatively small series have been published with reasonable chance of compatible samples. Reliable experience has been achieved in our tertiary center and a large sample size has been collected during recent years. Therefore, the aim of the current retrospective study was to describe our experience with EEJ in adolescent cancer patients in order to evaluate the safety and efficacy of EEJ and to give updated data regarding its outcome.

MATERIALS AND METHODS

Population

In this retrospective study we scanned the charts of all adolescent (aged 12–18) cancer patients who underwent EEJ in Sheba Medical Center between January 1, 2002 and December 31, 2012. All included patients were referred to the andrology team for fertility preservation consultation in our tertiary center after being diagnosed as cancer patients. Some patients were treated by the oncological team of our hospital and others were referred from various hospitals in Israel. Routine evaluation included personal and family medical history with special attention to malignancy type with regard to the scheduled anti-cancer treatments. Another focus regarded pubertal status including Tanner stage and the ability to masturbate. All included patients were diagnosed as Tanner stage $\geq$2 and did not attempt to masturbate mainly due to psychological difficulties (in a minority of cases masturbation was not considered because of religious beliefs); therefore EEJ was indicated. Complete physical examination was performed including comprehensive urological evaluation without any documented varicoceles in that cohort.

Electroejaculation

Our policy was to offer EEJ in all patients who demonstrated pubertal development (Tanner stages II–V) and were not able to masturbate. Therefore, patient referral to EEJ was based on clinical judgment. Testis ultrasound and hormonal profile were not included routinely in our evaluation for two main reasons. First, in these circumstances we provided a liberal approach regarding EEJ performance due to the great importance of SCP in that population. Therefore these tests had limited practical clinical implications. Second, in many instances we had to perform EEJ as fast as possible because of oncological considerations.

In order to minimize the need for anesthesia, in most cases EEJ performance was combined with bone marrow aspiration or insertion of intravenous catheter prior to initiation of gonadotoxic treatments. Before EEJ all patients parents gave their informed procedural consent with comprehensive understanding of EEJ and its possible complications. Patients were instructed to empty their bladder before the procedure. Under general anesthesia with the patient placed in a lateral decubitus position, a rectal probe (Seager electrical stimulator 11/8, G&S Instrument Co., Duncanaville, TX) was inserted with its electrodes facing anteriorly. The used voltage was 10–15 V for 4–5 seconds. When there was no response to initial stimulation, 2–3 additional stimulations were performed using 5–10 V lasting 3–5 seconds; according to patient age up to four stimulations could be given. Patients who did not ejaculate after repeated electrical stimulations were catheterized in order to rule out retrograde ejaculation. In cases of rectal temperature (monitored by the device) of 39.9°C, the electrical current was discontinued automatically in order to avoid thermal injury. In order to detect complications as early as possible vital signs were continuously monitored and physical examination was performed immediately after the procedure was completed.

Vibratory stimulation was not conducted in the current cohort for two reasons. First, EEJ is the preferred treatment in our institution for anejaculation due to various etiologies (spinal cord injury, retroperitoneal lymph node dissection, psychogenic anejaculation, etc.); therefore we have gained wide experience with that procedure. Second, some of our patients are Orthodox Jews, who prohibit vibratory stimulation as masturbation leading to "spilling seed," which is forbidden for religious reasons.

Outcome Measurements and Statistical Analysis

The primary endpoint was sperm recruitment for cryopreservation by EEJ. The secondary endpoint was sperm concentration and motility analyzed by a Makler chamber [20] according to the relevant WHO guidelines during EEJ performance [21] by the specialized laboratory team in our tertiary center. Semen parameters are presented as median and range because of skewed distributions. Continuous variables were compared using Wilcoxon Rank Sum test while categorical data were compared using the Fisher exact test as appropriate. A two sided P value <0.05 was considered significant.

Ethics Approval

The research was approved by the Hospital Research Ethics Board.

