Asian Journal of Medical Sciences 10(2): 6-12, 2019
DOI:10.19026/ajms.10.6013
ISSN:2040-8765; e-ISSN: 2040-8773

Submitted: August 2, 2018 Accepted: October 1, 2018 Published: July 25, 2019

Research Article

Interpretational Tool for Fertility Hormonal Profile Established from a Healthy Adult Kenyan Population

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Abstract: Infertility is a reproductive disorder that affects both male and female and prevents them from bearing desired child. Clinical laboratory plays a very important role in the process of investigating this disorder. A prospective study carried out in clinical chemistry laboratory of Kenyatta National Hospital involving 491 healthy individuals between 18-60 years. Reference ranges were constructed by using the parametric methods to estimate 2.5 and 97.5 percentiles of distribution as lower and upper reference limits. The fertility hormonal profile of the adult healthy Kenyan population was carried out by investigating luitenizing (LH), follicle stimulating (FSH), estradiol ii (EII), prolactin (PRL), progesterone PRG) and testosterone (TESTO) hormones; Four hundred and ninety-one voluntarily study subjects comprising of 221 males and 270 females were recruited in the study. Sex related reference values were established as follows:- TESTO [male (18-39 yrs [3.5-12.7 ng/mL], (40-50 yrs [2.5-6.9 ng/mL), (51-61 yrs [1.8-5.6 ng/mL] female: follicular and luteal phases [0-2 ng/mL], ovulation phase [0.7-3.3 ng/mL] and Menopause [0-1.17 ng/ml, FSH [male;[0.7-7.5 (μU/mL] female: follicular phase [4-16μU/mL, ovulation phase [5-20 μU/mL], luteal phases [2.5-7 μU/mL] and Menopause [0.11-1.89 μU/mL], LH [male;[0.5-5.7 μU/mL] female: follicular phase [0.7-8.6 μU/mL, ovulation phase [7-83μU/mL], luteal phase [0.4-6.8 μU/mL] and Menopause [0.13-2.18μU/mL], EII [male;[0-25 pg/mL]female: follicular phase[0-200 pg/mL], ovulation phase [134-467 pg/mL], luteal phases [75-351 pg/mL] and Menopause [1-17 pg/mL], PRG [male;[0.12-1.02 ng/mL]female: follicular phase [0.04-0.72 ng/mL], ovulation phase [0-5 ng/mL], luteal phases [0-0.38 ng/mL] and Menopause [0-0.38 ng/mL], PRL [male;[0-17 ng/mL]female: menstrual [1.7-23 ng/mL] and menopausal [8-29 ng/mL]. Bleeding period [menstrual [2-5 days].Sex specific reference ranges for the assessment of fertility and management of infertility disorders has been established. Age is an important factor in the interpretation of testosterone hormone in males. In females, the ovarian and pituitary fertility hormones have different reference ranges based on the phase of the menstrual cycle. These reference ranges are different from those reported in the literature, therefore clinical chemistry laboratories within the same geographical region should establish their own based on the population they serve.

Keywords: Hormones, infertility, kenyatta national hospital, kenyan, laboratory, reference range

INTRODUCTION

According to World Health Organization, definition of infertility in human beings is a disease condition of the reproductive system where there is evidence of failure for a woman to achieve clinical pregnancy after twelve months of more of regular unprotected sexual intercourse. Infertility is divided into two categories that is primary and secondary. Primary infertility is related to a couple who have never had a child despite the desire to do so. Secondary infertility is failure for a woman to conceive following a previous pregnancy. This disorder is known to affect both adult male and female equally (Gurunath*et al*., 2011). Infertility has been associated with both societal and psychological effects. Infertility may have psychological effects. Anxiety to conceive causes couples to have increased sexual dysfunction. Couples are known to suffer from emotional stress and marital difficulties. In some cultural setup, failure to conceive brings about stigmatization and a degree of rejection by the couple. Infertility has been considered as a type of disability in some societies (Khetarpal and Singh, 2012).

