Research Article
Cancer Diagnostic Tool Derived from Adult Kenyan Population

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Abstract: To establish the reference ranges of tumour makers for adult Kenyan population. A prospective study carried out in clinical chemistry laboratory of Kenyatta National Hospital involving 613 healthy individuals between 18-61 years. Parametric methods were used to constructed reference ranges by estimating 2.5 and 97.5% of distribution as lower and upper reference limits. Results for 596 voluntarily study subjects comprising of 360 male and 236 females were used for the establishment of reference ranges of: CA125, CA15-3, CA 19-9 and CEA. Tumour makers reference ranges established were as follows: CA125 (0-23) U/L, CA15-3 (0-19) U/L, CA 19-9 (0-16) U/L and CEA (0-4) U/L. TPSA was the only male oriented tumour maker studied. Results for 360 adult males were used for the establishment of TPSA whose established reference range was age group specific. Age group specific TPSA reference ranges established were as follows: Age group 18-28 year (0.1-1.4) ng/mL, 29-39 years (0.1-2.6) ng/mL, 40-50 years (1.1-3.5) ng/mL and 51-61 years (1.1-6.3) ng/mL. Common and sex-specific reference ranges for adult Kenyan population has been established. These reference ranges are different from those reported in literature, therefore each clinical chemistry laboratory should establish its own.

Keywords: Adult Kenyan, Kenyatta national hospital, reference range, tumour markers

INTRODUCTION

Cancer is a pathological disorder in which a group of cells display uncontrolled growth, invasion that intrudes upon and destroys adjacent tissues and sometimes metastasis, via lymph or blood. These three distinct properties of cancers differentiate them from benign tumors, which do not invade or metastasize (Anand *et al*., 2008). Environmental and hereditary genetic have been identified by researchers as the two main causes of cancer (Gorlova *et al*., 2007; Paola *et al*., 2002). There are different kinds of cancers and some of the most common includes:- breast, ovarian, prostatic, pancreatic, colorectal, brain, leukemia, testicular and lung cancer (Desai *et al*., 2001). Cancer affects people of all ages but a few types are more common in children. The overall risk of developing cancer increases with age. Cancer is a leading cause of death. In 2007 cancer caused about 13% of all human deaths worldwide (7.9 million) and the number of cases is rising as more people live to old age (WHO, 2011).

The presence of cancer can be suspected on the basis of symptoms, or radiological findings. Definitive diagnosis of cancer, however, involves the use of laboratory investigative tools which includes analysis of tumour makers and microscopic examination of a biopsy specimen. Tumor markers are antigenic substances that are found elevated in the body (usually in the blood or urine) when cancer is present. Almost everyone has a small amount of these markers in their blood, a reference used in the interpretation of tumour maker report (Sturgeon, 2002). Tumour markers are used to determine risk, screen for early cancer, establish diagnosis, estimate prognosis, predict that a specific therapy will work, or monitor for disease recurrence or progression. There are many different tumor markers. Some are seen only in a single type of cancer, while others can be found in many types of cancer. Some of the tumor makers in great use in clinical practice includes: Cancer Antigen 125 (CA 125), Cancer Antigen 15-3 (CA 15-3), Cancer Antigen 19-9 (CA 19-9), Carcinoembryonic Antigen (CEA) and Total Prostatic Specific Antigen (TPSA) (Tsao *et al*., 2006).

There are no published reference ranges for tumour markers for Kenyan adult population. Clinical Chemistry Laboratory of Kenyatta National Hospital relies on reagent manufacturers' published reference ranges, which may not adequately represent Kenyan adult population. The objective of this study was therefore to establish reference ranges for some tumour markers for adult Kenyan population. It was necessary to determine whether the levels of the studied tumour

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markers had any gender differences despite the prescribed use in diagnostic laboratory. Kenyatta National Hospital research and ethical committee approved the study (p342/11/2007).

MATERIALS AND METHODS

Study site: Nairobi Metropolitan Region of Kenya. Department of Clinical Chemistry Kenyatta National Hospital was the main analytical centre.

Study period: April 2010-December 2011.

Selection of study population: Prior to the recruitment of the study population, sensitization exercise was carried out through public lectures and advertisements in various institutions which included churches, mosques, medical colleges, universities and hospitals within Nairobi Metropolitan Region of Kenya. Six hundred and thirteen individuals comprising of 237 females and 376 males fulfilled the inclusion/exclusion criteria which were: Kenyan citizens between 18-60 years of age, normotensive, non-alcoholic, non-smokers, non-tobacco users, no medication and not pregnant. A questionnaire was administered to consenting study subjects to gather some socio-demographic data.

