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CLSI Guided Reference Interval Limits for Cancer Biomarkers for Adults and Geriatrics

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Abstract

Reference interval limits for cancer biomarkers in geriatrics are rare because priority is given to the development of reference interval limits for those in the age range of 18–60 years, which are normally used for clinical trials study. The aim of this study was therefore to develop gender and age-specific reference interval limits for cancer markers CA19-9, CEA, CA 15-3, CA 125, and PSA for adults and geriatrics in Taita-Taveta County, Kenya, using the CLSI CA28-A3 guideline. This prospective cross-sectional study involved 244 healthy referents, including 124 females and 120 males of ages 50–95, between May 2015 and December 2017 at the Department of Clinical Chemistry of Moi Subcounty Hospital, Voi, Kenya. Serum CA 19-9, CEA, CA 15-3, CA 125, and PSA of the 244 referents were measured using a well-calibrated, quality controlled Clinical Chemistry AutoAnalyzer. Gender differences in the measured values of the biomarkers were assessed using the Mann-Whitney U test, while age differences were assessed using the Kruskal-Wallis H test followed by the Mann-Whitney U test with an adjusted significant ρ-value of less than 0.0167. Reference interval limits for the measured cancer biomarkers were expressed in terms of medians and ranged between 2.5 and 97.5 percentiles. The established 95% reference interval limits were: 0-58 U/mL males and 0-42.8 U/mL females for CA 19–9, 0–7 ng/mL for CEA, 0-56.9 U/mL for CA 15–3, 0–25 ng/mL for CA 125, and 0–6.8 ng/mL for PSA. Gender-related biomarker values were developed for CA 19-9 adults and geriatrics (60–70 years), CEA for geriatrics (60–70 years), and CA 15-3 for adults. Age-related biomarker values were developed for CA 19–9 males and not for females. In conclusion, gender-related 95% reference interval limits were developed for CA 19-9, CEA, CA 15-3, CA 125, and PSA, and age-related 95% reference interval limits were established for CA 19-9. CA 19-9 decreased from adulthood to the early elderly and increased in the more elderly population. These developed reference interval limits for these biomarkers, which differed from those reported in previous literature, could be adopted for use in Taita-Taveta County, Kenya, for better medical care.

Keywords: Geriatric; Cancer Markers; Reference Intervals; Taita Taveta; Kenya.

1. Introduction

Reference intervals for cancer biomarker analytes are developed using a minimum of 120 referent individuals selected using specific inclusion and exclusion criteria and include the 2.5 percentile and 97.5 percentile of the measured analyte level data. The measured analyte levels are assessed for normality and non-normality to define whether the data is either expressed descriptively as median and range or mean and standard deviation. The normality or non-normality

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of the level of analytes determines whether the level of analytes is compared using parametric or non-parametric statistics. The measured analyte reference intervals are also stratified by age and gender where necessary. The reference intervals for the selected tumor biomarkers were performed using CLSI EP28-A3c guidelines, which recommend the use of nonparametric statistical approaches on the medians of the referent data regardless of the data distribution [1].

Reference intervals for tumor biomarkers such as carbohydrate antigen 15-3 (CA 15-3, breast cancer), carbohydrate antigen 125 (CA 125, ovarian cancer), carbohydrate antigen 19-9 (CA 19-9, pancreatic cancer), carcinoembryonic antigen (CEA, cervical cancer), and prostate-specific antigen (PSA, prostate cancer) are commonly used for diagnosing tumors, initiating treatment, and monitoring post-treatment outcomes. CA19-9 is also a screening tool for colorectal cancer. It can also be used to diagnose ovarian cysts, ovarian serous carcinoma, and mucinous adenocarcinomas. CEA is a diagnostic tool for digestive tract cancer, lung cancer, and some ovarian cancers. CA125 is a screening tool for malignant pelvic cancers, ovarian cysts, uterine fibroids, endometriosis, and other gynecological diseases [2]. Reference intervals for CA 19-9, CEA, CA 15-3, CA 125, and PSA are affected by many factors, including age (infants, children, adolescents, adults, geriatrics), sex (male, female), physical activity (sedentary or active), life-style (smoker or non-smoker, alcoholic or non-alcoholic), dietary habits such as taking carcinogen foods, environment (urban, peri-urban, or rural, contaminants, radiation, infections), geographical location (longitude and latitude, altitude, relative location), method and reagent used for detection, socioeconomic status (education, income, occupation, wealth, deprivation), and inclusion criteria used in the selection of the referent individual, among others. Despite the fact that there cannot be universal reference interval limits for biological parameters for all populations of the world, most African countries, including Kenya, use reference intervals for cancer biomarkers developed in Western countries (Europe and North America) for populations with technologies that have changed and populations whose diets and lifestyles have equally changed. These cancer biomarker reference interval limits, which are reported in medical literature (medical books, refereed articles in medical journals, and manufacturers of diagnostic test inserts and reagent kits), are inappropriate for use by Kenyan hospitals and laboratories without validation and verification of their accuracy. Thus, the International Federation of Clinical Chemistry and Laboratory Medicine and the Clinical and Laboratory Standards Institute (CLSI) recommend that each clinical laboratory develop its own reference intervals for tumor markers using the local population [1].

The locally developed cancer biomarker reference interval limits will improve the diagnostic accuracy of defining the histopathological classification and stage of disease; prognostically predict the development of disease and the prospect of recovery. There are also few reference interval limits for cancer biomarkers for geriatrics in Kenya, even though it is known that some of them change with age. Further, laboratory medicine reports for cancer biomarkers for geriatrics in Kenya are commonly referenced against the adult reference interval limits, which could lead to misdiagnosis, overuse of irrelevant medicines in managing incorrectly diagnosed diseases, and subjecting patients to unnecessary medical procedures for diseases they are not suffering from. The aim of this study therefore was to establish age and sex-specific 95% (double-sided) reference interval limits for the five selected tumor biomarkers for adults and geriatrics in Taita-Taveta County, Kenya, using the CLSI EP28-A3 guideline [1]. In addition, the established reference interval limits for tumor biomarkers were compared with the previously reported cancer biomarkers in the medical literature of similar populations from other parts of the world.