RESULTS

Patients' Characteristics

Between January 1, 2002 and December 31, 2012, forty-five 12–18 year-old cancer patients underwent EEJ in Sheba Medical Center, Ramat Gan, Israel. The mean age of our cohort was 14.96 ± 1.84 years. No complications were documented throughout EEJ performance. Thirty-one patients (68.9%) suffered from hematological malignancies compared to 13 patients (28.9%) who had solid tumors (two of them suffered from testicular cancer). In this retrospective study we did not have the oncological diagnosis of one patient.

Sperm Retrieval by Electroejaculation and Ejaculate Parameters

Sperm was successfully obtained in 30 (66.7%) patients. Retrieval failures were ejaculates with no sperm cells in 8 patients (17.8%) and no ejaculate in 7 patients (15.5%) (Fig. 1). The successfully obtained sperm cohort was older than the non-obtained sperm group (15.27 ± 1.91 and 14.33 ± 1.59 years, respectively) without significant difference ($P = 0.11$). Comparison of hematological versus solid malignancy rates in these groups did not reveal significant difference as well.

Investigation of the cryopreserved semen parameters revealed severe asthenospermia (<40% progressive motility [21]) with relatively normal sperm concentration and low volume: the median and range of volume, concentration, total sperm count, and motility

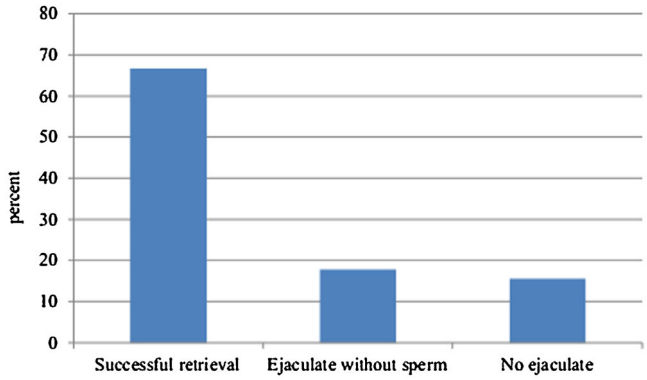

Fig. 1. Sperm recruitment by EEJ in adolescent cancer patients.

in the obtained ejaculate were 0.32 (0.05–5.6) ml, 19 (1–300) $\times$ 10^6/ml, 6.75 (0.05–840) $\times$ 10^6/ml, and 3.5 (0–45)%, respectively. One exceptional sperm count of 3,000 $\times$ 10^6/ml which we believe was not reliable and significantly diverted statistical analysis was excluded during sperm parameter analysis. Interestingly, 10 ejaculates had no motile sperm compared to the other 19 patients with motile obtained sperm. In order to evaluate these subgroups we compared ejaculate characteristics. Ejaculate volume as well as sperm concentration and total count were significantly lower in the non-motile sperm group compared to the motile sperm cohort (Table I).

The Possible Effect of Primary Malignancy on Retrieved Sperm

In order to investigate the optional influence of malignancy type on sperm quality we compared semen parameters between solid and hematological malignancies among the cryopreserved sperm cohort. There were 19 patients who suffered from hematological cancer, mostly HL (7 patients) followed by acute leukemia (5 ALL and 1 AML), NHL (5 patients), and aplastic anemia (1 patient). Eleven patients had solid tumors (including the sperm sample excluded due to non-reliable sperm count), 2 of them had testes cancer. The average age of the hematological malignancies group was significantly older than the solid tumor group, accompanied by higher median ejaculate volume. No differences were found regarding other semen parameters (Table II).

DISCUSSION

In this retrospective study we evaluated the safety and feasibility of EEJ for SCP in adolescent cancer patients who were not able to masturbate. EEJ resulted with obtained sperm in two-thirds of our cohort without complications. This success rate is reasonable and acceptable without any complications or additional risks when combined with bone marrow aspiration or central venous catheter insertion. Moreover, cryopreservation is extremely important in that adolescent cancer patients, who stand at great risk for future gonadal failure due to gonadotoxic cancer treatments and suffer from psychological obstacles that may diminish their ability to cryopreserve sperm obtained by masturbation.

