OCCURRENCE OF GONADAL DYSFUNCTION AMONG MALE PATIENTS SURVIVING CANCER THERAPY AT KENYATTA NATIONAL HOSPITAL

BY

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DECLARATION

I certify that this is my original work and has not been presented for a degree at any other university

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Thank you all and God bless you.

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LIST OF ABBREVIATIONS

ACTH.....Adreno-corticotropic hormone

ALL..... Acute lymphoblastic leukeamia

AML ..... Acute myeloid leukaemia

Ca ..... Carcinoma

ChlVPP.....Chlorambucil, Vinblastine, Prednisone,Procarbazine

CKD ..... Chronic Kidney disease

CLL ..... Chronic lymphocytic leukaemia

DNA..... Deoxyribonucleic acid

EIA .....Enzyme immunosorbent assay

e.g..... For example

EQC..... External Quality Control

FSH..... Follicle stimulating hormone

g..... Gram

GH.....Growth hormone

GnRH..... Gonadotropin Releasing Hormone

Gy..... Gray

HD..... Hodgkin's disease

ICSI.....Intra cytoplasmic sperm injection

IQC..... Internal quality control

IVF..... In vitro fertilization

KNH..... Kenyatta National Hospital

l ..... Liter

LH ..... Luteinizing Hormone

m ..... Meter

PI ..... Principal investigator

SD..... Standard deviation

TBI ..... Total Body Irradiation

TESE.....Testicular sperm extraction

USA ..... United States of America

RESULTS:

A total of 98 patients who had completed therapy for different types of cancers were studied. The mean age (SD) of the study population at recruitment was 46.88(18.44) years, with mean age (SD) at commencement of therapy being 43.40(19.47) years. The mean duration of time since last dose of therapy (SD) was 22.73(40.93) months. The types of cancers were varied with solid tumors accounting for 51.2 % and hematological and lymphoproliferative malignancies accounting for 48.8%. The study participants had been exposed to various anti-cancer therapeutic modalities; 44.9% had used chemotherapy alone while 23.5 % had used chemotherapy plus radiotherapy.9.2 % of the respondents had had pelvic irradiation, 17.3% head irradiation whereas 8.2 % had radiotherapy to other sites. Thirty nine point eight percent of the subjects had normal gonadal status as assessed by assay of FSH, LH and Testosterone, with 10.2 % having an atypical profile. Nineteen point four percent had primary testicular failure with 30.6 % having secondary testicular failure (1% hypogonadotropic hypogonadism and 29.6 % normogonadotropic hypogonadism). There was no significant association between type of cancer therapy used and the occurrence of gonadal dysfunction except for chemotherapy, OR 0.310 (95%CI 0.127-0.756) $p<0.01$.However when stratified into three age groups, age was noted to be an effect modifier. Younger age was noted to be protective among those who were exposed to chemotherapy but it was associated with increases risk of hypogonadism in those who had head irradiation.

CONCLUSION

Half (50%) of the male patients who survive cancer therapy at KNH were found to have gonadal dysfunction. Advanced age was associated with higher risk of gonadal toxicity in those who had chemotherapy, whereas in those who had head irradiation younger age was associated with increased risk of gonadal dysfunction. There is thus need for pretreatment counseling in patients about to commence cancer therapy on the possible toxicity to the gonads and for availing fertility preservation options for such patients.

2 LITERATURE REVIEW

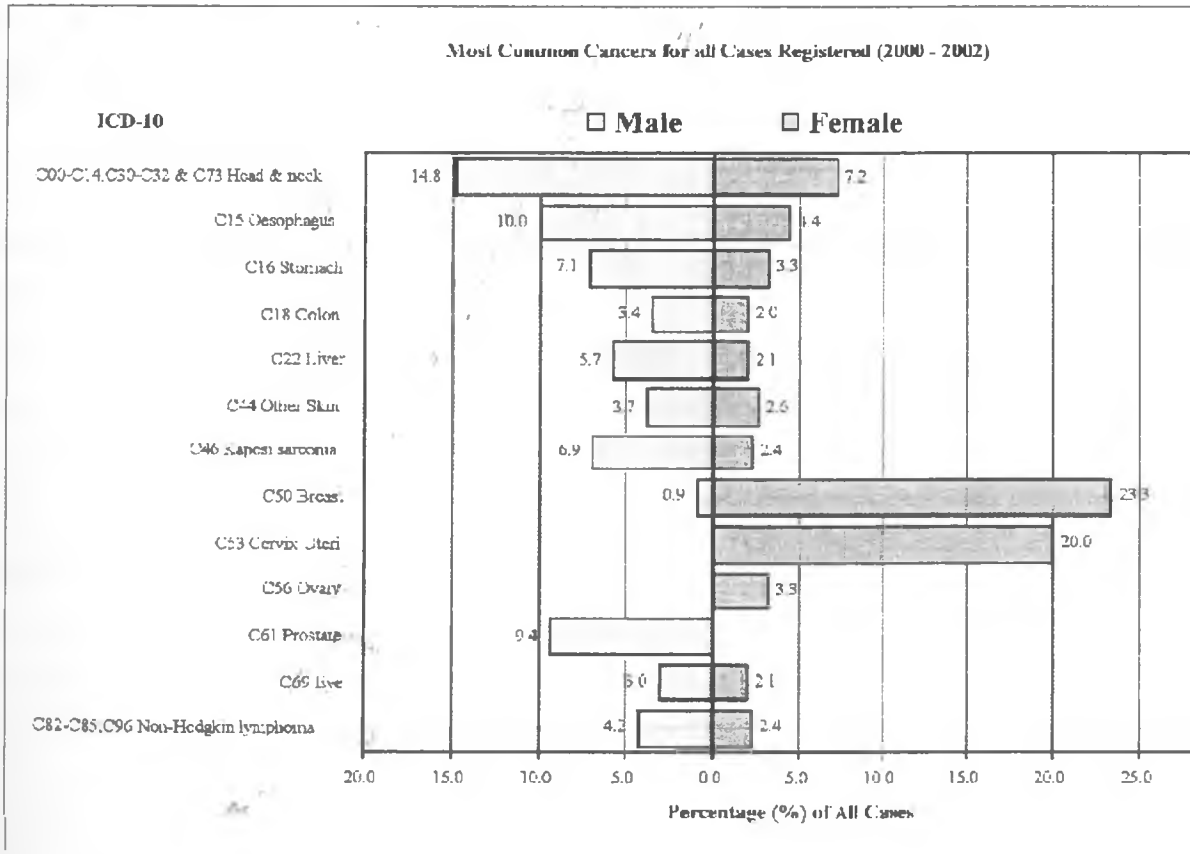
BACKGROUND

Survival rate for most types of cancer have improved dramatically over the years. For patients who have cancer, the success of treatment with regimens, that are toxic to gonadal function, has made infertility an important problem. Sterility from these therapies may be temporary or permanent.

The occurrence of this toxicity is related to a number of factors including the individual's age at the time of treatment, type of therapeutic agent, radiation field, total dose, single versus multiple agents, and length of time since treatment [1].

The Nairobi Cancer registry (2000-2002) reported 1511 adult males diagnosed with cancer over the 3 year period and 1799 adult females over the same period of time. [2]

Figure 2.1: Most common cancers (2000-2002)-Nairobi Cancer registry



In the USA, 17,000 men aged 15 to 45 years are diagnosed each year with Hodgkin's disease, lymphoma, bone and soft tissue sarcomas, testicular cancer or leukaemia.[1] In addition 12000 children under 15 years are diagnosed each year with cancer, including leukaemia, nervous system tumors, lymphomas and renal and other solid tumors. Survival is approaching 80% and because 85% of them receive chemotherapy, or gonadal or pituitary irradiation, their subsequent reproductive function is a significant concern.[1]

Testicular damage caused by cancer therapy was first described in humans in 1948, when azoospermia was reported in 27 of 30 men following treatment with nitrogen mustard[3].

A survey conducted by Schover and associates [4] revealed that 51% of men with cancer wanted children in the future, including 77% of men who were childless when their cancer was diagnosed.

Although newer chemotherapy regimens have resulted in a lower degree of infertility [5], the incidence of azoospermia after treatment remains high, with only 20% – 50% of these men having some recovery of spermatogenesis [6].

In an analysis of 30 published studies which examined gonadal function after various chemotherapy regimes, and which included 116 males who had been treated by various regimes that included cyclophosphamide, gonadal function and/or histology was assessed by a number of methods; semen analysis, basal LH and FSH concentrations and testicular biopsy. Fifty two of the 116 patients (45%) had evidence of testicular dysfunction following treatment [7].

Because it is difficult to predict which patients with cancer will survive or become sterile after treatment, sperm banking is strongly recommended for all patients with malignant disease who wish to preserve their fertility potential [8]

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EFFECTS OF CYTOTOXIC AGENTS ON ADULT MEN:

Testis consist of the semineferous (or germinal) epithelium arranged in tubules and endocrine components (testosterone producing leydig cells) in the interstitial region between the tubules[9]_The semineferous tubules contain germ cells, which consist of germ cells and differentiating spermatogonia, spermatocytes, spermatids and spermatozoa; and the sertoli cells, which support and regulate germ cell within the tubules [9]_The testicular vasculature is permeable and drugs can freely reach the leydig and sertoli cell barrier and damage late stage germ cells[9]

Among germ cells, the differentiating spermatogonia proliferate most actively and are extremely susceptible to cytotoxic agents, In contrast, leydig and sertoli cells, which do not proliferate in adults, survive most cytotoxic therapies. These cells, however suffer functional damage [9]

The loss of germ cells has secondary effects on the hypothalamic-pituitary-gonadal axis. Inhibin, secreted by sertoli cells declines, and because inhibin limits FSH secretion by the pituitary, serum FSH rises.[10]_Germinal aplasia reduces testis size and testicular blood flow is consequentially reduced, which results in distribution of less testosterone into circulation[10]

Because testosterone is a negative regulator of LH secretion by the pituitary and LH is the primary stimulator of testosterone synthesis by the leydig cells, LH increases to maintain testosterone levels[11] Germinal aplasia results in decrease levels of inhibin B and increase levels of FSH. After cytotoxic therapy, LH levels tend to be elevated and serum testosterone levels are in the low normal range[11, 12]

The induced azoospermia can be either prolonged or temporary, depending on the survival of stem spermatogonia and their ability to proliferate, differentiate and produce spermatozoa, which in turn depends on the nature of cytotoxic agent and dose.

If treatment involves agents that kill spermatogonia or affect differentiation are used, long periods of azoospermia ensue.

Although probability that spermatogenesis will recover decreases with the duration of azoospermia, few men have recovered spermatogenesis after as long as 20 years [11]

When sperm count returns, sperm motility appears normal [12], and fertility is generally restored. However when azoospermia is prolonged, sperm count may sometimes plateau at less than 1 million/ml [13] and sperm may have morphological abnormalities incompatible with fertility[12]

To evaluate the effects of cytotoxic therapy, one must consider the initial gonadal status. Men with testicular germ cell tumors have impaired semen quality even before institution of cytotoxic therapy[14]

INDIVIDUAL DRUGS:

Most sterilizing drugs are the alkylating agents (with the exception of dacarbazine) and cisplatin. The cumulative dose appears to be more important than the dose rate in determining whether or not sperm production will recover[15]

Regimens including high doses of alkylating agents are the most gonadotoxic, with prolonged azoospermia in greater than 90% of men who receive the highest doses of cyclophosphamide, procarbazine and chlorambcil. Platinum compounds can cause prolonged azoospermia in up to 50% of men[9]

The gonadal toxicities of other agents have generally been deduced from the effects of combination regimens; the doses given might be underestimates because there may be additive contributions from other drugs in the regimen[15].