Several causes have been attributed to infertility. Anti-sperm antibodies an example of Immune infertility

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is known to contribute to 10 to 30% of infertility in infertile couples. These antibodies are directed against surface antigens on the sperm, which impairs sperm motility and transport through the female reproductive tract (Restrepo and Cardona-Maya, 2013). Infection with organisms such as *chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* which are sexually transmitted infections have been known to be a cause of infertility in male and female (Ljubin-Sternak and Mestrovic, 2014; Lis *et al*., 2015). Severe forms of infertility such as azoospermia and oligozoospermia has been associated with the mutations to NR5A1 gene encoding steroidogenic factor-1(SF-1) in some men. Smoking and chemotherapy have been related to DNA damage that affect female ovocytes and male sperms (Ferraz-de-Souza *et al*., 2011; Zenzes, 2000). Diabetes mellitus and thyroid disorders are pathological conditions that have been associated with infertility in both male and female (Jangir and Jain, 2014; Livshits and Seidman, 2009; Andreeva, 2014; van den Boogaard *et al*., 2011).

Hyperprolactinemia even without any other underlying cause has been attributed to infertility in both male and female. Specific causes of female infertility include: an ovulatory, blockage of the fallopian tube, advance in age, tubal ligation and endometriosis. On the other hand, the specific causes of male infertility include: low semen quality, low sperm count, testicular malformation and low testosterone hormone levels. Environmental factors such as toxins (volatile organic solvents, chemical dusts and pesticides have been found to contribute to infertility in both male and female. Other studies have shown that tobacco consumers are more likely to suffer from infertility than non-tobacco consumers (Mendiola *et al*., 2008).

When all the symptoms points at infertility it is mandatory to confirm these clinical impressions by making proper and adequate diagnosis based on all the diagnostic methods available. Some of the diagnostic tools includes radiologic and clinical laboratory procedures. Fertility hormonal profile analysis plays an important role in the diagnosis and management of various pathological disorders that contribute to infertility in an individual. To investigate primary and secondary infertility that affect both male and female the following diagnostic hormonal profile parameters are carried out: Testosterone (TESTO), Prolactin (PRL), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol 2 (EII) and Progesterone (PRG).

The key aim of the current study was to establish the interpretation tool for fertility hormonal profile using a healthy adult Kenyan population. Immunochemical reaction techniques were used to determine the concentration of the studied fertility hormones. The Kenyatta National Hospital Research and Ethical Committee approved the study (P342/11/2007).

METHODOLOGY

The study was carried out among 491 healthy Kenyan adults comprising of 221 males and 270 females between the age 18-60 years. The study site was Nairobi Metropolitan region and the department of laboratory medicine, clinical chemistry laboratory of Kenyatta National Hospital children was the main analytical centre. Using a 5 mL syringe, 4 mL of venous blood was collected and put in a plain bottle labeled within the subject's identification details. It was allowed to clot at room temperature for 1 h and then centrifuged at 3000 g for five minutes. Separation of serum was done using a pasteur pipette for each specimen and transferred into specific vials labelled with subject's identification details. Specimens were stored at -20°C awaiting analysis. Mini Vidas (Biomerieux, Lyon, France)a closed system machine was used for analysis of the studied parameters. Reagents strips and solid phase receptacles employing immunochemical reaction analytical principle were used in Mini Vidas machine for the analysis of hormones. The assayed multisera normal was used for the quality control of the analytical work during the study period. The lower and upper limits of the reference intervals was obtained using the following formula: x- 1.96SD, x + 1.96SD where x = Mean and SD = standard deviation. The collected analytical data was statistically analyzed using the statistical package for social sciences (SPSS version 21). T-test was used for means comparison, while ANOVA and post-Anova-tests were used for multiple comparison of means. The tests were conducted at 95% confidence interval and significance level of 5%; p less than or equal to 0.05 was considered statistically significant.

RESULTS

Fertility hormones (TESTO, LH, FSH, EII, PRG) reference ranges for male and female were determined separately since they are dependent on the menstrual cycle phases in females. Therefore, no gender differences were determined for these fertility hormones. PRL reference range was constructed independently since it is not influenced by the menstrual cycle phases in female.