2. Materials and Methods

2.1. Study Site

This was carried out at the Department of Clinical Chemistry, Moi Subcounty Hospital, Voi, Kenya, from May 2015 to December 2017.

2.2. Study Design

This was a cross-sectional study design involving 244 referents including 124 females and 120 males of age 50-95 years.

2.3. Study Participants

The 244 study participants were recruited from Taita-Taveta County, Kenya, by the researcher, a nurse, and a clinical officer. The socioeconomic status and healthy history of each of the participants were obtained by use of a questionnaire. The importance and benefits of the study were explained to the participants by the researcher, who was then physically and clinically examined by the nurse and the clinical officer attached to the study. Those who accepted to participate were enrolled in the study, and those who had clinical conditions that could not allow them to participate in the study were referred for further clinical investigation and medical care. Potential participants who were negative for hepatitis B virus surface antigens, syphilis, and HIV/AIDS were enrolled in the study. Potential participants with any form of cancer were not recruited for the study. Participants outside the age 50-95 years and those who refused to participate were excluded from the study. Those with any other illnesses that could affect the level of the studied biomarkers were also excluded from the study.

2.4. Blood Collection and Laboratory Analysis

Two hundred and forty-four serum specimens were generated from the five millitres blood samples collected from each of the referent individuals. The levels of each of the five measured cancer biomarkers in the 244 serum specimens were analyzed using a well-calibrated, quality-controlled Chemwell Auto-Analyzer machine whose working principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (enzyme-linked fluorescent assay (ELFA)).

2.5. Blood Screening

Screening for Human Immunodeficiency Virus (HIV): An immunochromatographic reagent strip (Determine HIV-1/2, Tokyo, Japan) was used for screening of HIV 1 and 2. A sample of 50 µL was applied to the sample pad. After 1 minute, chase buffer was applied to the sample pad, and the test results were read within 15 minutes. Positive results were indicated by the appearance of two red bars on the control window and on the patient window. Negative results were indicated by the appearance of only one red bar at the control window.

2.6. Screening for Venereal Disease Research Laboratory (VDRL)

The syphilis ultra-rapid test, which is a qualitative membrane strip-based immunoassay (Treponema pallidum Strip, Beijing, China), was used for the screening of Treponema pallidum, which is the causative agent of the venereal disease, syphilis. A sample of 50 µl was placed on the sample pad, followed by 1 drop of buffer. The result was read after 10 minutes. A positive result was indicated by the appearance of two red lines, one on the control region and the other on the test region. A negative result was indicated by the appearance of one red line in the control region.

2.7. Screening for Hepatitis B Surface Antigen (HBs-Ag)

The HBs-Ag one-step hepatitis B Surface Antigen Test Strip (HBs-Ag, Beijing, China) was used for the screening of HBs-Ag. This was a qualitative lateral flow immunoassay test. The test strip was immersed in a tube containing the serum for screening for 10 to 15 minutes. It was then removed and placed on a non-absorbent flat surface, and the results were read within 15 minutes. A positive result was indicated by the appearance of two distinct red bars, one on the control region and the other on the test region. Negative results were indicated by the appearance of only one red bar at the control window.

2.8. Ethical Approval

The ethical approval to carry out this study was given by Kenyatta University Ethical Review Committee (KU-ERC) Ref Number I84/31987/15/ PKU/ 22096/1661.

2.9. Data Management and Statistical Analysis

Results generated in the cancer biomarkers study were initially recorded in the laboratory notebook before being entered into the Excel spreadsheet for cleaning. The clean data was exported to the SPSS software for descriptive statistics analysis. The descriptive statistics output included mean, variance, standard deviation, median, mode, skew, standard error of skewness (SES), kurtosis, standard error of kurtosis (SEK), range, minimum, maximum, 2.5, and 97.5 percentiles. These outputs were used to assess the normality of the cancer biomarker data. They were also used to define the mean ± standard deviation (SD), median, and 2.5 and 97.5 percentiles, the descriptive statistics used in the results tables. Differences between male and female values of each of the measured biomarkers were assessed using the Mann-Whitney U test, with a p-value of less than 0.05 being considered significant. A difference between each of the measured biomarkers within and between the three age groups was carried out using the Kruskal-Wallis H test followed by the Mann-Whitney U test with a significant ρ-value of 0.0167.

3. Results

The results for quality control material for cancer biomarkers are reported in Table 1. Results indicate that the quality control material for cancer biomarkers were within the expected range. This therefore implies that the analytical process was working normally and the results released during analysis on the referents cancer biomarkers are accurate and reliable.

Table 1. Results for quality control material for cancer biomarkers

Analyte (unit)	Assigned QC Report			Study QC Report		
	Mean	SD	CV (%)	Mean	SD	CV (%)
CA 19-9 (U/mL)	5.5	0.18	3.3	5.4	0.11	2.0
CA 15-3 (U/mL)	9.5	0.20	2.1	9.6	0.22	2.3
CEA (ng/mL)	4.5	0.14	3.1	4.6	0.13	2.8
CA 125 (U/mL)	15	0.61	4.1	14.7	0.63	4.2
PSA (ng/mL)	2.2	0.08	3.6	2.3	0.07	3.0

3.1. Results for Normality Statistics for the Measured Biomarkers for Cancers for Adults and Geriatrics of Taita-Taveta County, Kenya

Results for normality statistics for the measured biomarkers for cancer in adults and geriatrics in Taita-Taveta County, Kenya, are presented in Table 2. Results indicate that for carbohydrate antigen 19-9 (CA19-9), the data for the whole sample (50–95 years) and that of age category one (50–60 years) consisting of combined males and females and separate males and females is nonparametric based on the values of mean, median, mode, skewness, and kurtosis. Further, the CA19-9 data for age category two (60–70 years) is nonparametric for combined males and females and separate females based on the values of mean, median, mode, skewness, and kurtosis but parametric for separate males, while that for age category three (70–95 years) is nonparametric based on the value of skewness. Thus, a parametric subsample can be derived from a nonparametric sample.