Procarbazine [16] and cisplatin in high doses [17] are also highly sterilizing. Busulfan has an additive effect on the sterility resulting from cyclophosphamide treatment. [18] Addition of high doses of ifosfamide (46 g/m^2) to cisplatin-containing chemotherapy regimens significantly reduced the recovery of spermatogenic function. [19] Carboplatin, an analogue of cisplatin, appeared in the same combination regimens to produce less sterility than cisplatin [20]

Hormonal and biological agents are also used in treatment of cancer. Although effects of hormones on adults should be reversible approximately 40% of men who had more than 2 years of treatment with gonadotropin releasing hormone(GnRH) agonist and antiandrogen still had castrate levels of testosterone 1 year after cessation of treatment [21]

Treatment with corticosteroid prednisone or cytokine interferon-alpha has not produced any negative effect on male gonadal function [12]

RADIATION THERAPY:

The effects of radiation on the testes depend on the fractionation regimen. Whereas in all other organ systems, fractionation of radiation reduces the damage, radiation doses to the germinal epithelium of the testis given in 3 to 7 week fractionated courses cause more gonadal damage than single doses [22]

In the usual fractionated regimens, doses to the testes above 0.15 Gy are required to produce any reduction in sperm count. Doses between 0.15 and 0.5 Gy cause oligospermia. The nadir of sperm count occurs 4 to 6 months after the end of treatment, and 10 to 18 months are required for complete recovery [23]

Above 0.6 Gy, azoospermia occurs. The duration of azoospermia is dose dependent, and recovery can begin within 1 year after doses of less than 1 Gy, but not until more than 2 years after delivery of 2 Gy. Cumulative doses of fractionated radiotherapy of more than 2.5 Gy generally result in prolonged and likely permanent azoospermia. [24,25]

The onset and severity of radiation-induced hypopituitarism primarily depends on the total radiation dose, the fraction size and the time allowed between fractions for tissue repair (i.e. duration of the radiation schedule). [26] Deficiency of gonadotropin hormones (and other anterior pituitary hormones) occurs mainly after intensive irradiation (greater than 60 Gy) usually used to treat nasopharyngeal carcinoma and skull-base tumors. [27] Nevertheless, multiple anterior-pituitary hormone deficiencies can occur soon after doses of less than 40 Gy in patients whose neuronal integrity and reserve is reduced at the outset by tumors and/or previous surgery

EFFECT OF AGE ON GONADAL TOXICITY AND RECOVERY

It has been suggested that prepubertal boys are less susceptible to effects of chemotherapy than adult men, but there is no convincing evidence to support this argument. Testicular function as assessed in patients treated for HD in childhood, as indicated by raised gonadotropin concentrations, was found in a significant proportion of a cohort of 46 male patients treated with ChlVPP [24], with 89% and 24% having raised FSH and LH concentrations respectively.

Similarly high rates of toxicity were also reported in a small group of boys treated with cyclophosphamide and TBI before puberty [28], with raised LH concentration in five out of six, and raised FSH concentration in all six that had reached pubertal age.

Studies in adult male patients have shown that the likelihood of gonadal failure and infertility increases with the patient's age at the time of treatment. [15, 24]

Studies among patients exposed to head irradiation have however shown higher rates of secondary gonadal failure in the younger ages. Early observations on the frequency of gonadotropin deficiency suggested that younger age (less than 18 years) increases vulnerability to radiation damage. Gonadotropin deficiency was frequently seen in

children treated by total-body irradiation,[29] but not in any of the adults who had received comparable total-body irradiation schedules.[30] Young children who receive prophylactic cranial irradiation for acute lymphoblastic leukemia might be more susceptible to radiation-induced gonadotropin deficiency than older (postpubertal) children.[31] Similarly, in a cohort of 166 patients aged 6–80 years who had received high-dose irradiation for tumors of the head and neck, children younger than 15 years old had a higher incidence of gonadotropin deficiency soon after radiotherapy than did older patients.[32]

Age at treatment is though not a major factor in recovery from gonadal damage in men. Most studies have failed to show any effect, but a few have indicated increased testicular damage in older men[33,34]

LEUKEMIAS AND LYMPHOMAS

Approximately 22.6% of leukemia diagnoses occur in individuals younger than 45 years. The corresponding numbers are 65.3% and 15.8%, respectively, for Hodgkin disease and non-Hodgkin lymphoma. Collectively, there are more than 13,400 people younger than 45 diagnosed with these 3 cancers in the United States each year[35]

The leukemias most common in this age group (e.g., acute lymphoblastic leukemia and acute myeloid leukemia), Hodgkin disease, and non-Hodgkin lymphoma are typically treated with multiagent cyclic chemotherapy, which can cause temporary or permanent oligospermia or azoospermia in men and cessation of menses or premature ovarian failure in women. Lymphoma may also be treated with radiation therapy (up to 25–50 Gy) to sites of local disease that may encompass the testis, contributing to infertility. Cranial irradiation was used commonly in the past for acute lymphoblastic leukemia, although this treatment has been largely replaced in modern practice by intrathecal chemotherapy[35]

The overall fertility of male acute lymphoblastic leukemia survivors from children's cancer group centre was not significantly different from that of sibling controls. However,

in comparison with controls, the fertility rate for married survivors treated with cranial irradiation before age 10 was reduced by 91%.[36]

Survivors of lymphoma (non-Hodgkin lymphoma and particularly Hodgkin lymphoma) have been among the most comprehensively studied with regard to fertility, because they tend to present at a young age and with a favorable prognosis. Although the diversity of tumor subtypes and chemotherapeutic regimens is substantial, there is a clear consensus that older regimens that include higher doses of alkylating agents (particularly procarbazine) such as mechlorethamine, vincristine, prednisone, and procarbazine or cyclophosphamide, vincristine, prednisone, and procarbazine cause more severe and prolonged reproductive toxicity to both women and men than do regimens such as doxorubicin, bleomycin, vinblastine, and dacarbazine; mitoxantrone, vincristine, vinblastine, and prednisone; or methotrexate, doxorubicin, vincristine, prednisone, and bleomycin.[36]

It appears that lower fertility of male Hodgkin disease survivors is due at least in part to the disease itself, rather than its treatment. Oligospermia and abnormalities of sperm structure/function are apparent in some of these patients even before treatment and are associated with advanced stage and elevated erythrocyte sedimentation rate.[37]

TESTICULAR CANCER

Testicular tumors are among the most common tumors that affect young men, and more than 4,400 cases—are diagnosed in the United States annually among men younger than 45 years.[33] Patients with testicular cancer have an excellent prognosis, with a relative 5-year survival exceeding 95%, and fertility is one of the main concerns of survivors.[38] The first challenge regarding fertility of testicular cancer survivors is that many have quantitative and qualitative deficiencies in spermatogenesis. These have been frequently observed in men without cryptorchidism or other apparent anatomic explanations.[9, 33, 38]

Virtually all patients will undergo surgical removal of the affected testicle and receive postoperative chemotherapy and/or radiotherapy. The usual regimens are bleomycin, etoposide, and cisplatin or etoposide and cisplatin. Regimens including ifosfamide, vinblastine, cisplatin, and/or paclitaxel may be used to treat recurrent or residual disease. Platinum-based regimens have documented testicular toxicity.

In a prospective determination of the hormonal response after cessation of luteinizing hormone–releasing hormone agonist treatment in patients with prostate cancer among men who were normospermic before treatment, 16% were oligospermic and 20% azoospermic 1 year after treatment. Spermatogenesis continued to improve over the subsequent 5 years.[21]

Radiotherapy (recommended for some men with seminoma) has a much more deleterious effect on fertility, compared with chemotherapy alone. In a study of 451 patients after testicular cancer treatments, 91.2% who had tried to father a child before treatment were successful, as compared with 67.1% who tried after treatment. Among couples seeking pregnancy, the 5-year cumulative incidence of pregnancy for partners of men treated with surgery and chemotherapy only was approximately 85%; when treatment includes radiotherapy, the incidence was less then 65%.[39] Among men who became azoospermic after radiotherapy (32 Gy), recovery of spermatogenesis occurred 30 to 80 weeks after start of treatment.[5]

In an attempt to delineate which abnormalities result from cytotoxic chemotherapy, several studies also examined pretreatment testicular function, or compared chemotherapy- treated patients with those who underwent orchidectomy alone. Lampe et al., analyzed data relating to 170 patients with testicular germ cell cancers, who underwent treatment with either cisplatin or Carboplatin based chemotherapy. Forty patients (24%) were oligospermic. At a median follow-up of 30 months after completion of chemotherapy, only 64% of those who were normospermic before therapy remained normospermic, while 54 (32%) of the total cohort were azoospermic and 43 (25%) were oligospermic.[20]

GONADOTROPINS AS SURROGATE MARKERS FOR SPERMATOGENESIS

S. Ramesh Babu et al, [40] showed that gonadotropin (FSH and LH) levels were significantly elevated in infertile males when compared with the levels in proven fertile controls.

These results are in accordance with several other studies [41,42,43,44] which showed elevated levels of both FSH and LH in infertile males. Elevated levels of LH in oligozoospermic and azoospermic males when compared to normal fertile men were also reported [45, 46].

However in the same study, S. Ramesh Babu et al showed that the difference in the mean serum testosterone levels between fertile and infertile men were insignificant. Similar observations were made by Smith et al. [47] and Subhan et al.[44] In the same study, the mean serum FSH and LH levels were significantly elevated in infertile men with varicocele when compared with the controls.

Rege et al. [48] and Gorelic and Goldstein [49]showed elevated serum FSH levels and Nagao et al. [50]showed elevated levels of LH in infertile men with varicocele. In infertile males with abnormal histopathology (Sertoli cell only syndrome, hypo spermatogenesis, and spermatid arrest), the mean FSH levels were significantly elevated compared to the control group [50]

OPTIONS FOR FERTILITY PRESERVATION IN MALES.

There are various methods available for fertility preservation in male patients who are about to undergo potential gonadotoxic treatment regimens. These include:

- *Sperm cryopreservation*; Due to recent advances in IVF technology and sperm banking procedures, even men with extremely reduced sperm count and motility are candidates for sperm cryopreservation.It is strongly recommended that sperm be collected before initiation of cancer therapy because the quality of the sample and sperm DNA integrity may be compromised even after a single treatment session. De Mas et al [51] have reported a significant increase in the frequency of sperm aneuploidy that may be persistent up to 18 months or more after the initiation of chemotherapy. Therefore, cryopreservation should ideally be performed before initiation of chemotherapy or radiotherapy. Previously, patients who were persistently azoospermic after chemotherapy and who did not bank sperm before therapy were considered sterile and able to have children only through adoption or donor insemination. The recent success of testicular sperm extraction (TESE) combined with ICSI for patients suffering from non-obstructive azoospermia [52] indicate that the combination offers a potential new treatment option for these couples. In 2001, Chan et al [53] first reported successful sperm retrieval in 9 of 17 (53%) men with post chemotherapy azoospermia by using TESE in combination with ICSI [54]. A total of 20 attempts using TESE-ICSI resulted in clinical pregnancy in three of nine couples (33%) and in live delivery in two of nine couples (22%) [54]
- Gonadal shielding during radiation therapy

- Testicular suppression with GnRH agonists or antagonists. The efficacy of gonadoprotection through hormonal manipulations has only been evaluated in very small studies in cancer patients which do not support the effectiveness of this method[51] .

3 JUSTIFICATION OF STUDY

Gonadal dysfunction is a common complication of cancer therapy. Studies have shown that many patients who survive cancer therapy, after there cancer is treated, desire to get children of their own.