Specimen analysis: Four milliliters of blood was collected from each study subject. Serum was separated by centrifugation at 3000 g for 3 min. Studied tumour markers were determined by enzyme linked fluorescent assay method using Mini Vidas (Biomerieux, Lyon, France) machine. Specific assayed normal sera was used for the quality control of analytical work during study period.

RESULTS

Differences between male and female population was determined in order to construct separate or combined reference ranges of the studied parameters. Mean difference for CA 125, CA 15-3, CA 19-9,CA 15-3 and CEA were not statistically significant for both sexes with p-value greater than 0.05, therefore a combined reference range of each parameter was established as shown in Table 1. Test results for 469 adults (male: 233, female: 236) were used to construct reference ranges of CA 125, CA 15-3, CA 19-9 and CEA using mean±1.96 S.D. as shown in Table 1.

Three hundred and sixty male study participants were categorized in four groups i.e., group 1 (18-28), group 2 (29-39), group 3 (40-50) and group 4 (51-61) years. Multiple means comparison test of the four male age categories showed statistically significant differences between all the age group categories (p<0.001) as shown in Table 2. Therefore TPSA reference ranges were constructed for each specific age group category as shown in Table 3.

Comparison of established adult Kenyan reference ranges for the five studied tumour markers with some literature quoted reference ranges showed regional differences as shown in Table 4.

Table 1: Reference ranges for tumour markers for the studied healthy adult Kenyans

Parameter (unit)	Sex	N	x (S.D.)	x-1.96 S.D.	x+1.96 S.D.	Rr	t-value	Sig.*
CA 125 (U/L)	M	233	8.10 (6.80)	0	22	0-23	-0.906	0.365
	F	236	7.70 (6.40)	0	21			
	M/F	469	7.90 (6.60)	0	23			
CA 15-3 (U/L)	M	233	6.90 (5.40)	0	18	0-20	1.088	0.277
	F	236	7.50 (6.40)	0	21			
	M/F	469	7.20 (6.00)	0	20			
CA 19-9 (U/L)	M	233	6.10 (5.40)	0	17	0-16	-0.155	0.108
	F	236	6.01 (4.60)	0	15			
	M/F	469	6.10 (5.00)	0	16			
CEA (U/L)	M	233	1.50 (0.96)	0	3.5	0-3.5	-0.105	0.917
	F	236	1.49 (0.96)	0	3.5			
	M/F	469	1.50 (0.96)	0	3.5			

Results are expressed as mean (x) ±Standard Deviation (S.D.) for the number of subjects shown in the column labeled N; M: For male; F: Female; M/F: Combined male and female values; Rr: Reference range; p<0.05 is considered statistically significant by 2-tailed t-test; S.D.: Standard deviation

Table 2: TPSA means comparison for the four male age group categories

Age groups (I)	Age groups (J)	Mean difference (I-J)	S.E.	Sig.	95% CI Lower	95% CI Upper
18-28	29-39	-0.5756 (*)	0.1312	0.001	-0.9144	-0.2368
	40-50	-1.5640 (*)	0.1329	0.028	-1.9047	-1.2182
	50-60	-2.9471 (*)	0.1256	0.000	-3.2712	-2.6229
29-39	40-50	-0.9858 (*)	0.1345	0.015	-1.3331	-0.6385
	50-60	-2.3715 (*)	0.1272	0.037	-2.6999	-2.0431
40-50	51-61	-1.3857 (*)	0.1289	0.004	-1.7187	-1.0527

*: The mean difference significance at 0.05 level; Sig.: Significance; CI: Confidence interval; S.E.: Standard error

Table 3: Established reference ranges for TPSA for the studied healthy adult male Kenyans

Age group (year)	N	x	x (S.D.)	x-1.96 S.D.	x+1.96 S.D.	Rr (ng/mL)
18-28	85	0.73	0.73 (0.33)	0.1	1.4	0.1-1.4
29-39	81	1.30	1.30 (0.64)[a]	0.1	2.6	0.1-2.6
40-50	77	2.30	2.30 (0.61)[bd]	1.1	3.5	1.1-3.5
51-61	97	3.70	3.70 (1.33)[cef]	1.1	6.3	1.1-6.3