Table 2. Results of the normality statistics of cancer markers of adult and geriatric population of Taita-Taveta County, Kenya

Statistics		**Cancer biomarkers**									
≥50-95 years	**Gender**	**N**	**CA 19-9**	**N**	**CA 15-3**	**N**	**CEA**	**N**	**CA 125**	**N**	**PSA**
	M&F	244	12.80	244	23.55	244	1.85				
Mean	F	124	15.50	124	23.86	121	1.59	126	15.39		
	M	120	10.00	120	23.23	123	2.11			139	4.60
	M&F	244	0.926	244	0.912	244	0.123				
SEM	F	124	1.486	124	1.246	121	0.145	126	1.701		
	M	120	1.036	120	1.340	123	0.196			139	0.988
	M&F	244	**11**	244	**22**	244	**2**				
Median	F	124	**11**	124	**23**	121	**1**	126	**9**		
	M	120	**9**	120	**22**	123	**2**			139	**1**
	M&F	244	**0**	244	**1**	244	2				
Mode	F	124	**0**	124	**22**	121	1	126	**0**		
	M	120	**0**	120	**1**	123	2			139	**0**
	M&F	244	14.466	244	14.249	244	1.924				
SD	F	124	16.546	124	13.874	121	1.600	126	19.089		
	M	120	11.346	120	14.678	123	2.172			139	11.652
	M&F	244	209.258	244	203.047	244	3.701				
Variance	F	124	273.878	124	192.493	121	2.561	126	364.400		
	M	120	128.723	120	215.453	123	4.718			139	135.764
	M&F	244	**1.973**	244	**1.165**	244	**3.217**				
Skewness	F	124	**1.796**	124	**1.986**	121	**2.017**	126	**2.174**		
	M	120	**1.811**	120	0.469	123	**3.530**			139	**3.395**
	M&F	244	0.156	244	0.156	244	0.156				
SES	F	124	0.217	124	0.217	121	0.220	126	0.216		
	M	120	0.221	120	0.221	123	0.218			139	0.205
	M&F	244	**5.832**	244	**3.874**	244	**21.092**				
Kurtosis	F	124	**4.522**	124	**8.510**	121	**6.815**	126	**6.879**		
	M	120	**5.412**	120	0.094	123	**22.896**			139	**10.486**
	M&F	244	0.310	244	0.310	244	0.310				
SEK	F	124	0.431	124	0.431	121	0.437	126	0.428		
	M	120	0.438	120	0.438	123	0.433			139	0.408
	M&F	244	87	244	97	244	18				
Range	F	124	87	124	97	121	10	126	115		
	M	120	66	120	61	123	18			139	51
	M&F	244	0	244	0	244	**0**				
Minimum	F	124	0	124	0	121	0	126	0		
	M	120	0	120	0	123	0			139	0

	M&F	244	87	244	97	244	18				
Maximum	F	124	87	124	97	121	10	126	115		
	M	120	66	120	61	123	18			139	51
Percentiles											
	M&F	244	0	244	0	244	0				
2.5	F	124	0	124	0	121	0	126	0		
	M	120	0	120	0	123	0			139	0
	M&F	244	**56.38**	244	**56.88**	244	**7**				
97.5	F	124	**58**	124	**65.50**	121	**6.9**	126	**25**		
	M	120	**42.77**	120	**56.98**	123	**7**			139	**6.84**
≥50-60 years	**Gender**	**N**	**CA 19-9**	**N**	**CA 15-3**	**N**	**CEA**	**N**	**CA 125**	**N**	**PSA**
	M&F	92	13.09	79	23.48	67	1.76				
Mean	F	45	16.44	38	26.71	34	1.79	38	14.53		
	M	47	9.87	41	20.49	33	1.73			27	4.44
	M&F	92	1.514	79	1.744	67	0.198				
SEM	F	45	2.492	38	2.544	34	0.326	38	3.849		
	M	47	1.652	41	2.326	33	0.227			27	1.989
	M&F	92	11	79	23	67	2				
Median	F	45	12	38	25.5	34	1.5	38	4.5		
	M	47	10	41	20	33	2			27	1
	M&F	91		79	23	67	0				
Mode	F	45	0	38	26	34	1	38	0		
	M	47	0	41	1	33	3			27	0
	M&F	92	14.523	79	15.498	67	1.625				
SD	F	45	16.716	38	15.683	34	1.903	38	23.728		
	M	47	11.328	41	14.893	33	1.306			27	10.334
	M&F	92	210.915	79	240.202	67	2.639				
Variance	F	45	279.480	38	245.941	34	3.623	38	563.013		
	M	47	128.331	41	221.806	33	1.705			27	106.795
	M&F	92	**2.407**	79	**1.370**	67	**1.995**				
Skewness	F	45	**2.124**	38	**2.374**	34	**2.527**	38	**2.817**		
	M	47	**2.685**	41	0.463	33	0.004			27	**3.732**
	M&F	92	0.251	79	0.271	67	0.293				
SES	F	45	0.354	38	0.383	34	0.403	38	0.383		
	M	47	0.347	41	0.369	33	0.409			27	0.448
	M&F	92	**8.538**	79	**5.572**	67	**8.594**				
Kurtosis	F	45	**6.549**	38	**10.569**	34	**9.723**	38	**9.300**		
	M	47	**12.179**	41	-0.051	33	-1.320			27	**15.363**
	M&F	92	0.498	79	0.535	67	0.578				
SEK	F	45	0.695	38	0.750	34	0.788	38	0.750		
	M	47	0.681	41	0.724	33	0.798			27	0.872
	M&F	92	87	79	97	67	10				
Range	F	45	87	38	97	34	10	38	115		
	M	47	66	41	57	33	4			27	50
	M&F	92	0	79	0	67	0				
Minimum	F	45	0	38	0	34	0	38	0		
	M	47	0	41	0	33	0			27	0
	M&F	92	87	79	97	67	10				
Maximum	F	45	87	38	97	34	10	38	115		
	M	47	66	41	57	33	4			27	50