Establishment of male fertility hormones reference ranges for the studied adult Kenyans: Testosterone test results for 320 adult males 18-28 years (104), 29-39 years (78), 40-50 years (92) and 51-61 years (46) were used to construct TESTO reference ranges. Multiple means comparison of the four male age categories showed no statistical significant differences between age category 18-28 years and 29-39 years ($p = 0.562$), whilst as statistically significant difference ($p<0.05$) was observed in the other age categories. The mean and Standard Deviation (SD) results for FSH, LH, EII, PRG and PRL for 221 adult males were: {4.1 µU/mL and 1.74 µU/Ml}, {3.14 µU/mL and 1.31 µU/Ml}, {10.76

Table 1: Age specific male fertility hormones reference ranges for the studied healthy adult Kenyans

Parameter (unit)	Age group (yrs)	N	x(SD)	x-1.96SD	x+1.96SD	Rr
TESTO (ng/mL)	18-28	85	8.13(2.2)	3.5	12.7	3.5-12.7
	29-39	97	8.08(2.3)	3.5	12.6	
	40-50	92	4.67(1.11)	2.5	6.9	2.5-6.9
	51-60	46	3.71(0.97)	1.8	5.6	1.8-5.6
FSH (µU/mL)	18-60	221	4.1(1.74)	0.7	7.5	0.7-7.5
LH (µU/mL)	18-60	221	3.14(1.31)	0.5	5.7	0.5-5.7
E2 (pg/mL)	18-60	221	10.76(7.22)	0	25	0-25
PRG (ng/mL)	18-60	221	0.57(0.28)	0.12	1.02	0.12-1.02
PRL (ng/mL)	18-60	221	8.07(4.57)	0	17	0-17

pg/mL and 7.22 pg/Ml}, {0.57 ng/mL and 0.28 ng/mL}and {8.07 ng/mL and 4.57 ng/Ml} respectively. All the above results are as shown in Table 1.

Results are expressed as Mean (x) ± Standard Deviation (SD) for the number of subjects shown in the column labeled N; Rr = reference range. Age category 18-28 years and 29-39 years for TESTO had similar Mean (x) ± SD and were therefore combined to produce one reference range.

Establishment of female fertility hormones reference ranges for the studied adult Kenyans: In the establishment of female fertility hormones, menstrual cycle phases (follicular, ovulation, luteal), menstrual bleeding period and menopause period were considered in the studied adult female Kenyans.

Female TESTO reference ranges were constructed using 270 (follicular phase (79), ovulation phase (75), luteal phase (78), menopause (38)) test results. Female TESTO levels in follicular phase, luteal phase and menopause were not statistically significant (follicular phase/luteal phase ($p = 1.000$), follicular phase/menopause ($p = 0.439$) and luteal phase/menopause ($p = 0.287$). Therefore, same reference range was constructed for follicular phase, luteal phase and menopause. Mean and Standard Deviation (SD) were 0.75 ng/mL and 0.64 ng/mL, respectively. Ovulation phase TESTO levels was statistically different from the other phases with a mean and standard deviation of 2.02 ng/mL and 0.66 ng/mL, respectively. Female FSH reference ranges were constructed using 270 follicular phase (79), ovulation phase (75), luteal phase (78), menopause (28)} test results. Multiple comparison of the means difference for the phases using post hoc test (Tukey HSD) was found to be statistically significant ($p<0.001$). Therefore, reference ranges were constructed for each specific phase, luteal phase and menopause. Mean and standard deviation (SD) were follicular phase (10.14 µU/mL and 3.1 µU/mL, ovulation phase (12.62 µU/mL and 3.79 µU/mL), luteal phase (4.96 µU/mL and 1.27 µU/mL) and menopause (49.5 µU/mL and 20 µU/mL) respectively. Female LH reference ranges were constructed using 270 {follicular phase (79), ovulation phase (75), luteal phase (78), menopause (38)} test results. Multiple comparison of the means difference for the phases using post hoc test (Tukey HSD) was found to be statistically significant ($p<0.001$). Therefore, reference ranges were constructed for each specific phase. Mean and standard deviation (SD) were: -Follicular phase (4.63 µIU/mL and 2 µU/Ml), ovulation phase (44.73 µU/mL and 19.44 µU/mL), luteal phase 3.59 µU/mL and 1.61 µU/mL) and menopause (19.5 µU/mL and 6.6 µU/mL) respectively. Female EII reference ranges were constructed using 270 {follicular phase (79), ovulation phase (75), luteal phase (78), menopause (38)} test results. Comparison of the means difference by Tukey HSD was found to be statistically significant ($p = 0.001$). Therefore, reference ranges were constructed for each specific phase. Mean and Standard Deviation (SD) were:-follicular phase (100.05pg/mL and 51.04 pg/mL, ovulation phase (300.88 pg/mL and 84.9 pg/mL), luteal phase (214 pg/mL and 70.3 pg/mL) and menopause (15.8 pg/mL and 10 pg/mL), respectively. Female PRG reference ranges were constructed using 270 {follicular phase (79), ovulation phase (75), luteal phase (78), menopause (38)} test results. Comparison of the means difference by Tukey HSD was found to be statistically significant ($p<0.001$). Therefore, reference ranges were constructed for each specific phase. Mean and standard deviation (SD) were follicular phase (0.38 ng/mL and 0.17 ng/mL, ovulation phase (2.52 ng/mL and 5 ng/mL), luteal phase (3.73 ng/mL and 4.48 ng/mL) and menopause (0.18 ng/mL and 0.1 ng/mL, respectively. Female PRL reference ranges were constructed for both menstruating and menopausal females. Results for 232 female adults were used to construct PRL reference range for menstruating group. Mean and Standard Deviation (SD) was 12.37 ng/mL and 5.47 ng/mL, respectively. Lower and upper reference limits were 1.7 ng/m/L and 23 ng/mL, respectively. Results for 38 female adults were used to construct PRL reference range for menopausal female group. Mean and Standard Deviation (SD) was 18.3 ng/mL and 5.5ng/mL, respectively.