Results are expressed as mean (x) ±Standard Deviation (S.D.) for the number of subjects shown in the column labeled N; Rr: Reference range; [a]: $p<0.05$ indicates significance differences between age category 18-28 and 29-39 years; [b]: $p<0.05$ indicates significance differences between age category 18-28 and 40-50 years; [c]: $p<0.05$ indicates significance differences between age category 18-28 and 51-61 years; [d]: $p<0.05$ indicates significant differences between age category 29-39 and 40-50 years; [e]: $p<0.05$ indicates significant differences between age category 29-39 and 51-61 years; [f]: $p<0.05$ indicates significant differences between age category 40-50 and 51-61 years; S.D.: Standard deviation

Table 4: Comparison of established Kenyan adult reference ranges for tumour makers with some reference ranges quoted in literature

Parameter (units)	Gender	Kenyan	French	German
CA125 (U/mL)	M/F	0-23	5-35	0-35
CA15.3 (U/mL)	M/F	0-20	0-30	0-22
CA19.9 (U/mL)	M/F	0-16	0-27	<37
CEA (ng/mL)	M/F	0-3.5	0-5	<4.6
TPSA (ng/mL)	M (18-28 year)	0.1-1.4	0.2-1.7	0-1.3
	M (29-39 year)	0.1-2.6	-	-
	M (40-50 year)	1.1-3.5	0.3-2.2	0-2
	M (51-61 year)	1.1-6.3	0.3-3.4	0-3

French (Vidas 30418, 2004) German (Heil *et al*., 2002)

DISCUSSION

This study established reference ranges for CA 125, CA 15-3, CA 19-9, CEA and TPSA. A common reference range for each parameter was established since male and female had no statistical difference ($p>0.05$). In the diagnosis and management of various cancer related pathological disorders, upper reference range limit of specific tumour marker is a major tool for interpretation. Lower reference range limit of any tumour marker is of no clinical significance but a sign of good health. It is worth to mention that clinical chemistry quantitative laboratory results alone cannot make a conclusive cancer diagnosis. Other tools such as histological tissue examinations and radiological procedures are used in conjunction with the clinical chemistry quantitative laboratory results. Currently there are more than 20 different tumor markers that have been characterized and are in clinical use. Some are associated with only one type of cancer, whereas others are associated with two or more cancer types. There is no "universal" tumor marker that can detect any type of cancer. Current study established reference ranges for CA 125 and CA 153 both male and female even for those tumour markers whose principal utility is in diagnosing and monitoring therapy in female patients with specific type of cancer e.g., while CA-125 is best known as a marker for ovarian cancer, it may also be elevated in other cancers such as endometrial, fallopian tube, lung, breast and gastrointestinal (Bast *et al*., 1998). Levels of tumuor marker CA153 can also be higher in other cancers, like lung, colon, pancreas and ovarian and in some non-cancerous conditions, like benign breast conditions, ovarian disease, endometriosis and hepatitis. The greatest challenge in dealing with cancer is failure of diagnosing at an early stage, resulting with majority of patients with advanced stage of carcinoma dying of the disease. Studies on

cancer have been geared into improving diagnostic tools for early cancer detection. Population based reference range is one of these diagnostic tools used for early cancer detection.

Since cancer of the ovary is female oriented, current study established reference ranges of CA125 for adult female Kenyan population as 0-23 U/mL. An adult Kenyan female being investigated for ovarian cancer would be diagnosed at CA 125 levels above 23 U/mL. CA 125 upper reference range limit of the Kenyan adult population is lower than that of the French and German populations which is 35 U/mL for both populations. Incidentally, French and Germans CA 125 tumour maker reference ranges quoted in diagnostic kits are the ones used for diagnosis and management of ovarian cancer in the Kenyan health institutions. Consequently, the female Kenyan population has always been over diagnosed by the use of reference range for CA 125 quoted in the diagnostic kits. The findings of this study will revolutionize the interpretation of CA 125 laboratory report by making it independent from literature based reference ranges.

CA 15-3 is another female oriented tumour maker whose principal utility is in monitoring therapy; used in the management of patients with breast cancer. Current study established reference range for CA15-3 as 0-20 U/mL. An adult Kenyan female being investigated for breast cancer would be diagnosed at CA 15-3 levels above 20 U/mL. CA 15-3 upper reference range limit of the Kenyan adult population is lower than that of the French and German populations which are 30 and 22 U/mL, respectively. The use of literature based CA 15-3 reference ranges quoted in diagnostic kits from Germany and America under diagnoses the Kenyan female population being investigated for breast cancer. The established CA 153 reference range for the adult female Kenyan will revolutionize the diagnosis and management of breast cancer in Kenya. This Kenyan

based CA 153 reference range would be an asset for future research work on breast cancer in Kenya.