Percentiles											
	M&F	92	0	79	0	67	0				
2.5	F	45	0	38	0	34	0	38	0		
	M	47	0	41	0	33	0			27	0
	M&F	91	**63.08**	79	**57**	67	**5.8**				
97.5	F	45	**82.5**	38	**32.65**	34	**2.46**	38	**24.25**		
	M	47	**58**	41	**56.65**	33	**3**			27	**9.18**
≥60-70 years	**Gender**	**N**	**CA 19-9**	**N**	**CA 15-3**	**N**	**CEA**	**N**	**CA 125**	**N**	**PSA**
	M&F	92	10.42	96	24.80	104	2.05				
Mean	F	48	14.02	53	24.30	43	1.60	54	14.87		
	M	44	6.50	43	25.42	61	2.36			63	4.35
	M&F	92	1.387	96	1.428	104	0.219				
SEM	F	48	2.345	53	2.004	43	0.243	54	2.441		
	M	44	1.126	43	2.040	61	0.327			63	1.52
	M&F	92	11	96	23.50	104	2				
Median	F	48	11	53	23	43	1	54	11		
	M	44	1	43	23	61	2			63	0
	M&F	92	0	96	24	104	2				
Mode	F	48	0	53	24	43	0	54	0		
	M	44	0	43	1	61	2			63	0
	M&F	92	13.304	96	13.995	104	2.231				
SD	F	48	16.250	53	14.587	43	1.591	54	17.937		
	M	44	7.466	43	13.374	61	2.556			63	12.066
	M&F	92	176.994	96	195.866	104	4.978				
Variance	F	48	264.063	53	212.792	43	2.530	54	321.738		
	M	44	55.744	43	178.868	61	6.534			63	145.586
	M&F	92	**2.454**	96	**1.333**	104	**3.810**				
Skewness	F	48	**2.060**	53	**1.827**	43	**1.289**	54	**1.790**		
	M	44	0.646	43	0.162	61	**3.964**			63	**3.404**
	M&F	92	0.251	96	0.246	104	0.237				
SES	F	48	0.343	53	0.327	43	0.361	54	0.325		
	M	44	0.357	43	0.361	61	0.306			63	0.302
	M&F	92	**10.407**	96	**3.296**	104	**24.674**				
Kurtosis	F	48	**6.866**	53	**5.393**	43	1.941	54	**3.712**		
	M	44	-1.014	43	0.445	61	**23.099**			63	**10.441**
	M&F	92	0.498	96	0.488	104	0.469				
SEK	F	48	0.674	53	0.644	43	0.709	54	0.639		
	M	44	0.702	43	0.709	61	0.604			63	0.595
	M&F	92	86	96	83	104	18				
Range	F	48	86	53	83	43	7	54	83		
	M	44	24	43	55	61	18			63	51
	M&F	92	0	96	0	104	0				
Minimum	F	48	0	53	0	43	0	54	0		
	M	44	0	43	1	61	0			63	0
	M&F	92	86	96	83	104	18				
Maximum	F	48	86	53	83	43	7	54	83		
	M	44	24	43	56	61	18			63	51

Percentiles											
	M&F	92	0	96	1	104	0				
2.5	F	48	0	53	0.35	43	0	54	0		
	M	44	0	43	1	61	0			63	0
	M&F	92	**37.68**	96	**62.33**	104	**7**				
97.5	F	48	**75.2**	53	**77.40**	43	**6.8**	54	**20.5**		
	M	44	**23.75**	43	**55.9**	61	**11.95**			63	**7.84**
≥70-95 years	**Gender**	**N**	**CA 19-9**	**N**	**CA 15-3**	**N**	**CEA**	**N**	**CA 125**	**N**	**PSA**
	M&F	60	15.98	69	21.88	73	1.64				
Mean	F	31	16.42	33	19.88	29	2.00	34	17.18		
	M	29	15.52	36	23.72	44	1.41			49	5.00
	M&F	60	2.018	69	1.576	73	0.197				
SEM	F	31	3.077	33	1.590	29	0.384	34	2.588		
	M	29	2.625	36	2.632	44	0.204			49	1.715
	M&F	60	**12.50**	69	**21**	73	**1**				
Median	F	31	**11**	33	**21**	29	**2**	34	15		
	M	29	**15**	36	**21.5**	44	**1**			49	1
	M&F	60	**0**	69	**19**	73	**0**				
Mode	F	31	**0**	33	**22**	29	**0**	34	0		
	M	29	**0**	36	**1**	44	**1**			49	0
	M&F	60	15.631	69	13.092	73	1.686				
SD	F	31	17.134	33	9.134	29	2.070	34	15.091		
	M	29	14.136	36	15.794	44	1.352			49	12.007
	M&F	60	244.322	69	171.398	73	2.844				
Variance	F	31	293.585	33	83.422	29	4.286	34	227.725		
	M	29	199.830	36	249.463	44	1.829			49	144.167
	M&F	60	**1.045**	69	0.519	73	**1.674**				
Skewness	F	31	**1.172**	33	-0.980	29	**1.297**	34	0.506		
	M	29	0.793	36	0,517	44	**1.857**			49	**3.411**
	M&F	60	0.309	69	0.289	73	0.281				
SES	F	31	0.421	33	0.409	29	0.434	34	0.404		
	M	29	0.434	36	0.393	44	0.357			49	0.340
	M&F	60	0.513	69	**1.242**	73	**3.647**				
Kurtosis	F	31	0.611	33	0.770	29	1.797	34	-0.877		
	M	29	0.142	36	0.180	44	**5.777**			49	**10.830**
	M&F	60	0.608	69	0.570	73	0.555				
SEK	F	31	0.821	33	0.798	29	0.845	34	0.788		
	M	29	0.845	36	0.768	44	0.702			49	0.668
	M&F	60	58	69	61	73	8				
Range	F	31	58	33	36	29	8	34	51		
	M	29	52	36	61	44	7			49	51
	M&F	60	0	69	0	73	0				
Minimum	F	31	0	33	0	29	0	34	0		
	M	29	0	36	0	44	0			49	0
	M&F	60	58	69	61	73	8				
Maximum	F	31	58	33	36	29	8	34	51		
	M	29	52	36	61	44	7			49	51
Percentiles											
	M&F	60	0	69	0	73	0				
2.5	F	31	0	33	0	29	0	34	0		
	M	29	0	36	0	44	0			49	0
	M&F	60	**58**	69	**59.5**	73	**7.15**				
97.5	F	31	**24**	33	**26**	29	**3**	34	**31**		
	M	29	**26**	36	**33.75**	44	**6.63**			49	**8.97**

For carbohydrate antigen 15-3 (CA15-3), the data for the whole sample (50–95 years) and that for age categories one (50–60 years) and two (60–70 years) consisting of combined males and females and separate males and females is nonparametric based on the values of mean, median, mode, skewness, and kurtosis, but that for age category three is parametric. Thus, a parametric subsample can be derived from a nonparametric sample.