One of the advantages of EEJ is its relatively low complication rate. Although rectal mucosal injury, autonomic dysreflexia, and rectal discomfort were previously described [16,22], EEJ is considered a safe and efficient procedure [16,17,23]. Rectal examination prior to the procedure in order to exclude pelvic mass or mucosal atrophy is mandatory to reduce the risk for rectal perforation. Close vital signs monitoring during EEJ and early intervention with calcium channel blocker are acceptable to cope with autonomic dysreflexia [16]. In the current study no complications were documented during the procedure, similar to a previous report regarding EEJ in psychogenic anejaculation [17]. These results emphasize the safety of EEJ in addition to its efficacy.

Although EEJ is a well-known method for fertility preservation among young cancer patients [4,24], only small series had been published. To our best knowledge this is the largest described cohort published regarding EEJ in an adolescent cancer population. Hagenas et al. [3] performed EEJ in 11 patients (mean age 14.4 years) and vibratory stimulation to one patient who could not deliver semen samples by masturbation. Only six samples were cryopreserved without motility description, while four samples included one low volume sample without sperm and two samples that contained no motile sperm. The distribution of semen characteristics is similar to that of our cohort. Therefore, the current study strengthens a previous EEJ outcome report in a smaller adolescent cancer patient cohort. However, contrary to Hagenas et al., we cryopreserved non-motile sperm. Rajasekaran et al. [25] have emphasized that electrical stimulation such as EEJ generates reactive oxygen species and affects superoxide dismutase activity, which may be responsible for inadequate sperm motion and viability. However, lack of motility does not have to be irreversible. Moreover, our policy is based on previous reports of established pregnancies using non-motile cryopreserved sperm retrieved by testicular sperm retrieval (TSR), resulting in the conclusion that lack of motility before cryopreservation does not exclude favorable outcome [26]. In conclusion, we believe that the absence of motility

TABLE I. Comparison of Motile and Non-Motile Sperm Groups

	Motile cryopreserved sperm (n = 19)	Non-motile cryopreserved sperm (n = 10)	*P*
Volume (ml)[a]	0.5 (0.05–5.6)	0.005 (0.05–1.6)	0.007
Sperm concentration ($\times 10^6$/ml)[a]	42 (2–300)	1 (1–22)	<0.001
Total sperm count ($\times 10^6$)[a]	28.4 (0.4–840)	0.05 (0.05–4.5)	<0.001
Motility (%)[a]	5 (2–45)	0	

[a]Median and range.

TABLE II. Sperm Parameters Related to Malignancy

	Hematological cancer (n = 19)	Solid tumors (n = 10)	*P*
Age (years)	15.97 ± 1.65	14.05 ± 1.83	0.008
Volume (ml)[a]	0.65 (0.05–5.6)	0.2 (0.05–4.4)	0.04
Sperm concentration ($\times 10^6$/ml)[a]	20 (1–300)	18 (1–170)	0.44
Total sperm count ($\times 10^6$)[a]	25.05 (0.05–840)	4.25 (0.05–88)	0.25
Motility (%)[a]	5 (0–35)	2 (0–45)	0.52

[a]Median and range.

immediately after EEJ may be temporary due to procedure electrical and thermal effects and does not preclude the possibility of future ovum fertilization, especially when ICSI is performed.