A total of 229 menstrual female subjects were categorized in three age groups: 18-28 yrs (97), 29-39 yrs (55) and 40-50 yrs (77). Comparison of the means difference by Tukey HSD was found to be statistically insignificant ($p = 0.759$). Therefore, reference range was constructed for the whole female group. Mean and standard deviation (SD) was 3 days and 0.81 days, respectively. All the above results are as shown in Table 2.

Table 2: Established female fertility hormones reference ranges for the studied adult Kenyans

Parameter (unit)	N	Fertility cycle	x(SD)	x-1.96 SD	x+1.96 SD	Rr
TESTO (ng/mL)	79	Follicular	0.75(0.64)	0	2.05	0-2
	78	Luteal	0.73(0.66)	0	2.03	
	157	Fol/Lut	0.79(0.66)	0	2.04	
	75	Ovulation	2.02(0.66)	0.7	3.3	0.7-3.3
FSH (μU/mL)	79	Follicular	10.14(1.3)	4	16	4-16
	75	Ovulation	12.62(3.8)	5	20	5-20
	78	Luteal	4.96(1.3)	2.5	7	2.5-7
	38	Menopause	49.5(20)	10	89	10-89
LH (μU/mL)	79	Follicular	4.63(2)	0.7	8.6	0.7-8.6
	75	Ovulation	44.7(19.4)	7	83	7-83
	78	Luteal	3.59(1.61)	0.4	6.8	0.4-6.8
	38	Menopause	19.5(6.6)	7	32	7-32
EII (pg/mL)	79	Follicular	100(51)	0	200	0-200
	75	Ovulation	301(85)	134	467	134-467
	78	Luteal	214(70)	75	351	75-351
	38	Menopause	16(10)	0	35	0-35
PRG (ng/mL)	79	Follicular	0.38(0.17)	0.04	0.72	0.04-0.72
	75	Ovulation	2.52(5)	0	5	0-5
	78	Luteal	3.73(4.5)	0	18	0-18
	38	Menopause	0.18(0.1)	0	0.38	0-0.38
PRL (ng/mL)	232	Menstrual	12.37(5.47	1.7	23	1.7-23
	38	Menopause	18.3(5.5)	8	29	8-29
Bleeding period (day)	229	Menstrual	3.00(0.81)	1.5	4.5	2-5

Results are expressed as Mean (x) ± Standard Deviation (SD) for the number of subjects shown in the column labeled N; Rr = Reference range. In theFollicular and Luteal stages of the fertility cycle, the levels of TESTO were similar and were therefore combined to produce one reference range.

DISCUSSION

Establishment of adult Kenyan male and female reference ranges for fertility hormones in the current study was important in an effort to address infertility problems affecting the Kenyan population. Separate male and female fertility hormones were established due to gender differences. The established female fertility hormones reference ranges had in consideration changes in hormonal patterns during the menstrual cycle. In this regard, female established fertility hormones were divided into four phases' i.e., follicular phase, ovulation phase, luteal phase and menopause.