In the diagnosis, treatment and management of cancer of the pancrease, CA 19-9 is the best parameter. A lot of information in literature on pancreatic cancer is based on the Americans, Germans and the Britons, since these populations use their own diagnostic and management tools based on their healthy populations. Developing countries, Kenya inclusive have very scarce information on pancreatic cancer. For example, Kenyan health institution use CA 19-9 literature quoted reference ranges for Germans; 0-37 U/mL and French; 0-27 U/mL, to diagnose and manage pancreatic cancer in the adult Kenyan population. Current study established adult Kenyan reference range for CA 19-9 as 0-16 U/mL. The upper reference range limit; 27 U/mL for the French adult population is above that established in this study. Considering the fact that CA 19-9 reference range for the French population has always been used to make diagnosis and manage pancreatic cancer in the adult Kenyan population, it can therefore be concluded that there has been under diagnosis of the Kenyan individuals. On the other hand, pancreatic cancer has always been under diagnosed whenever German based CA19-9 reference range upper limit; 37 U/mL have been used for interpretation of a laboratory report of a Kenyan individual. It is evident from the findings of this study that reference ranges are affected by the geographical locality of a population as shown by reference ranges of Kenyans, Germans and French populations. With the establishment of Kenyan based reference range of CA 19-9, proper diagnosis and management of pancreatic cancer would hence forth be achieved and any further dependency of literature based reference range will gradually cease.

Colorectal Cancer (CRC) is the third most common cancer worldwide. This cancer is diagnosed in clinical laboratory by the estimation of CEA (Strate and Syngal, 2005). Current study established the Kenyan adult population reference range of CEA as (0-3.5) ng/mL. The upper CEA reference range limit; 3.5 ng/mL established in the current study is lower than those from Germany and France. French adult population reference range of CEA is (0-5) ng/mL. A Kenyan individual would be under diagnosed for colorectal cancer in an event the French reference range is used for interpretation of laboratory report for a Kenyan individual. A similar situation of under diagnosis would be witnessed in an event the German reference range of CEA (0-4.6 ng/mL) is used to interpret CEA report of a Kenyan individual. These three different reference ranges for Kenyans, Germans and French adult populations is a clear indication of the effect geographical location have on reference ranges. It is therefore appropriate that every individual report should be interpreted using relevant CEA reference range, in order to avoid any misdiagnosis of colorectal cancer.

Prostate cancer is the most common tumor in men across the world. Prostate cancer tends to develop in men over the age of fifty and although it is one of the most prevalent types of cancer in men, many never have symptoms, undergo no therapy and eventually die of other causes. The current study developed reference ranges of TPSA based on the age group categories of adult male population. It is evident from the current study findings that TPSA concentration increases with age (Hsing and Chokkalingam, 2006). This is shown by the increase in mean TPSA concentration of each specific age group. The mean TPSA concentrations were as follows:- 18-28 years (0.73 ng/mL), 29-39 years (1.3 ng/mL), 40-50 years (2.3 ng/mL) and 51-61 years (3.7 ng/mL). This finding concurs with the findings of other studies carried out in other populations. Reference range interval of TPSA for age groups 18-39, 40-50 and 51-61 years for the French male population were 1.5, 1.9 and 3.1 ng/mL, respectively. On the other hand, German male population reference range interval in age groups 18-39, 40-50 and 51-61 years were 1.3, 2 and 3 ng/mL, respectively.

There are two differences observed between the Kenyan population and the other compared populations. First, upper reference range limit which is used to diagnose prostrate cancer, the levels of the Kenyan population are higher than those of the other compared populations as shown in Table 4. Secondly, the current study developed separate TPSA reference range for age groups 18-28 and 29-39 years which were (0.1-1.4) ng/mL and (0.1-2.6) ng/mL, respectively. The French and Germans populations' reference range for the two age groups were 0.2-1.7 and 0-1.3 ng/mL, respectively as shown in Table 4 (Heil *el al*., 2002). Since the diagnostic TPSA upper reference range limits for the Kenyan population is higher than the French and German populations, it therefore implies that the Kenyan male adult population would be over diagnosed by the use of French and German reference ranges for interpretation of Kenyans TPSA results.

CONCLUSION

In conclusion, this is the first study carried out in Kenya to establish tumour maker reference ranges that will make the diagnosis of cancer independent of literature quoted reference ranges. Consequently, the findings of this study will be used to determine risk, screen for early cancer, estimate prognosis, predict that a specific therapy will work, or monitor for disease recurrence or progression. Total prostatic specific antigen which is the male oriented tumour maker, showed an increasing trend as male advances in age. Interpretation of total prostatic specific antigen report for the diagnosis and management of prostate cancer would require the provision of the individual's age.

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