Various proactive alternatives and preventive measures are now available for patients at risk of gonadal dysfunction after cancer therapy .These are increasingly being offered to patients about to undergo therapy or to those who already have infertility as a result of gonadal dysfunction. (These measures are not routinely undertaken in our local facilities)

Counseling is an important part of the decision-making process for patients, on choice of proactive alternatives and preventive measures. Again counseling on possibility of gonadal toxicity in patients about to undergo therapy is not routinely undertaken at KNH, probably due to lack of local data to highlight the magnitude of the problem.

There is no local or regional data on prevalence of gonadal dysfunction among male patients surviving cancer therapy. This study will thus provide insight into the magnitude of the problem in the local set up and provide informed basis for pre-treatment counseling of patients. The study also hopes to guide policy decisions about availing various fertility preservation options for patients about to undergo cancer therapy.

RESEARCH QUESTION

What are the socio-demographic and treatment variables, associated with occurrence of gonadal dysfunction due to cancer therapy in male patients treated for cancer at KNH?

4 STUDY OBJECTIVES

4:1 GENERAL OBJECTIVE:

The broad objective was to determine gonadal dysfunction and exposure factors (socio-demographic and treatment variables) in male patients surviving cancer therapy at KNH.

4:2 SPECIFIC OBJECTIVES:

These were to:

1. Determine the proportion of gonadal dysfunction among patients surviving cancer therapy at KNH
2. Determine relationship between therapy used (chemotherapy and/or radiotherapy) and occurrence of gonadal dysfunction
3. Determine relationship between occurrence of gonadal dysfunction and age of the study participants

4:3 SECONDARY OBJECTIVE:

Determine the perceived fertility of the study participants

5 METHODOLOGY

5.1 STUDY DESIGN:

This was a cross sectional study among male patients surviving cancer therapy at KNH.

5.2 STUDY POPULATION

The study population comprised of cancer patients who had undergone cancer therapy at KNH and completed therapy at least 6 months before recruitment into study.

5.3 STUDY SITES:

The study was conducted at two sites:

1. The medical oncology outpatient clinic at KNH- runs every Monday from 9.00am to 1.00 pm.
2. Cancer Treatment Center at KNH - runs 4 days a week, from Monday to Thursday, from 9.00am to 1.00 pm.

5.4 STUDY PERIOD:

The study was carried out over a four month period, from November, 2008 to February, 2009.

5.4 INCLUSION CRITERIA:

1. Male patients who had completed cancer therapy more than 6 months prior to study period.
2. Patients aged above 15

5.5 EXCLUSION CRITERIA:

1. Patients on palliative care
2. Patients with other endocrine disorders; hypothyroidism, Cushing's disease
3. Patients on testosterone and/or gonadotropin replacement therapy
4. Patients currently on corticosteroid therapy
5. Patients with known pre-existing gonadal dysfunction
6. Patients with chronic liver disease
7. Patients with chronic kidney disease
8. Patients who declined consent to study

5.6 SAMPLE SIZE ESTIMATION:

The sample size for the study was estimated using the following formula:

$$N = \frac{(Z\alpha)^2 \times P \times q}{D^2}$$

Where;

P; proportion of gonadal toxicity

q ; 1 – p

Zα; 1.96

D ; Precision 0.1

N ; calculated sample size

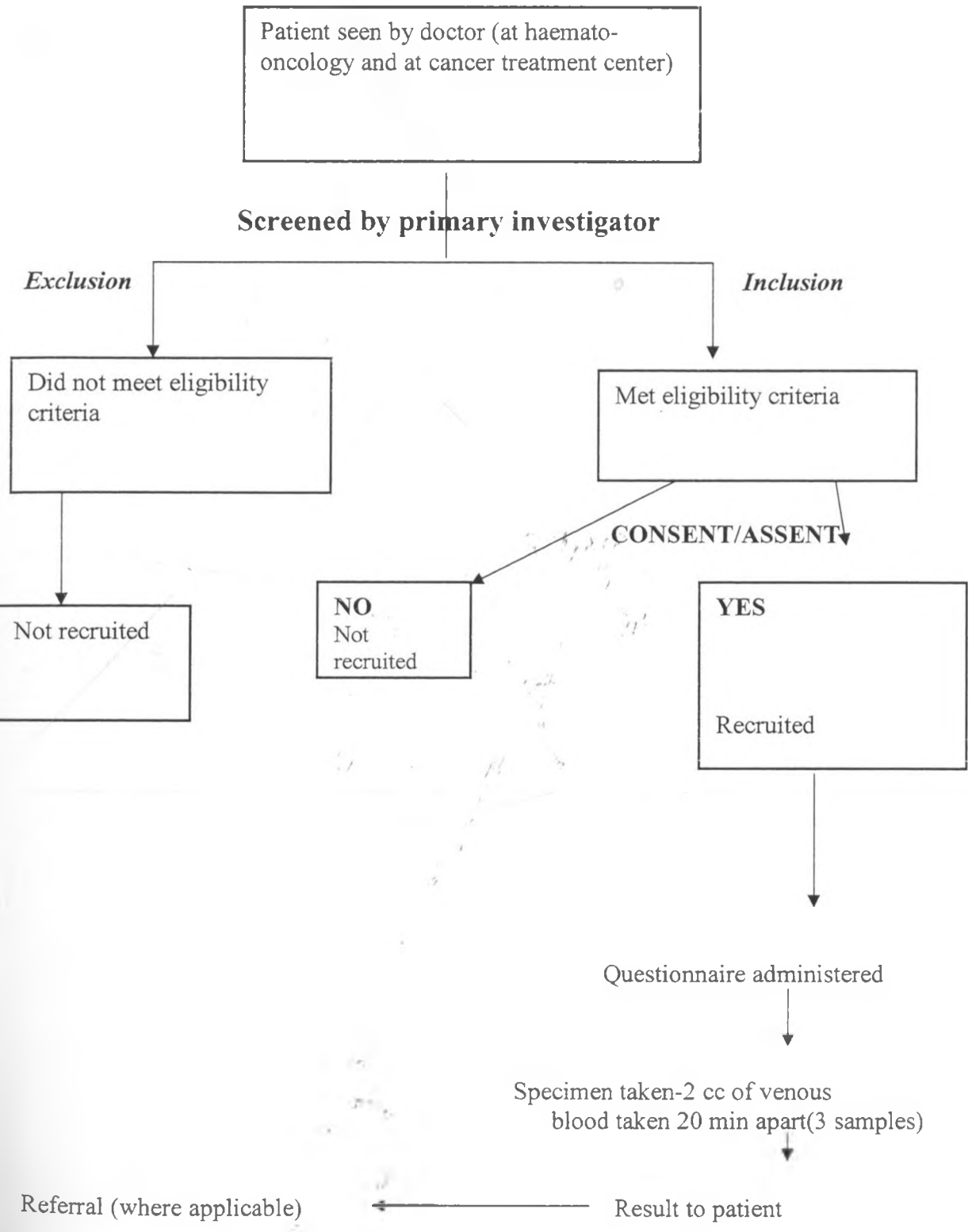
Sample size **96 patients**

Prevalence of 50% was used to calculate the sample size[5,6,8].

SAMPLING METHOD:

Participants from the two study sites were consecutively sampled and merged

PARTICIPANTS FLOW:



5.7 STUDY VARIABLES

Independent variables were:

- Age (in years) - at recruitment and at start of therapy.
- Marital status.
- Educational level.
- Occupation.
- Diagnosis/type of cancer.
- Mode of therapy; chemotherapy and/or radiotherapy (Pelvic and/or head irradiation).
- Duration (in months) since last dose of therapy.

Study outcomes/Dependent variables;

- Sex hormone profile (FSH, LH, Total testosterone).
- Perceived fertility.

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Study outcomes/Dependent variables;

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- Perceived fertility.

5.8 DEFINATION OF TERMINOLOGIES AND CONSEPTS:

The American Association of Clinical Endocrinologists (2002) defined **hypogonadism** as inadequate gonadal function as manifested by deficiencies in gametogenesis and/ or secretion of gonadal hormones. Thus hypogonadism in this study was defined as low gonadal hormone (testosterone).

Hormone profile:

Reference ranges (Males):

FSH ..... 1.0 - 14.0 IU/L (Human EIA test kit reference number 53020)

LH ..... 0.7-7.4 IU/L (Human EIA test kit reference number 53010)

Testosterone (total) ...12.20- 29.80 nmol/L (Human EIA test kit reference number 55010)

Gonadal dysfunction was defined as either primary testicular failure (hypergonadotropic hypogonadism), or secondary testicular failure (either hypogonadotropic hypogonadism or normogonadotropic hypogonadism).

The operational definition of **perceived fertility** was: ability of a cohabiting couple to achieve a pregnancy when there is a desire to do so[55].

Cancer was defined as any malignant tumor/proliferation i.e. carcinoma, lymphoma, leukaemia and sarcoma[9].

Cancer therapy was defined as treatment aimed at eradicating/ameliorating the cancer. This was radiotherapy, chemotherapy or both[9].

6 SCREENING, RECRUITMENT AND CLINICAL METHODS

The principal investigator/study assistant visited the oncology outpatient clinic every clinic day. He also visited the cancer treatment center on clinic days. Every male patient, aged above 15 years who completed cancer therapy more than 6 months before study period, was considered a potential recruit. Those who met the eligibility criteria were informed about the study and recruited after consenting (or assenting) to the study.

The principal investigator went through patients' records at the central registry, obtained contacts of patients with long clinic booking (beyond anticipated study period) and contacted all patients who met eligibility criteria. These patients were asked to come to the clinic (transport costs borne by principal investigator) and recruited into the study after meeting the eligibility criteria and duly giving consent (or assent) to the study.

For each recruited patient, a physical examination was done. A questionnaire (appendix 1) was administered, to inquire about the demographic characteristics, diagnosis, treatment history and to assess perceived fertility. Their fertility was assessed in terms of pregnancies achieved prior to start of therapy and any achieved after commencing therapy(if a desire to achieve a pregnancy existed).The patients' records were reviewed to establish the diagnosis and treatment history. To prevent double recruitment, the patients' files were marked with an adhesive sticker.

Specimen collection, transportation and storage: Three samples of 2 cc venous blood was drawn, in sterile disposable syringes, 20 minute apart, to allow for effects of hormone secretory pulses (GnRH is released in discrete pulses approximately every 2 h, resulting in corresponding pulses of LH and FSH [39]).All samples were taken before 11.00 am (peak testosterone secretion in males occurs between 0800 and 1100 hours[39]). The samples were then taken to Department of Obstetrics and Gynecology laboratory, in plain bottles, for hormone assays. The blood was transferred to clean labeled bottles and serum allowed to retract. Once retracted, serum was separated by centrifugation, and frozen at -20 degree Celsius until time of analysis.

Specimen analysis: The samples were assayed for FSH, LH, and testosterone (total), using commercially available kits (Human EIA test kit - Germany), through EIA techniques. The EIA was performed as an indirect solid phase sandwich type immunoassay. The microwells used were coated with monoclonal anti-LH/FSH/Testosterone, followed by blocking the unreacted sites to reduce non-specific binding. The test principles are :

Step 1; Antigens present in calibrators and patient samples bind to the coated antibody

Step 2; The antigen-antibody complex reacts with enzyme labeled monoclonal antibody conjugate resulting in the hormone antigen being sandwiched between the solid phase antibodies and the enzyme conjugate

Step 3; The enzyme converts added substrate to form a colored solution

Step 4; The concentration of color change is proportional to the concentration of antigens present in the samples and was read by a microplate reader at 450 nm.

Quality control: Each assay was subjected to IQC (available in the respective test kits).EQC was performed on selected samples. The tests were conducted by qualified technicians in Department of Obstetrics and Gynecology laboratory.