Current study established male testosterone reference ranges in relation to various age groups. No differences that were observed between age group 18-28 yrs and 29-39 yrs therefore a common reference range was established. Separate reference ranges were established for age groups 40-50 yrs and 51-61 yrs due to the difference observed in this study. Testosterone which is predominantly a male fertility hormone decreases in advance with age. This decrease was evident in this study, considering the mean decrease from 8.13 ng/mL in age group 18-39 yrs to 3.71 ng/mL in age group 51-61 yrs. Investigation of hypogonadism disorders in adult Kenyan male population is achieved by considering the testosterone reference range lower limits of each age group. Levels above the established male testosterone reference range upper limit are not of clinical significance since there are no known pathological disorders associated with hypogonadisms in adult males. There was no agreement between the findings of the current study with studies carried out elsewhere in terms of age group differences. Heil *et al*. (2002), established a common male testosterone reference range for German population as opposed to Kenyan age group-based testosterone reference ranges.

Current study established female testosterone reference range based on the phases of the menstrual cycle. There was no difference in testosterone levels for follicular phase, luteal phase and menopause. Therefore, a common adult female testosterone reference range was established. Ovulatory phase testosterone levels were different from menstrual and non-menstrual phases of an adult female. This difference leads to the establishment of ovulatory phase testosterone reference range. High levels of ovulatory testosterone are physiologically important to aid the ovulation process in healthy adult female. Study findings from other geographical regions did not express this physiological importance of testosterone during ovulation. Common female adult testosterone reference range was established for German and French adult female populations. The German and French adult female testosterone reference ranges quoted in literature are some of the references made in the interpretation of laboratory testosterone reports in Kenyan health institutions.

Prolactin reference ranges for the Kenyan female subjects was established for the menstrual and menopausal female groups. Differences in prolactin concentration for these groups were observed whereby it was increased in the menopausal females. In menstrual female the reference range upper limit is clinically important when dealing with the effects of hyperprolactinemia. Based on the findings of this study, a prolactin concentration of greater than 23 ng/mL in a Kenyan female of reproductive age is an indicator of

infertility. On the other hand, hyperprolactinemia which means a value of below 1.7 ng/mL would be of no clinical importance in a menstrual Kenyan female. Established prolactin reference range upper limit of Kenyan adult female population is lower than French and German adult females as quoted in literature. Using the French and German prolactin reference range to diagnose a case of hyperprolactinemia in a female Kenyan would result with under diagnosing the individual. Such individual would have all the clinical features of hyperprolactinemia with misleading laboratory results, since the literature-based reference range does not represent the female Kenyan population.

Although prolactin is not a predominant male fertility hormone, hyperprolactinemia is known to cause infertility even without any other abnormal clinical presentation. According to the established male prolactin reference range for the Kenyan population, hyperprolactinemia is indicated by concentrations above 17 ng/mL. As in female's hyperprolactinemia in males is of no clinical importance.

Current study established reference range of FSH based on the menstrual cycle phases. Kenyan female population shows a rise of FSH in the follicular and ovulation phases of the menstrual cycle leading to a major decline in the luteal phase. The increase of FSH in the follicular phase is of clinical importance since the hormone has a responsibility of controlling the follicular phase of the menstrual cycle. Considering the FSH means for the three menstrual cycle phase i.e., follicular, ovulation and luteal phase respectively, there is a pattern of increase in the follicular phase then a pick in the ovulation phase and a decline in the luteal phase. This pattern corresponds with FSH physiological activity pattern within the menstrual cycle. Hypogonadism and hypogonadism would be detected at different levels of the menstrual cycle of the Kenyan adult female population based on the upper and lower reference range limits. Other populations compared with the Kenyan population also express the same physiological patterns but differing in the FSH levels of specific phase of the menstrual cycle.

Current study findings of high levels of FSH in circulation during menopause due to elimination of the negative feedback effect on the pituitary by the failure of ovarian estrogen production, is in agreement with other studies reported in literature(Burger, 1994).Despite being predominantly a female reproductive hormone, FSH plays a great role in male adult by stimulating the process of spermatogenesis. Primary hypogonadism in adult Kenyan male (e.g., impotence) would be detected at FSH levels less than 0.7 μIU/mL or a value greater than 7.5 μU/mL. Low level of FSH in male interferes with the process of spermatogenesis whilst high levels affects testosterone functions.