For carcinoembryonic antigen (CEA), the data for the whole sample (50–95 years), age category one (50–60 years) consisting of combined males and females and separate females, age category two (60–70 years) consisting of combined males and females and separate males, and age category three (70–95 years) consisting of combined males and females and separate males and females are non-parametric based on the values of mean, median, mode, skewness, and kurtosis.

For carbohydrate antigen 125 (CA 125), the data for the whole sample (50–95 years), age category one (50–60 years), and age category two (60–70 years) are nonparametric, but that of age category three (70–95 years) is parametric based on the values of mean, median, mode, skewness, and kurtosis. Thus, a parametric subsample can be derived from a nonparametric sample. For prostate-specific antigen (PSA), the data for the whole sample (50–95 years), age category one (50–60 years), age category two (60–70 years), and age category three (70–95 years) are nonparametric (Table 1.2). However, the Clinical Laboratory Standards Institute [1] recommends that reference interval limits be developed using non-parametric statistics descriptors such as median and range within the 2.5 percentile and 97.5 percentile, regardless of normality status.

3.2. Reference Interval Limits for Serum Cancer Biomarkers for Adults and Geriatrics of Taita-Taveta County, Kenya

The median reference interval limits for the five serum cancer biomarkers for adults and geriatrics in Taita-Taveta County, Kenya, are presented in Table 3. Results indicate that the reference intervals for CA 19–9 and CEA are significantly different between males and females, with females having higher and lower interval limits than males, respectively. Therefore, separate reference interval limits were established for these two cancer biomarkers. The established median reference interval limits for CA 19-9 for males are 9 (0-42.8) U/mL with a mean rank of 111.93, which is significantly lower than those for females of 11 (0-58) U/mL with mean rank of 132.73 (U = 6172, z = -2.324, $\rho = 0.020$, r = 0.1488), while that for CEA for males is 2 (0-7) ng/mL with mean rank of 112.5 is significantly lower than those for females of 1 (0-6.9) ng/mL with mean rank of 132.67 (U = 6211, z = -2.287, $\rho = 0.022$, r = 0.1464). The median reference interval limits for CA 15-3 for males are 22 (0–57) U/mL with a mean rank of 120.3, and for females they are 23 (0–65.5) U/mL with a mean rank of 124.63. They are statistically similar, and therefore combined reference interval limits were established. The established combined median reference interval limits for CA 15-3 for adults and geriatrics in Taita-Taveta County, Kenya, are 22 (0-56.9) U/mL (U = 7176, z = -0.479, $\rho = 0.632$, r = 0.0307). The established median reference interval limits for CA 125 for female adults and geriatrics in Taita-Taveta County, Kenya, are 9 (0–25) U/mL, while those for PSA are 1 (0–6.8) ng/mL.

Table 3. Reference interval limits for serum cancer biomarkers for adults and geriatrics of Taita-Taveta County, Kenya

Analyte (unit)	Sex	N	Median	Percentile		Reference Interval	IV	Difference between M&F	
				2.5th	97.5th			z-value	Sig.
CA-19-9 (U/mL)	M&F	244	12.80±14.47					-2.324	ρ = 0.020
			11	0	56.38	0-56.4	56.3		
	F	124	15.50±16.55						
			11	**0**	**58**	**0-58**	**58**		
	M	120	10.00±11.35						
			9	**0**	**42.77**	**0-42.8**	**42.8**		
CEA (ng/mL)	M&F	244	1.85±1.92					-2.287	ρ = 0.022
			2	0	7	0-7	7		
	F	121	1.59±1.60						
			1	**0**	**6.9**	**0-6.9**	**6.9**		
	M	123	2.11±2.17						
			2	**0**	**7**	**0-7**			
CA 15-3 (U/mL)	M&F	244	23.55±14.25					-0.479	ρ = 0.632
			22	**0**	**56.9**	**0-56.9**	**56.9**		
	F	124	23.86±15.50						
			23	0	65.5	0-65.5	65.5		
	M	120	23.23±14.68						
			22	0	56.98	0-57	57		
CA 125 (U/mL)	F	126	15.39±19.09						
			9	0	25	**0-25**	25		
PSA (ng/mL)	M	139	4.60±11.65						

Results are expressed as Mean ± standard deviation (SD), and Median and range for the number of referent participants in the column labeled N. Statistical comparisons of the median values between male and female referent participants were carried out using Mann-Whitney U test. Differences were considered significant at $\rho < 0.05$.

3.3. Effect of Age on the Reference Interval Limits for the Five Cancer Biomarkers for Adult and Geriatric Male and Female Population of Taita Taveta County, Kenya

The effect of age on the reference interval limits for cancer biomarkers CA 19-9, CEA, CA 15-3, CA 125, and PSA for adults and geriatrics in Taita Taveta County, Kenya, is reported in Table 1.4. Results for Kruskal-Wallis H test analysis indicates that the reference interval limits for cancer biomarkers, carbohydrate antigen 19-9 (CA 19-9) (χ^2 (2) = 5.744, ρ = 0.057 for combined males and females, and χ^2 (2) = 0.710, ρ = 0.701) for females), carcinoembryonic antigen (CEA) (χ^2 (2) = 2.270, ρ = 0.321 for combined males and females, χ^2 (2) = 0.727, ρ = 0.695 for males, and χ^2 (2) = 1.353, ρ = 0.508 for females), CA 15-3 (χ^2 (2) = 1.084, ρ = 0.582 for combined males and females, χ^2 (2) = 2.417, ρ = 0.325 for males, and χ^2 (2) = 4.952, ρ = 0.084 for females, carbohydrate antigen 125 (CA 125) (χ^2 (2) = 2.248, ρ = 0.299) and PSA (χ^2 (2) = 3.282, ρ = 0.194) for adults and geriatrics of Taita Taveta County, Kenya are not significantly affected by advancement in age ($\rho > 0.05$; Table 4). However, the reference interval limits for CA 19–9 for male adults and geriatrics in Taita-Taveta County, Kenya, are significantly affected by advancement in age (χ^2 (2) = 8.932, ρ = 0.011). Further pairwise comparisons using the Mann-Whitney U test with an adjusted significant ρ-value of 0.0167 indicate that the reference interval limits for CA 19–9 for male adults in their sixth decade (1 (0–23.75 U/mL) with a mean rank of 31.25 are significantly lower than those of their seventh decade onwards (15 (0–26) U/mL) with a mean rank of 45.72 (U = 3855, z = -2.900, ρ = 0.004, r = 0.3394). Reference interval limits for CA 19–9 for male adults and geriatrics in their fifth decade were similar to those of the sixth decade and the seventh decade onward.