Result interpretation:

1. Hypergonadotropic hypogonadism (primary testicular failure)- was defined as high gonadotropin (LH , FSH) with low testosterone[56]
2. Hypogonadotropic hypogonadism (secondary testicular failure) – was defined as low gonadotropin with low testosterone[56]
3. Normogonadotropic hypogonadism(secondary testicular failure) – was defined as normal gonadotropins with low testosterone[56]
4. Normal gonadal function – was considered if the results were within reference range[56]
5. Atypical profile – hormone profile not fitting into any of above profiles

The results of the blood assays were communicated to the patients. Where the results were found to be abnormal the patients were guided to receive appropriate medical attention (referred to fertility clinic, KNH).

Data analysis

Data was collected on study proforma /questionnaire and entered into MS access computer data base. The data was then cleaned and verified. Statistical analysis was done using SPSS version 15.0.

Data analysis was done as follows:

Descriptive analysis: Continuous data i.e. age ,time since last therapy and hormonal level was presented as means, standard deviations, medians, proportions and frequencies while categorical data such as pattern of gonadal dysfunction, type of cancer, type of cancer therapy, and perceived fertility, shall be presented in proportions, frequencies and percentages.

Bivariate analysis: to identify associations between exposure variables and pattern of gonadal dysfunction. Odds ratios was used as measures of association while p-value of less than or equal to 0.05 used to determine statistical significance. Associations between pattern of gonadal dysfunction and exposure variables (socio-demographic and treatment variables) was examined using the chi-square test for the categorical data while for continuous variables, the student–t test was used to determine statistical significance.

7 ETHICAL CONSIDERATION

The study was conducted after approval by the Department of Internal Medicine, University of Nairobi, and The Kenyatta National Hospital/ University of Nairobi Ethical Research Committee.

The objectives and purposes of the study were clearly explained to eligible participants in a language suitable to them prior to inclusion into the study. The eligible participants were also informed of the possible discomforts e.g. needle prick pain.

The study subjects were accorded full opportunity and encouragement to ask questions relating to the study.

The participants were informed of their right to refuse to participate and that they were informed that they were free to withdraw from research at any time without penalty or loss of benefits to which they were otherwise entitled to.

Patients noted to have deranged hormone profile were asked to come to hospital and referred to an andrologist (fertility clinic, KNH) for further management.

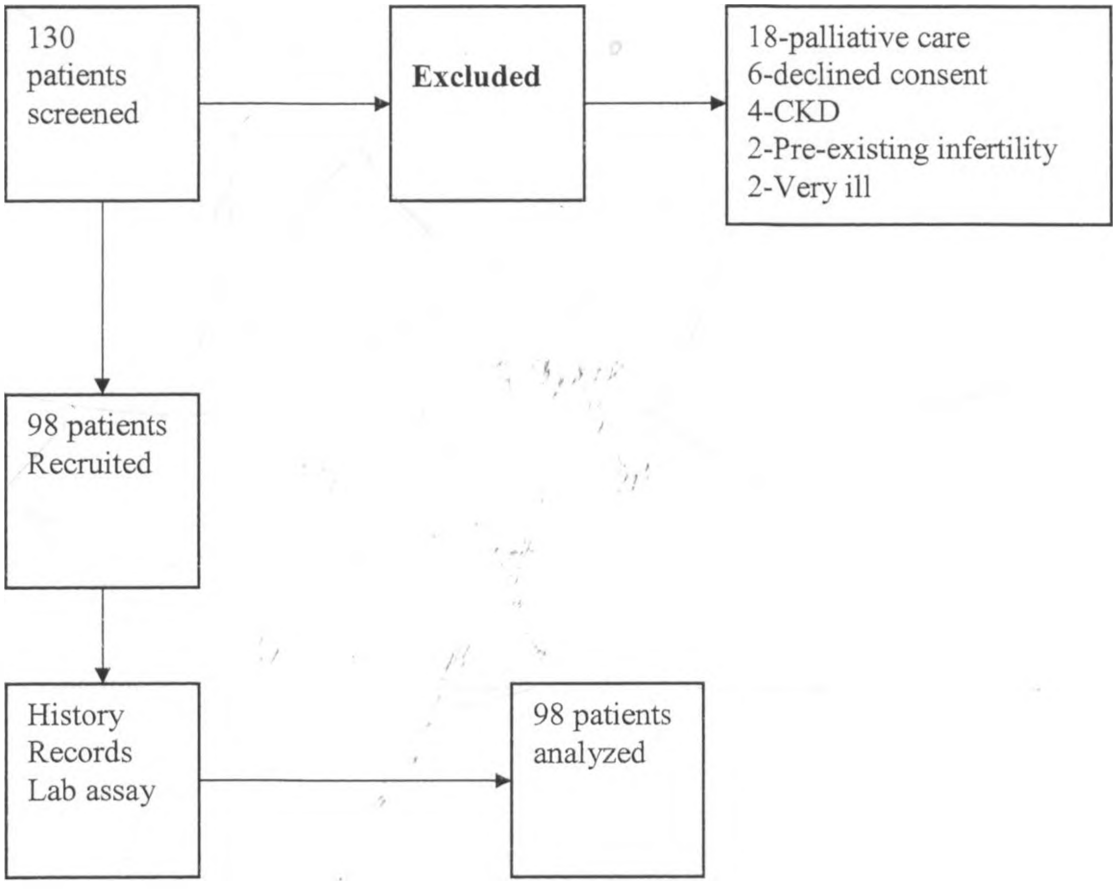
The full cost of the study was borne by the principal investigator.

8 RESULTS

8.1 FLOW OF PATIENTS

A total of 130 patients were screened, with 32 of them failing to meet eligibility criteria. Thus 98 subjects were recruited in the study and analyzed.(figure 9.1.1)

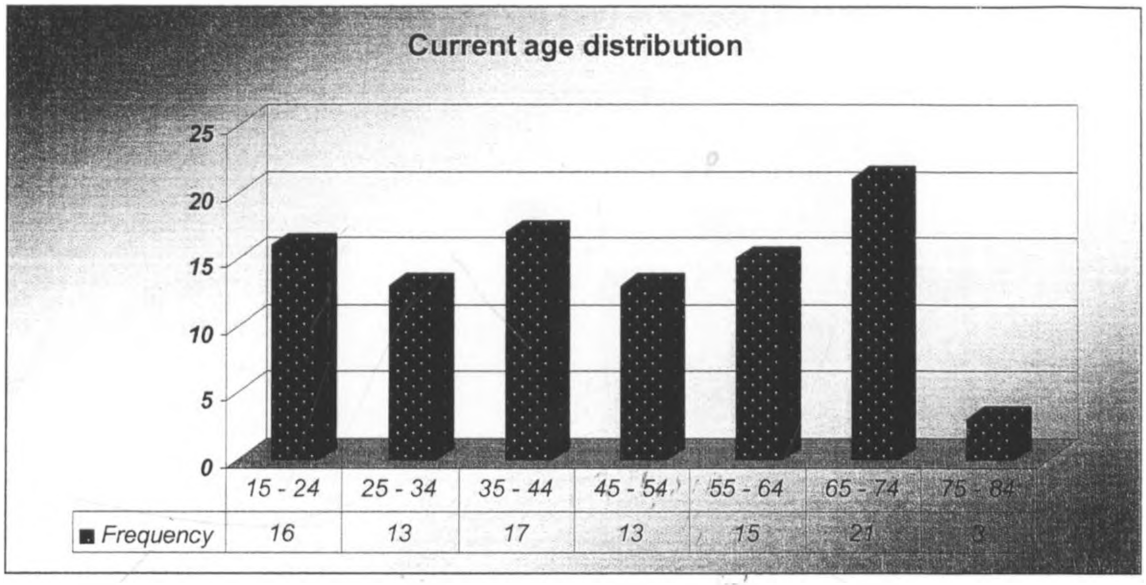
Figure 8.1.1: Flow of patients



8.2 BASELINE CHARECTERISTICS

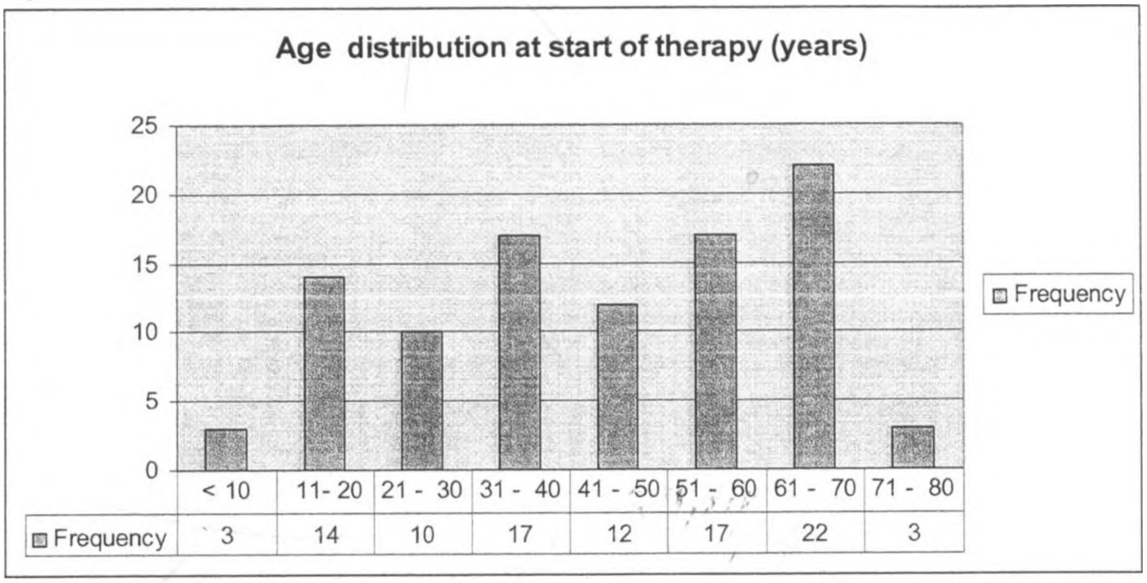
The mean (SD) age at recruitment of the study participants was 46.88 (18.44), median of 49 years, minimum age recruited being 15 years and 80 years as the oldest participant recruited. The participants were distributed across all age groups(figure 8.2.1) .

Figure 8.2.1: Graph of age at recruitment of study participants



The mean (SD) age at onset of therapy 43.40 (19.47), median age of 45 years. minimum age at start of therapy being 5 years and 78 years as highest age at start of therapy(figure 8.1.2).