Retrieved ejaculate in the current study was characterized by asthenospermia as previously described regarding EEJ [15,17,27]. Hovav et al. [14] performed 12 EEJ in 6 patients with mean motility of 14% (range 0–53%). However, the notable asthenospermia in different populations (such as spinal cord injury and psychogenic anejaculation) highlights the idea that the EEJ procedure is the cause for that phenomenon. An additional important factor in the studied population is age. Although age difference did not reach statistical significance between the obtained sperm cohort and the non-obtained sperm group, the *P* value of 0.11 in these small cohorts may represent an important contribution of older age to successful sperm retrieval. The possible effect of malignancy type on sperm recruitment was not clearly defined in the current study mainly due to small sample size. On one hand, in spite of the relatively small sample size, the cryopreserved sperm group had lower rates of hematological malignancies compared to the non-obtained sperm cohort. On the other hand, comparison of sperm parameters between the hematological and solid tumor patients failed to demonstrate any significant differences. That inconsistency was previously demonstrated. While Bahadur et al. [12] have demonstrated uniform sperm counts across hematological as well as solid tumors including HL, NHL, ALL, osteosarcoma, Ewing sarcoma, testicular cancer, and others, Bonetti et al. [28] and Williams et al. [29] found lower sperm concentration among testes cancer patients compared to hematological malignancies. However, gonadal dysfunction has recently been reported in adolescent patients with ALL, NHL, and HL as well [19].

The current study describes various results for EEJ in adolescent cancer patients. Although we divided our cohort to successful versus non-successful EEJ by the ability to cryopreserve sperm, one cannot ignore the fact that both cohorts were further divided to subgroups. The successful cohort was divided by motility to two groups that significantly differed by volume, sperm concentration, and total count values. The non-successful cohort included patients who had no ejaculate compared to ejaculate without sperm in others. Therefore, the wide variety of EEJ results may represent a continuum of pubertal changes in that population.

EEJ has been performed in our institution for more than 10 years in patients who presented androgen characteristics such as testicular growth, which is the first clinical sign of gonadal pubertal maturation [30]. The importance of pubertal stages on spermatogenesis in cancer patients was not initially as clear as it is today. The comprehensive assessment of gonadal function of boys and adolescents during cancer diagnosis was published only 1-year ago [19]. We adopted a liberal approach towards EEJ performance in that population because of the optional detrimental effect of oncological treatments on future spermatogenesis, therefore patients with Tanner stage $\geq$2 were included according to clinical judgment of a urologist infertility expert. The lack of Tanner stage documentation and hormonal investigation may be considered as a limitation of the study. However, it should be noted that the long investigation period enabled us to include the largest published cohort. Moreover, since a liberal approach should be adopted for the use of EEJ in adolescent cancer patients, the contribution of these examinations to clinical practice should be further investigated.

Van Casteren et al. demonstrated successful pregnancies in 49% of couples treated by ART with cryopreserved sperm in adult cancer survivors. Pregnancy rates for ICSI and IVF were significantly higher than those with IUI [8]. Although cryopreservation resulted with lower quality semen parameters in adult cancer patients, fertility outcome after ICSI was demonstrated as comparable to the average pregnancy rates achieved with other male factor patients [27]. Therefore, SCP is considered a reliable method for fertility preservation [10,31]. However, data regarding fertility outcome with ejaculate retrieved by EEJ in adolescents is lacking. The relatively low sperm quality may reduce fertility outcome with cryopreserved ejaculate. Fortunately, the high availability of ICSI makes even severe asthenospermia treatable [14]. We assume that these methods will be needed in future fertility treatments using cryopreserved ejaculate from adolescents.

The current study describes EEJ as a safe and feasible procedure for cryopreservation. Obtained ejaculate is characterized by quality variety between patients with notable asthenospermia as previously described in other populations. The use of additional urologic and endocrinology evaluations may bring light on the diverse outcome in that cohort. Oncology healthcare providers should discuss the impact of cancer treatment on fertility as well as fertility preservation options prior to initiation of gonadotoxic treatments, since many parents want to preserve their son's fertility even if the risk of becoming infertile or the chances of fertility restoration are low [24]. Another implication of the current results deals with the heterogeneous financial support covered by insurance companies in different countries and the optional need to reassess their policy regarding SCP as a part of cancer care. Patients who failed to recruit sperm present a special challenge, and additional approaches such as testicular sperm extraction (TESE) or testicular sperm aspiration (TESA) should be further investigated. Further studies regarding the need for ART and fertility outcome are needed.

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