Table 4. Effect of age on the reference interval limits for the five cancer biomarkers for adults and geriatrics of Taita Taveta County, Kenya

Analyte (Unit)	Gender	Changes in the concentration of cancer biomarker by age					
		N	≥50-60 years	N	≥60-70 years	N	≥70-95 years
CA 19-9 (U/mL)	M&F	92	13.09±14.52	92	10.42±13.30	60	15.98±15.63
			11 (0-63.08)		8 (0-37.68)		12.5 (0-58)
	M	47	9.87±11.33	44	6.50±7.47	29	15.52±14.14
			10 (0-63.08)*		**1 (0-23.75)***		**15 (0-26)^{bc}**
	F	45	16.44±16.72	48	14.02±16.25	31	16.42±17.13
			12 (0-82.5)		**11 (0-75.2)**		11 (0-24)
CEA (ng/mL)	M&F	67	1.76±1.63	104	2.05±2.23	73	1.64±1.69
			2 (0-5.8)		2 (0-7)		1 (0-7.15)
	M	33	1.73±1.31	61	2.36±2.56	29	2.00±2.07
			2 (0-3)		**2 (0-11.95)***		2 (0-3)
	F	34	1.79±1.90	43	1.60±1.59	44	1.41±1.35
			1.5 (0-2.46)		**1 (0-6.8)**		1 (0-6.63)
CA 15-3 (U/mL)	M&F	79	23.48±15.50	96	24.80±14.00	69	21.88±13.09
			23 (0-57)		23.5 (1-62.33)		21 (0-59.5)
	M	41	20.49±14.89	43	25.42±13.37	36	23.72±15.79
			20 (0-56.65)*		23 (1-55.90)		21.5(0-33.75)
	F	38	26.71±15.68	53	24.30±14.59	33	19.88±9.13
			25.5 (0-32.65)		24 (0.35-77.40)		21 (0-26)
CA 125 (U/mL)	F	38	14.53±23.73	54	14.87±17.94	34	17.18±15.09
			4.5 (0-24.25)		11 (0-20.5)		15 (0-31)
PSA (ng/mL)	M	27	4.44±10.33	63	4.35±12.07	49	5.00±12.01
			1 (0-9.18)		0 (0-7.84)		1 (0-8.97)

Results are expressed as Mean ± standard deviation (SD), and Median and range for the number of subjects indicated in the column labeled N. $^{*}\rho < 0.05$ when male reference interval limits are significantly different when compared to female reference interval limits for each age category, $^{a}\rho < 0.05$ when the reference interval limits for age range ≥ 50-60 years is significantly different when compared to the reference interval limits for age range ≥ 60-70 years, $^{b}\rho < 0.05$ when the reference interval limits for age range 50-60 years is significantly different when compared to the reference interval limits for age range ≥ 70-95 years, and $^{c}\rho < 0.05$ when the reference interval limits for age range ≥ 60-70 years is compared to the reference interval limit for age range ≥ 70-95 years.

However, an investigation on the effect of gender on the reference interval limits for biomarkers of cancer in specific age categories using the Mann-Whitney U test indicates that for CA 19–9, male adults in the fifth decade (10 (0–63.08) U/mL) with a mean rank of 40.85 are significantly lower than those of adult females (12 (0–82.5) U/mL) with a mean rank of 52.40 ($U = 792$, $z = -2.088$, $\rho = 0.037$, $r = 0.2177$), and geriatric males in the sixth decade (1 (0–23.75) U/mL) with a mean rank of 40.42 are significantly lower than those of geriatric females (11 (0–75.2) U/mL) with a mean rank of 52.07 ($U = 788.5$, $z = -2.134$, $\rho = 0.033$, $r = 0.2225$).

Further, for CEA male geriatrics, reference interval limits in their sixth decade (2 (0-11.95) ng/mL) with a mean rank of 57.33 are significantly higher than those of their female counterparts (1 (0-6.8) ng/mL) with a mean rank of 45.65 ($U = 1017$, $z = -1.987$, $\rho = 0.047$, $r = 0.1948$). In addition, for CA 15-3, adult male reference interval limits in their fifth decade (20 (0-56.65) U/mL) with a mean rank of 34.72 are significantly lower than those of their female counterparts (25.5 (0-32.65) U/mL) with a mean rank of 45.70 ($U = 562.5$, $z = -2.127$, $\rho = 0.033$, $r = 0.2393$).

3.4. Comparison of Developed Reference Interval Limits of the Five Selected Cancer Biomarkers for Adults and Geriatrics of Taita-Taveta County, Kenya with Those Reported in Medical Literature

A comparison of the developed reference intervals of the five selected cancer biomarkers for the adult and geriatric population of Taita-Taveta County, Kenya, with those reported in medical literature is presented in Table 1.5. Results indicate that the established reference interval limits for CA 19-9's lower limit for the male and female population of Taita-Taveta County, Kenya, are lower than those of the Caucasian population, while the upper reference interval limit is higher than that of the Caucasian population. Further, the established reference interval limits for the CEA lower limit for the male and female population of Taita-Taveta County, Kenya, are similar to those of the Scandinavian and Han populations, while the upper reference interval limit for the male and female population of Taita-Taveta County, Kenya, is higher than that of the Scandinavian population but similar to that of the Han population. Further, the established lower reference interval limit for CEA for the adult and geriatric male and female population of Taita-Taveta County, Kenya, was lower than that of the Shuyang population, but the upper reference interval limit was higher (Table 5).