Figure 8.2.2: graph of age at start of cancer therapy



A majority of the study participants were married (77.6%), with only 19.4 % of them being single.19.4 % of the respondents were employed, 24.5 % were retired, 32.7 % self employed, 14.3 % were in training institutions (students) and 9.2 % were not in any employment.1 % of the participants had no formal education with 30.6% having had primary education, 41.8% had secondary education and 26.5% had tertiary education. The study participants were from all the provinces (except North Eastern) province of Kenya. The sociodemographic characteristics of the participants are summarized below (table 8.2.1)

Table 8.2.1: Summary of demographic characteristics of study subjects

DEMOGRAPHIC CHARECTISTIC	MEASURE
Current age(at recruitment)	***Years***:
• Mean	46.88
• SD	18.44
• Median	49.00
• Minimum age	15
• Maximum age	80
Age at start of therapy	***Years***:
• Mean	43.40
• SD	19.47
• Median	45
• Minimum	5
• Maximum	78
Marital status :	***Percentage:***
• Single	19.4
• Married	77.6
• Cohabiting	1
• Divorced	1
• Widowed	1
Geographical Distribution (Provinces):	***Percentage:***
• Central	28
• Nairobi	21
• Eastern	17
• North Eastern	0
• Coast	3
• Rift Valley	15
• Nyanza	13
• Western	3
Educational level :	***Percentage:***
• None	1
• Primary	30.6
• Secondary	41.8
• Tertiary	26.5

8.3 PRIMARY DIAGNOSIS (TYPE OF CANCER)

The study participants were treated for various types of cancer. Non Hodgkin's lymphoma was the most frequent type of cancer accounting for 16.3 %, Kaposi's sarcoma accounted for 11.2%, prostate cancer 10.2 %, Hodgkin's lymphoma and bladder cancer accounting for 7.1 % each, CLL accounting for 6.1 % and the rest accounting for 41.8 %.These are shown in table below (table 8.3.1)

Table 8.3.1: Table showing type of cancer of study participants

Diagnosis/type of cancer (from patient records)	Frequency	Percent
NON HODGKINS LYMPHOMA	16	16.3%
KAPOSIS SARCOMA	11	11.2%
PROSTATE CA	10	10.2%
HODGKINS LYMPHOMA	7	7.1%
BLADDER CA	7	7.1%
CLL	6	6.1%
BREAST CA	4	4.1%
OESOPHAGUS CA	4	4.1%
TONGUE CA	4	4.1%
OSTEOGENIC SARCOMA	3	3.0%
POST NASAL SPACE CA	3	3.0%
AML	2	2.0%
GLIOMA	2	2.0%
FIBROSARCOMA	2	2.0%
BURKITTS LYMPHOMA	2	2.0%
LEIOMYOSARCOMA	2	2.0%
GLIOMA	2	2.0%
AAL	1	1.0%
COLON CA	1	1.0%
CONJUCTIVA CA	1	1.0%
MALIGNANT FIBROUS HISTIOCYTOMA	1	1.0%
MULTIPLE MYELOMA	1	1.0%
ORBITAL CA	1	1.0%
LARYNX CA	1	1.0%
SQUAMOUS CELL CA	1	1.0%
TESTICULAR CA	1	1.0%
CRANIOPHARYNGIOMA	1	1.0%
Total	98	100.0%

8.4 TYPE OF THERAPY USED

The study participants had been exposed to various anti-cancer therapeutic modalities. Forty four point nine percent had used chemotherapy alone while 23.5 % had used chemotherapy plus radiotherapy. Nine point two percent of the respondents had had pelvic irradiation, 17.3% head irradiation whereas 8.2 % had radiotherapy to other sites. Four point one percent of the respondents underwent orchidectomy as part of their cancer therapy. Eleven point two percent of the respondents were on other concurrent treatments (figure 8.4.1).The patients who had chemotherapy were exposed to various groups of chemotherapeutic agents (figure 8.4.2).

Figure 8.4.1: Graph of frequency of type therapy used

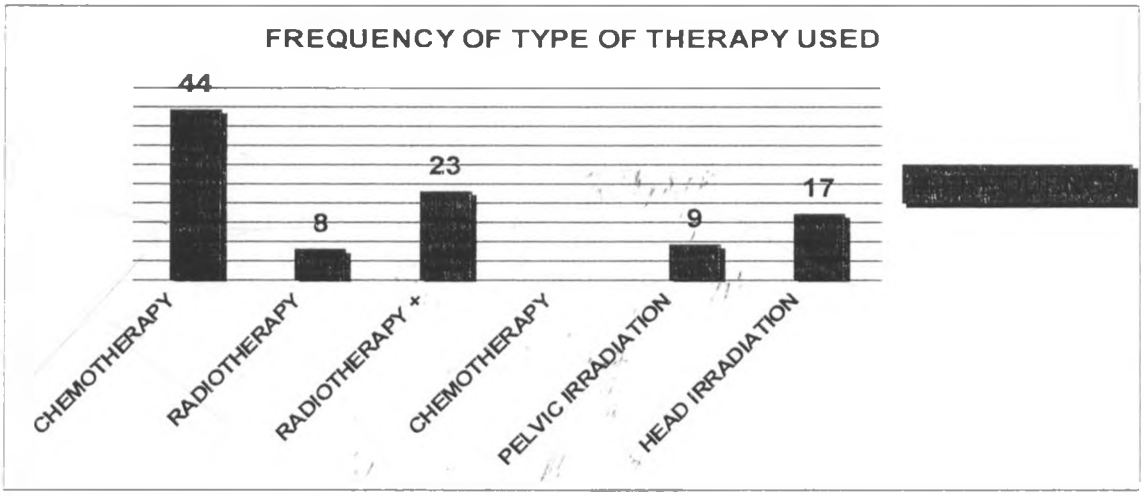
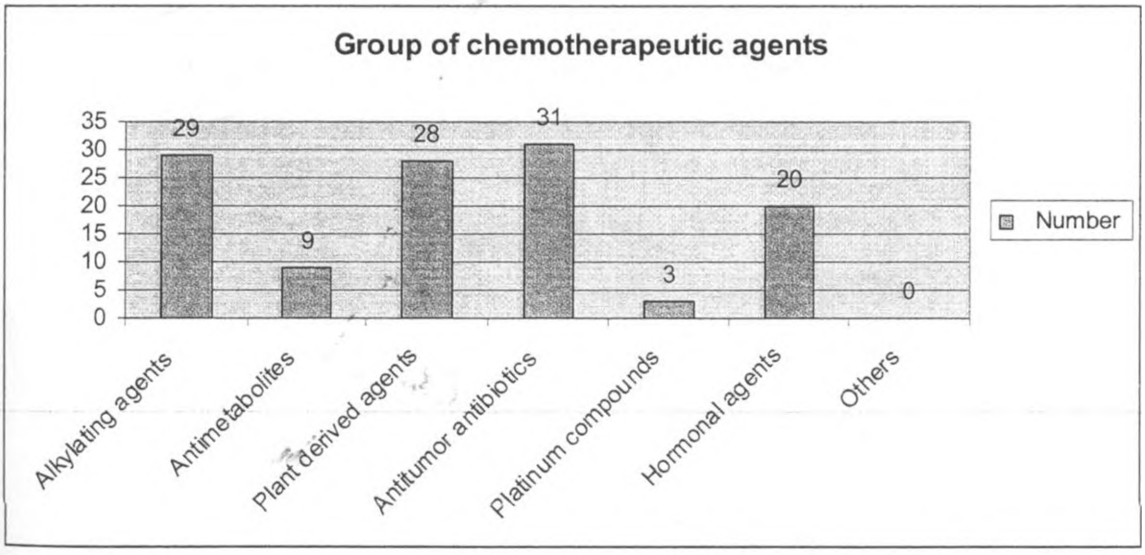


Figure 8.4.2: Graph of group of chemotherapeutic agent



8.5 GONADAL DYSFUNCTION

Gonadal dysfunction among the study participants was determined by their hormonal profile (FSH, LH and total testosterone).The figures and table below show the hormonal profile of the study participants:

Figures 8.5.1: Graph of FSH levels of study participants

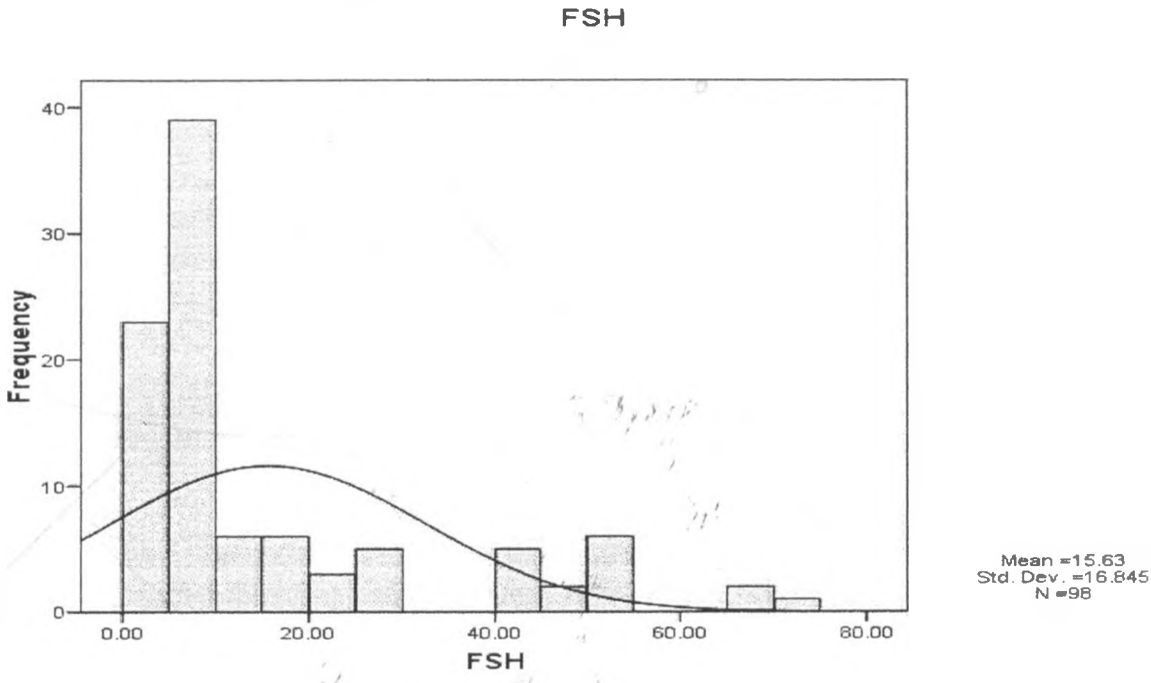


Figure 8.5.1 shows the distribution of FSH levels of the study participants. The reference range for FSH was 1-14 IU/L. The curve was skewed with more subjects having values below the upper reference limit. Thirty point six percent of the study participants had FSH levels above 14 IU/L.