It is evident from the findings of this study that the concentration of LH in a healthy female of reproductive age depends on the phases of a menstrual cycle. The LH concentration pattern produced by LH means for the three menstrual cycle is similar to that of FSH i.e., there is a pattern of increase in the follicular phase then a peak in the ovulation phase and a decline in the luteal phase. This pattern is in line with LH physiological activity pattern within the menstrual cycle. Hypogonadism and hypogonadisms would be detected at different levels of the menstrual cycle of the adult female Kenyan population based on the upper and lower reference range limits. Despite the differences in LH references ranges of the menstrual cycle phases, other studies have produced similar patterns to that of the current study (Heil *et al*., 2002). Current study findings of high levels of LH in circulation during menopause due to elimination of the negative feedback effect on the pituitary by the failure of ovarian estrogen production, is in agreement with other studies reported in literature (Burger, 1994).LH reference range established in this study for the adult Kenyan male population will detect primary hypogonadism in concentrations less than 7 μU/mL and above 32.7 μU/mL. Low level of LH in male interferes with the process of spermatogenesis whilst high levels affects testosterone functions.

It is evident from the current study that EII concentration levels are low during the follicular phase with a significant surge at ovulation phase and a decline in luteal phase. This pattern corresponds with the physiological activities of EII in a normal female of productive age. The same pattern is expressed by other studies carried out elsewhere in Germany (Heil*et al*., 2002). This study has established that the EII production decreases in menopause compared with the reproductive period of a female. The mean EII concentration during menopause was very low compared with the concentration in the phases of the menstrual cycle. This EII low concentration could be attributed to significant alterations of hypothalamic-pituitary feedback mechanisms in addition to decreased ovarian function.

Current study produced a PRG pattern of low concentrations in the follicular phase followed by a steady rise in the ovulation phase and a high peak in the luteal phase. This pattern corresponds with the physiological pattern of PRG as seen in a healthy adult female of reproductive age (Conneely *et al*., 2004). Similar studies reported in literature and compared with the findings of the current study have a similar PRG pattern despite the concentration levels of specific menstrual cycle phase. It is evident from the study findings that PRG concentrations in the menopausal female population are very low.

Adult males have PRG levels similar to those in women during the follicular phase of the menstrual

cycle (Dennerstein *et al*., 2003). Studies by Heil *et al*. (2002) in German population concurred with this similarity between PRG levels in male and in follicular phase in female. Current study differed with this since the PRG levels for males and female follicular phase were different ($p<0.001$). These differences could be due to the geographical localities of the three populations.

CONCLUSION

In conclusion, this is the first study to establish fertility hormonal reference ranges for adult Kenyan population. Separate reference ranges were established for both male and female study population. Age has been found to be a factor to consider especially when the testosterone is being interpreted for the adult male. The interpretation for the female fertility hormones has to be done based on the phase of the menstrual cycle apart from prolactin hormone.

RECOMMENDATIONS

- Clinical chemistry is a wide field having many biochemical parameters used in diagnosis of various pathological disorders. This study has managed to establish reference ranges for six biochemical parameters. There is great need to establish reference ranges for other parameters not included in this study. Once this is achieved, the Kenyan population will be dependent on local reference ranges other than literature based reference ranges.
- The age and gender of an individual must be included whenever a health provider makes a laboratory request. This is important since it has been established that some parameters reference ranges are dependent on the age of an individual such as testosterone. Gender of an individual must always be considered during analysis since the study has established gender differences in the studied fertility hormones.

ACKNOWLEDGMENT

The research team would like to acknowledge all the study subjects for their acceptance to participate in the study. Mount Kenya University for the facilitation in the publication of the research findings. The chief executive officer of Kenyatta National Hospital for the provision of the analytical equipment and a conducive environment for carrying out the research**.** Biomurex (France) and Hass Scientific (Kenya) for Provision of reagents and financial assistance. Clinical chemistry staff of Kenyatta National Hospital for the excellent technical assistance. Professor John Kihoro of Cooperative University for the statistical analysis of the study data. Irene Wanjiru Kaguru for the professional data handling.

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