Table 5. Comparison of developed reference interval limits of the five selected cancer biomarkers for adult and geriatric population of Taita-Taveta County, Kenya with those reported in medical literature

Analyte (Unit)	Sex	This study RI	Vestergaard et al. (1999) [3]	Bjerner et al. (2008) [4]	Qin et al. (2011) [5]	Zhang et al. (2016) [6].
CA-19-9 (U/mL)	M&F	0-56.4				
	F	**0-58***	5.2-30.2			
	M	**0-42.8**	3.6-23.0			
CEA (ng/mL)	M&F	0-7		0-4.12	0-6.94	
	F	**0-6.9***				0.43-4.26
	M	**0-7**				0.60-5.43
CA 15-3 (U/mL)	M&F	**0-56.9**				
	F					
	M					
CA 125 (U/mL)	F	0-25				
PSA (ng/mL)	M	0-6.8				

Caucasian population by Vestergaard et al. (1999) [3], Scandinavian population by Bjerner et al. (2008) [4], Han population by Qin et al. (2011) [5], Shuyang population by Zhang et al. (2016) [6]. CA 125 reference interval is 0-35 U/mL. The only tumor marker recommended for clinical use in the diagnosis and management of ovarian cancer.

3.5. Effect of Age on the Reference Intervals for PSA for Adult and Geriatric Male Population of Taita-Taveta County, Kenya with those Reported in Medical Literature

The effect of age on the reference intervals for PSA for the adult and geriatric male population of Taita-Taveta County, Kenya, compared with those reported in medical literature, is presented in Table 6. Results show that this study's lower reference interval limit for PSA for the adult and geriatric male population of Taita-Taveta County, Kenya, for all the compared age categories was similar to those of the European, Beijing, Caucasian, African-American, Japanese, Chinese, and Taiwanese populations, but lower than the lower reference interval limit reported for the Bangladesh population. Further, this study's upper reference interval limit for PSA for the adult and geriatric male population of Taita-Taveta County, Kenya, for all the compared age categories is higher than those reported for European, Beijing, Bangladeshi, Caucasian, African-American, Japanese, Chinese, and Taiwanese (Table 6).

Table 6. Effect of age on the reference intervals for PSA for adult and geriatric male population of Taita-Taveta County, Kenya with those reported in medical literature

	Population/Age	Changes in PSA concentration with age (years)			
		50-59	60-69	70-79	80-95
PSA (ng/mL)	The study RI		**0-6.8**		
	European population	0-2.27	0-3.46	0-4.26	
	Beijing	0-2.92	0.0-4.11	**0-5.59**	0-7.29
	Bangladeshi 1	2.1-3.7	2.6-3.4	**3.6-6.0**	**Up to 7.29**
	Bangladeshi 2	2.1-3.7	2.6-3.4	**3.6-6.0**	**0.9-9.5**
	Caucasians	0-3.5	0-4.5	**0-6.5**	
	African Americans	0-4.0	0-4.5	0-5.5	
	Japanese	0-3.65	0-4.06	0-5.09	0-5.66
	Chinese	0-2.35	0-3.20	0-3.39	0-3.39
	Taiwanese	0-3.31	0-5.05	0-5.73	
	Singapore	0-2.3	0-4.0	**0-6.3**	**0-6.6**
	Korean	0-2.5	0-3.9	0-5.8	
	Iranian	0-2.61	0-3.59	0-4.83	

African-American population by Cho et al. (2005) [7]; European population by Luboldt et al. (2007) [8]; Iranian population by Khezri et al. (2009) [9]; Chinese population by Qin et al. (2011) [5]; Taiwanese, Singapore and Korea by Park et al. (2012) [10]; Beijing population by Liu et al. (2013) [11]; Bangladeshi 1 and 2 by Rahman et al. (2014) [12].

4. Discussion, Conclusions and Recommendations

Results indicating that the CA 15-3 (breast cancer biomarker) reference intervals for the adult and geriatric male and female populations of Taita-Taveta County, Kenya, are not significantly different imply that this parameter is gender independent. The developed reference interval for CA 15-3 for the adult and geriatric male and female population of Taita-Taveta County, Kenya, for this parameter is 22.4 (0–56.9) U/mL. This developed upper reference interval limit of CA 15-3 in this study differs from the upper reference interval limit for this parameter reported in the literature of 30 U/mL; this difference could partly be due to the matrix effects of the detection method used. Slev et al. (2006) [13] obtained different reference intervals for CA 15-3 using seven different detection methods from serum samples from the same 120 referent individuals (Access 2': 97.5% upper reference limit of 23.3 U/mL; ADVIA Centaur: 97.5% upper reference limit of 30.8 U/mL; ARCHITECT i2000: 97.5% upper reference limit of 29.2 U/mL; AxSYM: 97.5% upper reference limit of 30.6 U/mL; Elecsys 2010: 97.5% upper reference limit of 41.2 U/mL; IMMULITE 2000: 97.5% upper reference limit of 42.3 U/mL; VITROS ECi: 97.5% upper reference limit of 51.7 U/mL). Hayes et al. (1989) [14] established an age- and gender-independent reference interval for CA 15-3 of 1.5–25.1 U/mL, as reported by Duffy (1999) [15]. The difference between the developed reference intervals for CA 15-3 for adult and geriatric males and females of Taita-Taveta County, Kenya, in this study and the previously developed reference interval limits by other researchers could also be explained by differences in race, ethnicity, lifestyle, and geographical location of the referent individual used.