Figures 8.5.2: Graph of LH levels of study participants

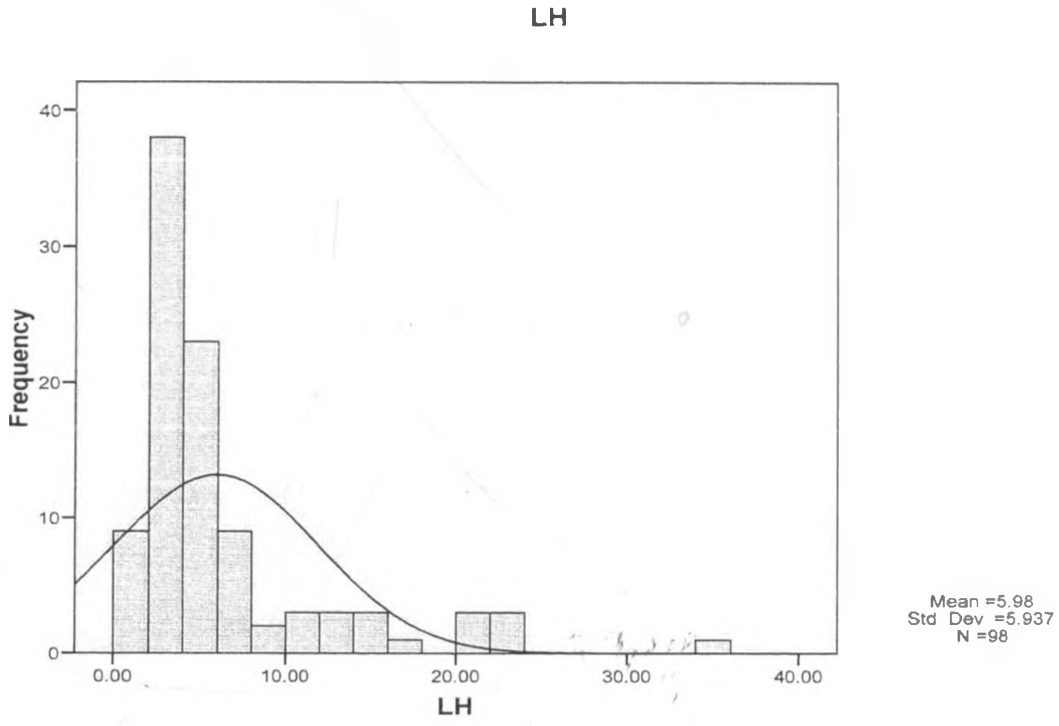
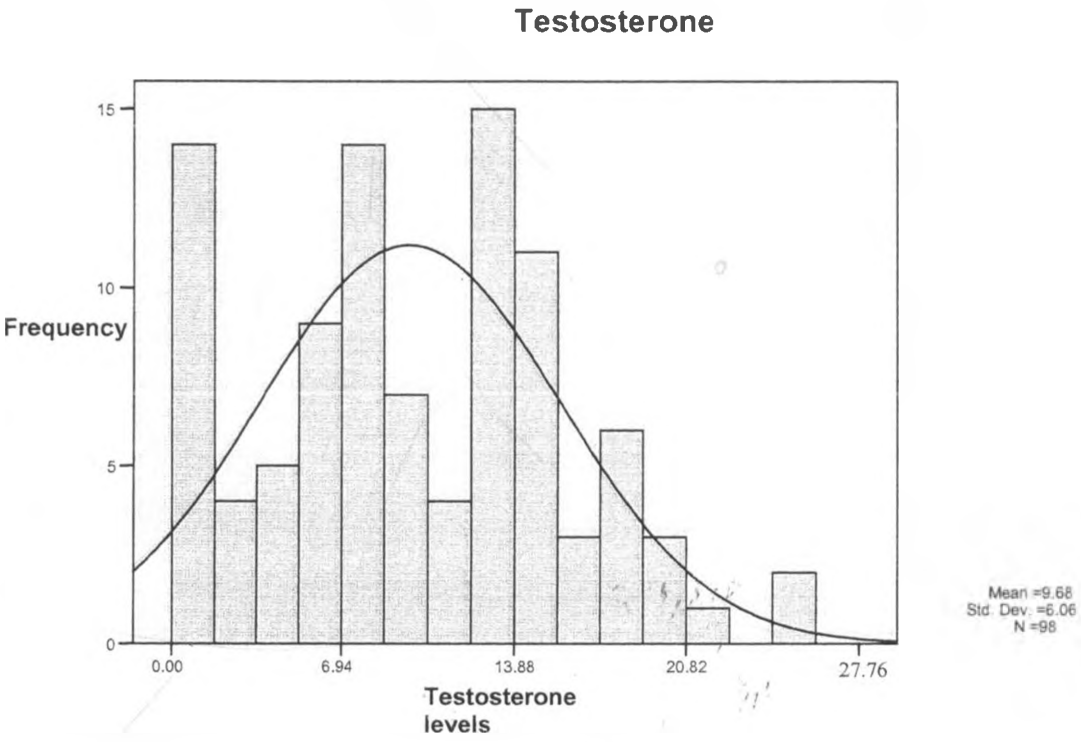


Figure 8.5.2 shows the LH levels of the study participants. Reference range for LH in this study was 0.7-7.4 IU/L. The distribution curve was skewed with majority of the values being below the upper reference limit. Twenty one point four percent of the subjects had LH levels above the upper limit.

Figures 8.5.3: Graph of Testosterone levels of study participants



Testosterone levels of the study participants are shown in figure 9.5.3.The reference range for testosterone was 12.20- 29.80 nmol/L. More than half (58.2 % of the subjects had values lower than the lower limit.

A summary of the hormonal profile of the study participants is shown in table 8.5.1

Table 8.5.1: Summary of hormonal profile of participants

	MEAN	MEDIAN	SD	MINIMUM	MAXIMUM
FSH(IU/l)	15.63	8	16.85	2.2	74
LH(IU/L)	5.98	4	5.94	0.8	35
TESTOSTERONE(nmol/L)	9.68	9.37	6.06	0.35	25.68

Based on the individual hormonal level and reference ranges for the various hormones (see above) the participants were categorized into the various diseases processes. Thirty nine point eight percent had a normal profile with 10.2 % having an atypical profile. Of those who had hypogonadism; 1% of all the subjects, had hypogonadotropic hypogonadism, 29.6 % had normogonadotropic hypogonadism and 19.4 % had primary testicular failure (hypergonadotropic hypogonadism).Gonadal dysfunction in this study was thus **50 %** (1 + 19.4 + 29.6) .This is shown in table below (table 8.5.2):

Table 8.5.2: Gonadal status of study participants

Diagnosis	**Frequency**	**Percent**
Normal profile	39	39.8 %
Hypogonadotropic hypogonadism	1	1.0 %
Normogonadotropic hypogonadism	29	29.6 %
Hypergonadotropic hypogonadism	19	19.4 %
Atypical profile	10	10.2 %
Total	98	100.00%

Difference in the means was then ascertained between those with normal profile and those with hypogonadism using the student t test. Age at start of therapy, age at recruitment, were significantly different between the two groups. There was however no significant difference in duration since last cancer therapy in the two groups. (Table 8.5.3)

Table 8.5.3: table showing difference in the means of the dependent variables

	Hypogonadism N=49	**Normal profile. N=39**	**P – value(chi square)**
1. Age at start of therapy (years):			
• Mean.....	46.2245	36.75	
• SD.....	18.626	19.83	
• Median.....	48.00	36.50	0.027*
• Minimum.....	12	5	
• Maximum.....	73	74	
2. Current age (years):			
• Mean	49.20	41.06	
• SD.....	18.255	17.86	
• Median.....	54	38.50	0.043*
• Minimum.....	15	15	
• Maximum.....	75	76	
3. Duration since last dose of therapy(months):			
• Mean.....	15.63	36.19	
• SD.....	13.959	63.80	0.214
• Median.....	12.00	11.50	
• Minimum.....	6	6	
• Maximum.....	72	227	

* Significant ($p < 0.05$)

Table 8.6.2: Association between Group of chemotherapeutic agent and gonadal dysfunction

Group of chemotherapeutic agent	**OR (95% CI)**	**P value**
Alkylating agents	0.519 (0.211-1.276)	0.176
Antimetabolites	0.296 (0.084-1.050)	0.070
Plant derived agents	0.275 (0.110-0.689)	0.007*
Antitumor antibiotics	0.421 (0.174-1.017)	0.076
Platinum compounds	0.519 (0.151- 1.786)	0.356
Hormonal agents	0.651 (0.251-1.693)	0.466

*Significant ($p < 0.05$)

8.7 EFFECT OF AGE ON OCCURRENCE OF GONADAL DYSFUNCTION AFTER CANCER THERAPY

Both age at start of therapy and age at recruitment were significantly different between those with gonadal dysfunction and those who had normal function p values 0.027 and 0.043 respectively (table 8.5.3).

The subjects were then stratified into three age groups (below 30 years, 30 to 60 years and above 60 years to check for the effect of age on occurrence of gonadal dysfunction. Younger age (below 30 years) among those who had chemotherapy was protective against gonadal dysfunction stratum OR 0.056 (95% CI 0.008-0.450). This association was statistically significant, p=0.003.In the older age groups, chemotherapy showed no significant effect. Head irradiation was significantly associated with gonadal dysfunction among the younger age group, OR (95%CI) 3.000(1.638-5.493), p=0.012, as compared to the older age groups where the association was not significant. The other modes of therapy showed no significant associations with gonadal dysfunction in the three age groups (Table 9.7.1). The Mantel Haenszel common OR was then used to calculate the adjusted OR. The adjusted OR was similar to the crude OR of the various modalities of therapy, thus age is an effect modifier (Tables 8.6.1 & 8.7.2).

Tables 8.7.1: Table showing stratification into three age groups

AGE GROUP:	**< 30 YEARS**		**30 – 60 YEARS**		**>60 YEARS**	
	OR(95%CI)	P value	OR(95%CI)	P value	OR(95%CI)	P value
Chemotherapy	0.056 (0.008-0450)	0.003*	0.125 (0.324-3.903)	0.552	0.269 (0.014-5.267)	0.459
Chemotherapy + Radiotherapy	2.000 (0.240-14.58)	0.653	0.437 (0.105-1.871)	0.306	2.400 (0.215-26.82)	0.624
Head irradiation	3.000 (1.638-5.493)	0.012*	1.240 (0.665-2.312)	0.719	0.633 (0.155-2.582)	0.429
Pelvic irradiation	-	-	2.000 (0.167-23.96)	1.000	0.500 (0.006-4.153)	0.596
Radiotherapy(other Sites)	2.273 (1.460-3.537)	0.462	2.235 (1.570-3.183)	0.107	1.385 (1.040-1.844)	0.549

*Significant ($p < 0.05$)

Table 8.7.2: Table showing adjusted OR for the three strata

	Adjusted OR	**95% CI**
Chemotherapy	0.016	0.133-0.814
Radiotherapy + Chemotherapy	0.794	0.402-2.731
Head irradiation	0.106	0.821-7.892
Pelvic irradiation	0.837	0.250-5.539
Radiotherapy (other site)	0.733	0.159-3.642

8.8 PERCEIVED FERTILITY

Perception of fertility was assessed in terms of ability to achieve a pregnancy if a desire to do so existed. Majority of the respondents were married/cohabiting (78.6%) and 68.4% of the respondents were sexually active. Sixty nine point four percent reported that their libido had gone down since starting therapy.

Only 23(23.5%) of the respondents desired to achieve pregnancy with their spouses since start of cancer therapy. Only 3 participants were successful in achieving a pregnancy.

Table 8.8.1: Table showing success in attempt to achieve pregnancy

Success of attempt to achieve pregnancy	**Frequency**	**Percent**
1 Successful	3	13.0%
2 Not successful	20	87.0%
Total	23	100.0%

9 DISCUSSION

Knowledge on the occurrence of gonadal dysfunction in cancer patients surviving cancer therapy is limited in the local set up. This is because there is no local or even regional data available to ascertain the magnitude of this problem among this patient group. This study set out to determine the occurrence of gonadal toxicity among male cancer patients surviving cancer therapy at KNH. Ninety eight male patients were enrolled after meeting eligibility criteria and consenting/assenting to the study. The participants were recruited over a study period of 4 months, from November 2008 to February 2009.

Mean age of the study participants at recruitment was 46.88 ± 18.44 years, with their mean age at commencement of therapy being 43.40 ± 19.47 years. There are no other similarly designed studies for comparison of ages of the study participants. The study participants were from a diverse geographical distribution across Kenya. This was because the study site being a tertiary referral institution, patients are referred in from across the country.

The prevalence of gonadal dysfunction in this patient population was 50%, with 19.4% of the subjects having primary testicular failure, and 30.6% having secondary testicular failure. The prevalence rates of hypogonadism after cancer therapy vary in various studies. This variability is due to assessment of different treatment regimes. Rivkees et al reported 45% gonadal dysfunction in 116 patients who underwent chemotherapy regimes that included cyclophosphamide[7]. Hall et al reported 36% testicular dysfunction among patients with testicular cancer treated with LHRH agonist after cessation of therapy [21]. Lampe et al, in a follow up study of 170 patients with testicular germ cell cancers, after a median 30 month follow up period, 57% of the total cohort had testicular dysfunction[20]. This though included some patients who had been shown to have pre-existing hypogonadism. Naysmith et al in an analysis of 30 studies published examining gonadal function after various chemotherapeutic regimes, reported 50-70 % occurrence of

gonadal dysfunction in those with normal pretreatment gonadal function [8]. The resultant prevalence in this study thus compares well with other studies. However there are no similarly designed studies that may give a better comparison, moreover there is no local/regional data for comparison.

It is important to note that the gonadal function can be impaired by the primary cancer. Patients who were known to have pre-existing infertility were excluded from the study. However it was difficult to ascertain the proportion of the study participants who had hypogonadism as a result of their primary malignancy. Studies that demonstrated gonadal dysfunction before therapy, showed significant gonadal toxicity in those who had normal pretreatment gonadal function[8].

In the analysis of the relationship of various anticancer therapeutic options that the participants were exposed to and gonadal dysfunction; radiotherapy, radiotherapy plus chemotherapy, head irradiation and pelvic irradiation, showed trends of association with gonadal dysfunction, although these associations were not statistically significant. The lack of association might be because of use of testicular shields during radiotherapy. Various studies have shown a strong association between radiotherapy and gonadal dysfunction. Huyghe and collegues [39] in an analysis of fertility among men with testicular cancer, demonstrated period fertility of less than 65% in men who had radiotherapy as opposed to more than 85% in those who had either surgery or chemotherapy.

Use of chemotherapy alone was though noted to have a gonadal function sparing effect, OR 0.310(95% CI 0.127-0.756).This association was statistically significant $p<0.01$.The explanation for this finding was that these effects were confounded by another factor. Clearly age at onset of therapy (discussed below) was shown to be an effect modifier. Those patients who had their cancers exclusively treated by chemotherapy tended to be younger and thus the effect modification aspect of age cannot be ignored in this cohort.

Among the various classes of chemotherapeutic agents, only the plant derived agents were significantly associated with occurrence of gonadal dysfunction OR 0.275 (95 % CI 0.110 -0.689) p 0.007. It is difficult to be sure of this effect because of use of combination treatment modalities. The commonest of these agents, as used in this patient population, is vincristine which is a component of regimes used in the treatment of lymphomas. Since lymphomas tend to occur in the younger age groups then likewise the effect of age can be responsible for this sparing effect. Moreover gonadal toxicity is not a common documented effect of these agents[15].However studies need to be carried out on possible mechanisms of the effect of these agents on the gonadal function.

Age at start of therapy was significantly (p=0.027) different between those who had hypogonadism and the subjects with a normal profile. When the subjects were stratified into three age groups i.e. under 30 years, 30 to 60 years and over 60 years, use of chemotherapy in the younger age (under 30 years) was protective, OR 0.056(95 % CI 0.008 – 0.45) against occurrence of gonadal dysfunction. This effect was lost in the older age groups. This sparing effect is due to the effect modification effect of age (discussed below). This finding is in keeping with the findings of Marina et al and Klein et al who demonstrated that the likelihood of gonadal failure and infertility increased with the patients' age at the time of treatment[15, 25].

Head irradiation was more harmful in the younger (below 30 years), OR 3.00 (95% CI 1.638-5.493).Mechanisms for this effect are not clear, but a possible explanation can be because of increased gonadotropin and other anterior pituitary hormone activity at the peripubertal period, the gonadotrophs may be more susceptible because of increased cell division. This hypothesis is however speculative and further research is needed on the exact mechanism. This finding is consistent with other studies which have demonstrated higher incidence of hypopituitarism among the younger patient population exposed to head irradiation. In a cohort of 166 patients aged 6–80 years who had received high-dose irradiation for tumors of the head and neck, children younger than 15 years of age had a

As a secondary outcome, perceived fertility of the study participants was assessed as the ability of one to achieve a pregnancy in the spouse when a desire to do so existed. Majority of the patients were married (78.6%), with 68.4% of the respondents being sexually active. Only 23(23.5%) desired to achieve a pregnancy and only 3 (13%) of them were successful in achieving a pregnancy. The low number of those who desired fertility could be due to the fact that a good number of the study participants were elderly and thus may have had all the children they desired to have. Cancer being a debilitating condition it is possible that faced with the condition, many patients did not prioritize getting another child .A survey conducted by Schover et al [3] revealed that 51% of men with cancer wanted children in the future, including 77% of men who were childless when their cancer was diagnosed. Huyghe E. et al [39] demonstrated a 5 year cumulative fertility of 67.1% after treatment .Our point fertility was 13%.In order to demonstrate the fertility of this cohort, 5 year fertility would be desirable, thus follow-up study on this cohort is necessary to conclusively demonstrate their fertility.

10 CONCLUSION

1. Fifty percent gonadal dysfunction occurred among male patients surviving cancer therapy at KNH
2. Chemotherapy has gonadal sparing effect on patients younger than 30 years of age
3. Higher risk of gonadal dysfunction among the younger (< 30 years) in those patients exposed to head irradiation

11 STUDY LIMITATIONS

1. Use of composite modes of therapy in the analysis
2. Inability to establish gonadal dysfunction attributable to primary cancer.
3. Recall bias

12 RECOMMENDATIONS

1. Routine pretreatment counseling of all cancer patients on possible gonadal toxicity as a result of cancer therapy, especially young patients with desire to have a family.
2. Setting up of a specialized fertility clinic at KNH that can provide fertility preservation measures e.g. sperm cryopreservation to patients about to undergo cancer therapy.
3. Long term prospective studies should be conducted in a similar cohort to ascertain fertility of the study population and any eventual recovery of gonadal function in those found to have had gonadal toxicity.

13 REFERENCES

1. American National Cancer Institute. *Comprehensive Cancer database*,2007; **4**:20
2. Cancer incidence report-Kenya Medical Research Institute;2000-2002
3. Spitz S.The histological effects of nitrogen mustard on human tumours and tissues.*Cancer* 1948 ; **1**:383-398
4. Schover LR, Brey K, Lichtin A, Lipshultz LI, Jeha S. Knowledge and experience regarding cancer, infertility, and sperm banking in younger male survivors. *J Clin Oncol.* 2002; **20**: 1880 –9.
5. Meirow D, Schenker JG. Cancer and male infertility. *Hum Reprod.* 1995; **10**: 2017.
6. Carson SA, Gentry WL, Smith AL, Buster JE. Feasibility of semen collection and cryopreservation during chemotherapy. *Hum Reprod.* 1999; **6**: 992.
7. Rivkees S.A. and Crawford J.D. The relationship of gonadal activity and chemotherapy-induced gonadal damage. *JAMA* 1988; **259**: 2123-2125.
8. Naysmith TE, Blake DA, Harvey VJ, and Johnson NP. Do men undergoing sterilizing cancer treatments have a fertile future? *Hum Reprod.* 1998; **13**: 3250 -5.
9. Meistrich ML, Vassilopoulou-Sellin R, Lipshultz LI. Gonadal Dysfunction, in DeVita VT, Heilman S, Rosenberg SA (eds). Cancer: Principles and Practice of Oncology. Philadelphia, PA: Lippincott Williams & Wilkins 2001: 2560–2574.
10. Wang J,Galil KAA,Setchell B.P.Changes in testicular blood flow and testosterone production during aspermogenesis after irradiation.*J.endocrinology* 1983;**98**:35
11. Marmor D,Grob-Mendez F,Duyck F,et al.Very late return of spermatogenesis after chlorumbucil therapy:case reports *Fertil Steril* 1992;**58**:845.
12. Pryzant RM,Meistrich ML,Wilson E,et al.Long term reduction in sperm count after chemotherapy,with or without radiation therapy for non-hodgkins lymphoma *Bone Marrow Transplant* 2002;**30**:447.
13. Meistrich ML,Wilson G,Brown BW,et al.Impact of cyclophosphamide on long term reduction in sperm count in men treated with combination chemotherapy for Ewing's and soft tissue sarcomas *Cancer* 1992;**70**:2703.
14. Jacobsen KD, Theodorsen L, Fossa SD. Spermatogenesis after unilateral orchiectomy for testicular cancer in patients following surveillance policy. *J Urol.* 2001;**165**:93

15. Marina S,Barcelo P,permanent sterility after immunosuppressive therapy *Int J Androl.* 1979;**2**:6.
16. da Cunha MF,Meistrich ML,Fuller LM et al.Recovery of spermatogenesis after treatment for Hodgkin's disease *J Clin Oncol.* 1984;**2**:571.
17. Hansen PV,Trykker H,Helkjaer PE,et al.Testicular function in patients with testicular cancer treated with ochiectomy alone or orchiectomy plus cisplatin based chemotherapy *J Natl Cancer Inst.* 1989;**81**:1246.
18. Sanders JE,Hawky J,Levy W ,et al.Pregnancies following high-dose cyclophosphomide with or without high dose busulphan or total body irradiation and bone marrow transplantation *Blood* 1996;**87**:3045.
19. Longhi A,Macchiagodema M,Vitali G ,et al.Fertility in the male patients treated with neoadjuvant chemotherapy for osteosarcomas *J Pediatr Hematol Oncol.* 2003; **25:**292.
20. Lampe H, Horwich A, Norman A, et al. Fertility after chemotherapy for testicular germ cell cancers. *J Clin Oncol.* 1997; **15**: 239–245.
21. Hall MC, Fritzsch RJ, Sagalowsky AI, et al. Prospective determination of the hormonal response after cessation of luteinizing hormone–releasing hormone agonist treatment in patients with prostate cancer. *Urology* 1999; **53**:898.
22. Meistrich ML, van Beek MEAB. Radiation sensitivity of the human testis. *Adv Radiat Biol* 1990; **14**:227.
23. Gordon W, Siegmund K, Stanisic TH, et al. A study of reproductive function in patients with seminoma treated with radiotherapy and orchidectomy. *Int J Radiat Oncol Biol Phys.* 1997;**38**:83
24. Mackie E.J., Radford M. and Shalet S.M.Gonadal dysfunction following chemotherapy for childhood Hodgkin's disease. *Med. Paediatr.Oncol.*1996;**27**:74-78
25. Klein CE. Gonadal Complications, in Kufe DW, Pollock R, Weichselbaum RR, et al. (eds). Cancer Medicine. 6th ed. Hamilton, Ontario: BC Decker, Inc.; 2003: 2189–2210.
26. Thames HD and Hendry JH (1987) Response of tissues to fractionated irradiation: effect of repair. In *Fractionation in Radiotherapy*, 53–99 (Eds. Thames HD and Hendry JH) London: Taylor and Francis

27. Chen MS *et al.* Prospective hormone study of hypothalamic–pituitary function in patients with nasopharyngeal carcinoma after high dose irradiation. *Jpn J Clin Oncol* .1989;**19**: 265–270
28. Leiper A.D., Stanhope R.,Lau T et al. The effect of total body irradiation and bone marrow transplantation during childhood and adolescence on growth and endocrine function.*Br J. Haematol.*1987;**67**:419-426
29. Ogilvy-Stuart AL *et al.* Endocrine deficit after fractionated total body irradiation. *Arch Dis Child.*1992; **67**: 1107–1110
30. Littley MD *et al.* Endocrine and reproductive dysfunction following fractionated total body irradiation in adults. *Q J Med.* 1991; **78**: 265–274
31. Brauner R *et al.* Greater susceptibility to hypothalamopituitary irradiation in younger children with acute lymphoblastic leukemia. *J Pediatr.* 1986; **108**: 332
32. Samaan NA *et al.* Endocrine complications after radiotherapy for tumors of the head and neck. *J Lab Clin Med.* 1987 **109**: 364–372
33. Hansen PV,Trykker,Svennakjaer IL,at al.Long term recovery of spermatogenesis after radiation in patients with testicular cancer *Radiother Oncol.* 1990;**18**: 117.
34. Anserini P,Chiodi S,Spinelli S,et al.Semen analysis following allogenic bone marrow transplantation *Bone Marrow Transplant.* 2002; **30**: 447.
35. Ries LAG, Eisner MP, Kosary CL, et al. (eds). SEER Cancer Statistics Review, 1975–2001. Bethesda, MD: National Cancer Institute.
36. Byrne J, Fears TR, Mills JL, et al. Fertility of long-term male survivors of acute lymphoblastic leukemia diagnosed during childhood. *Pediatr Blood Cancer* 2004; **42**: 364–372.
37. Chatterjee R, Haines GA, Perera DM, et al. Testicular and sperm DNA damage after treatment with fludarabine for chronic lymphocytic leukaemia. *Hum Reprod.* 2000; **15**: 762–766
38. Hendry WF, Stedronska J, Jones, CR, et al. Semen analysis in testicular cancer and Hodgkin's disease. Pre- and post-treatment findings and implications for cryopreservation. *Br J Urol.* 1983; **5**: 769–773.
39. Huyghe E, Matsuda T, Daudin M, et al. Fertility after testicular cancer treatments: results of a large multicenter study. *Cancer* 2004; **100**: 732–737

40. S.Ramesh Babu,M.D Sandhnani,M.Swarna,P. Padmavathi and P.P Reddy. Evaluation of FSH,LH, and Testosterone levels in different subgroups of infertile males. *Indian Journal of Clin. Biochem.* 2004;**19**:45-49

41. Sulthan, C., Craste-de-paulet, B., Audran, F.,Iqbal, Y. and Ville, C. Hormonal evaluation in male infertility. *Ann. Biol. Clin. Paris.*1985; **43** (1), 63-66.

42. Zabul, J., Mierzejewski, W. and Rogoza, A. Usefulness of examining gonadotropin hormones and testosterone in men with abnormal semen. *Ginekol-pol.*1994; **65** (2), 71-74.

43. Weinbauer, G.F. and Nieschlag, E. Gonadotropin control of testicular germ cell development. *Adv. Exp. Med. Biol.*1995; **317**, 55-65.

44. Subhan, F., Tahir, F., Ahmad, R. and Khan, Z.D Oligospermia and its relation with hormonal profile. *Pak. Med. Assoc.*1995; **45** (9), 246-247.

45. Hopkinson, C.R.N., Mauses, J., Schenk, B., Flitze,E. and Hauser, C.H. Some interrelationship between plasma levels of LH, FSH, oestradiol - 17B, androgens and semen analysis data in male infertility patients. *Andrologia.*1977 ; **9**, 216.

46. Merino, G.E.S., Camales, Vadillo, M.L. Forsbach, G., Solis, J. and Zarate, A. Abnormal prolactin levels in semen and seminal plasma in infertile men. *Arch. Androl.*1980; **4**, 353.

47. Smith. S.R., Thompson, S.G., Haines, A.P., Jeffcoate, S.L. and Hendry, W.F. Plasma concentrations of pituitary and testicular hormones of fertile and infertile men. *Clin. Reprod. Fertil.*1985; **3** (1), 37-48.

48. Rege, N.A., Phadke, Bhatt, J., Khatri, N., Sheth, A., Joshi, U. and Vaidya, R. Serum gonodotropins and testosterone in infertile patients with varicocele. *Fertil. Steril.*1979; **31** (4), 413- 416.

49. Nagao, R.R., Plymate, S.R., Berger, R.E., Perin, E.B. and Paulsen, A.C. Comparison of gonadal function between fertile and infertile men with varicocele. *Fertil. Steril.*1986; **46.** 930-933.

50. Yanam, O., Ozdiler, E., Seckiner, I. and Gogus, O. Significance of serum FSH levels and testicular morphology in infertile males. *Int. Urol. Nephrol.*1999; **31** (4), 519-523.

51. De Mas P, Daudin M, Vincent MC, Bourrouillou G, Calvas P, Mieusset R, Bujan L. Increased aneuploidy in spermatozoa from testicular tumour patients after chemotherapy with cisplatin, etoposide and bleomycin. *Hum Reprod* 2001; **16**: 1204 – 1208.

52. Devroey P, Liu J, Nagy Z, Goosens A, Tournaye H, Camus M, Van Steirteghem A, Silber S. Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. *Hum Reprod.* 1995; **10** : 1457 – 1460.
53. Tournaye H, Liu J, Nagy PZ, Camus M, Goossens A, Silber S, Van Steirteghem AC, Devroey P. Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular spermatozoa.*Hum Reprod.* 1996; **11**: 127 – 132.
54. Chan PTK, Palmero GD, Veeck LL, Rosenwaks Z, Schlegel PN. Testicular sperm extraction combined with intracytoplasmic sperm injection in the treatment of men with persistent azoospermia postchemotherapy. *Cancer* 2001 ;**92**: 1632 – 1637
55. Adedevoh, S. W(ed) 1984 Subfertility and infertility in Africa. Report of an international workshop, University of Ibadan, Nigeria.
56. H.L Verjens, H.J. van der Moles,K.B Eik-Nes. Relationship between circulating testosterone levels, LH and FSH in intact and castrate males. *Euro Jour of Endocrin.* 2002; **79** (2):380-386.

14 APPENDICES

14.1 APPENDIX 1: QUESTIONNAIRE

A. IDENTIFIERS:

Name …….. Date / /

IP No …… Contact (phone no) ……

Study No.

B. SOCIO-DEMOGRAPHIC VARIABLES:

Current age (Years and completed months) ……..

Age at start of therapy (Years and completed months) ……..

Marital status (mark appropriately):

Married	
Cohabiting	
Widowed	
Divorced/Separated	
Single	

Education level (mark appropriately)):

Tertiary	
Secondary	
Primary	
None	

Occupation (mark appropriately):

Self employed	
Employed	
Unemployed	
Retired	
Training/Student	
Others	

Usual residence ……..

C. PERCIEVED FERTILITY (Mark 1 or 2):

1. Sexually active ? .....

2. Children/pregnancies before start of therapy ? .....

3. Desire and attempt to achieve pregnancy since start of cancer therapy? .....

4. If yes to above, was it successful? .....

5. Is your libido the same now as before therapy? .....

   If not, has it gone down? .....

KEY: 1-YES 2-NO

D.MEDICAL AND TREATMENT HISTORY;

1. Duration (months) since completing therapy .....

2. Diagnosis/type of cancer (from patient records) .....

3. Stage of disease (TNM- from patient records) .....

Mode of therapy (mark appropriately):

Chemotherapy	
Radiotherapy	
Radiotherapy + Chemotherapy	
Head irradiation	
Pelvic irradiation	
Orchidectomy	
Hypophysectomy	

Other concurrent treatments (specify) .....

Chemotherapeutic agents:

Class 1 Alkylating agents

Name of drug	yes	No	Dosage	No of coarses	Duration (months)
Cyclophosphamide					
Mustine					
Ifosfamide					
Melphalan					
Chlorambucil					
Busulphan					
Carmustine					
Lomustine					
Dacarbazine					
Others					

Class 2 Antimetabolites

Name of drug	Yes	no	Dosage	No of coarses	Duration (months)
Methotrexate					
Cytosine arabinoside					
5-flourouracil					
6-thioguanine					
Fludarabine					
Others					

Class 3 Plant Derived agents

Name of drug	yes	No	Dosage	No of coarses	Duration (months)
Vinblastine					
Vincristine					
Etoposide					
Topotecan					
Others					

Class 4 Antitumor antibiotics

Name of drug	yes	no	Dosage	No of coarses	Duration (months)
Daunorubicin					
Doxorubicin					
Epirubicin					
Idarubicin					
Mitoxantrone					
Bleomycin					

Actinomycin-D					
Mitomycin-C					
Others					

Class 5 Platinum compounds

Name of drug	yes	No	Dosage	No of coarses	Duration (months)
Cisplatin					
Carboplatin					
Oxaplatin					
Others					

Class 5 Miscellaneous

Name of drug	Yes	no	Dosage	No of coarses	Duration (months)
L-asparaginase					
Procabazine					
Hydroxyurea					
Amsacrine					
Mitoguazone					
Others					

Class 6 Hormonal agents

Name of drug	yes	No	Dosage	No of coarses	Duration (months)
Prednisone/prednisolone					
Dexamethasone					
Oestrogens					
Progestins					
Oestrogen receptor modulators					
Oestrogen aromatase inhibitors					
Antinadrogens					
LHRH analogs					
Others					

Class 7 Cytokines

Name of drug	Yes	no	Dosage	No of coarses	Duration (months)
Interferone					
Interleukin 2					
Growth factors					
Others					

Class 8 Targeted compounds

Name of drug	Yes	no	Dosage	No of coarses	Duration (months)

Rituximab					
Alemtuzumab					
Imatinib					
Bevacizumab					
Others					

If on radiotherapy,

Total radiation dose.....

E.HORMONAL PROFILE

FSH (iu /l) ..... (1.0 - 14.0 iu/l)
LH (iu/l) ..... (0.7 – 7.4 iu/l)
Testosterone (nmol/l) ..... (9.36 – 37.10 nmol/l)

Diagnosis (tick one):
Primary testicular failure (hypergonadotropic hypogonadism) □
Hypogonadoptropic Hypogonadism □
Hypogonadotropic hypogonadism □
Normal hormonal profile □
Atypical profile (specify) .....

F TESTICULAR VOLUME:

Rt.....(cm^3) Lt..... (cm^3) (using prader ochiometer)

14.2 APPENDIX 2

CONSENT/ASSENT EXPLANATION

My name is Dr Fredrick Otieno, a postgraduate student at The University of Nairobi, conducting a study for my thesis. I would like to explain about the study I am carrying out. I intend to look at gonadal dysfunction in male patients who are surviving cancer treatment.

Treatment for cancer can at times affect the ability of one to get a child.

We do not know to what extent the above problem affects our patients, hence the necessity for this study.

In order to understand the magnitude of this problem among our patients, I will ask you some questions concerning your diagnosis, treatment given, fertility before and after treatment.2 ml of blood will also be drawn from your arm vein, and this will be sent to the lab to check your sex hormone profile.

You will not be coerced to answer the questions. Participation is purely voluntary. Your participation may however enable us to understand the extent of gonadal dysfunction in patients surviving cancer therapy. If you have any further questions, you are free to ask them any time. If you consent to be enrolled into the study, then you will be required to sign or put a thumb print in the space provided.

Risks:

A mild unpleasant sensation will be experienced during blood withdrawal for the above laboratory tests

Some of the questions will touch on your private life, but this information will not be disclosed to anyone else.

Benefits:

All above procedures will be done free of charge

The results of the tests conducted on you shall be communicated to you by the primary investigator and where the results are found to be abnormal you shall be referred to the fertility clinic for further management.

In case of any Queries contact Dr Otieno on 0722798216

Or KNH Ethics Committee Prof Guantai KNH 726300 ext 44355 or 44102

I _____ do hereby consent freely, without any form of coercion or inducement to take part in the above study and to be interviewed. Its purpose and nature has been fully explained to me by _____ and I understand that I can withdraw at any time should I change my mind.

Signed: _____ Date: _____

Witnessed By: _____ Date: _____

I _____(relationship) _____ do hereby assent freely, on behalf of ..... without any form of coercion or inducement to take part in the above study and to be interviewed. Its purpose and nature has been fully explained to me and him by _____ and I understand that he can withdraw at any time should he change his mind.

Signed: _____ Date: _____

Witnessed By: _____ Date: _____

14.3 APPENDIX 3

REFERRAL FORM

I am a postgraduate student in the Department of Medicine, conducting a study on gonadal dysfunction among male patients surviving cancer therapy at KNH.

I refer ..... IP No ..... ,who was treated for.....received(treatment).....
.....
.....
.....
.....He participated in the study and had following result:

LH .....
FSH .....
Testosterone .....
Testicular volume Rt ..... Lt .....

Assistance to him shall be highly appreciated

Signed .....

Date .....

Dr Otieno Fredrick,
Department of Internal Medicine,
University of Nairobi