Results indicating that the CA 19-9 (pancreatic cancer biomarker) and CEA (cervical cancer biomarker) reference intervals for adult and geriatric male and female populations of Taita-Taveta County, Kenya, were significantly different, with females having higher CA 19-9 levels than males and lower CEA levels than males, implies that these parameters are gender dependent. The developed age-independent but gender-dependent reference interval for adult and geriatric male and female populations of Taita-Taveta County, Kenya, for CA 19-9 in their fifth decade is 10 (0-63.08) U/mL for males and 12 (0-82.5) U/mL for females, and in their sixth decade is 1 (0-23.75) U/mL for males and 11 (0-75.2) U/mL for females, respectively, CEA in their sixth decade is 2 (0-11.95) ng/mL for males and 1 (0-6.8) ng/mL for females, and CA 15-3 in their fifth decade is 20 (0-56.65) ng/mL for males and 25.5 (0-32.65) ng/mL for females, respectively.

The higher levels of CA 19-9 observed in females relative to males in adults and geriatrics in Taita-Taveta County, Kenya, could be associated with sexual differentiation, which involves genetics and epigenetics in addition to acute sex hormone actions, as reported by Rubin et al. (2020) [16]. This same reason could also explain the higher levels of CA 15-3 and CEA in adult males and geriatrics, respectively, compared to females in Taita-Taveta County, Kenya [16]. The decrease in the levels of CA 19-9 with age for males and not females in this study is in contrast to its increase in the study reported by Zhang et al. (2018) [17] and could be related to the uterus and ovaries in females who are also releasing CA 15-3 and CA125 [17]).

The developed CA 19-9 reference interval in this study differs from the age-independent reference intervals reported by Vestergaard et al. (1999) [3] of 5.2 (upper limit of 30.2) U/mL for females and 3.6 (upper limit of 23.0) U/mL for males. This also differs from the reported age- and gender-independent literature reference interval limits for CA 19–9 of 35–37 U/mL. Bjerner et al. (2008) [4] also reported an age- and gender-independent upper limit reference interval for CA 19–9 of 28.3 U/mL. This difference between the developed reference interval limits for CA 19–9 for the Taita-Taveta population in this study from those previously reported by others (Vestergaard et al. (1999) [3]; Bjerner et al. (2008) [4]) could be attributed to the race, the geographical location of the referent individuals, and the detection method and reagents used.

La'ulu & Roberts (2007) [18] obtained different reference interval limits for CA 19-9 using five different detection methods from serum samples from the same 127 referent individuals (ADVIA Centaur: 9.7 (4.7-37.1) U/mL; ARCHITECT i2000: 4.4 (2.0-26.4) U/mL; IMMULITE 2000: 3.3 (2.5-17.0) U/mL; Elecsys E170: 6.9 (0.6-31.9) U/mL; UniCel Dxl 800: 7.0 (1.0-33.2) U/mL). The developed age-independent but gender-dependent reference interval for CEA in this study differs from the age-independent and gender-independent upper reference interval limit for CEA reported by Bjerner et al. (2008) [4] of 4.12 ng/mL for referent individuals aged 50–70 years. Qin et al. (2011) [5] also reported a reference interval upper limit of 6.94 ng/mL for CEA in Chinese males aged 45 to 70 years. Further, Zhang et al. (2016) [6] also reported gender-dependent reference interval limits for CEA of 1.88 (0.60–5.43) ng/mL for males and 1.44 (0.43–4.26) ng/mL for females of 50–90 years, with males having higher values than females. The difference between the developed reference interval for CEA in this study and those reported in medical literature previously by other researchers could be explained by the differences in race, lifestyle, and geographical location of the referent population and the differences in the detection methods and reagents used to assay this parameter.

The developed age-independent PSA (prostate cancer biomarker) reference interval limit for the adult and geriatric male population of Taita-Taveta County is 0–6.8 ng/mL, while the age-independent CA 125 reference interval limit for the female population of Taita-Taveta County is 0–25 U/mL. The age independence of the developed PSA reference interval limit for the male population for Taita-Taveta County could be due to a similar prostate gland volume and similar PSA density for ages 50–95. The prostate gland volume and prostate gland PSA density increase with age [9]. The age independence of the developed reference interval for PSA for the Taita-Taveta County male population differs from the findings of other researchers [8]. [upper limit: 2.27 ng/mL for 50–59 years, 3.46 ng/mL for 60–69 years, and 4.26 ng/mL for 70–79 years]; Liu et al. (2013) [11] [upper limit: 2.92 ng/mL for 50–59 years, 4.11 ng/mL for 60–69 years, 5.56 ng/mL for 70–79 years, 7.29 ng/mL for above 80 years]; Rahman et al. (2014) [12] [2.1–3.7 ng/mL for 50–59 years, 2.6–3.4 ng/mL for 60–69 years, 3.6–6.0 ng/mL for 70–79 years, and 0.9–9.5 ng/mL for 80–89 years]) who reported increasing PSA levels with advancing age. The age independence and upper limit difference of the present findings from the international age-dependent reference interval for PSA (3.5 ng/mL for 50–59 years, 4.5 ng/mL for 60–69 years, and 6.5 ng/mL for 70–79 years for Caucasians) could be explained by the genetic differences between the different races, the method and reagents used in its detection, and the geographical location of the referent individuals. Cho et al. (2005) [7] reported increasing PSA reference interval limits with advancing age from different races (African Americans [4.0 ng/mL for 50-59 years, 4.5 ng/mL for 60-69 years, 5.5 ng/mL for 70-79 years], Japanese [3.65 ng/mL for 50-59 years, 4.06 ng/mL for 60-69 years, 5.09 ng/mL for 70-79 years, 5.66 ng/mL for 80-89 years], Chinese [2.35 ng/mL for 50-59 years, 3.20 ng/mL for 60-69 years, 3.39 ng/mL for 70-79 years, 3.39 ng/mL for 80-89 years], Taiwanese [3.31 ng/mL for 50-59 years, 5.05 ng/mL for 60-69 years, 5.73 ng/mL for 70-79 years], Singaporeans [2.3 ng/mL for 50-59 years, 4.0 ng/mL for 60-69 years, 6.3 ng/mL for 70-79 years, 6.6 ng/mL for 80-89 years], and Koreans [2.5 ng/mL for 50-59 ng/mL, 3.9 ng/mL for 60-69 years, 5.8 ng/mL for 70-79 years]).

Khezri et al. (2009) [9] reported increasing PSA levels with advancing age for adult and geriatric Iranian men (2.61 ng/mL for 50–59 years, 3.59 ng/mL for 60–69 years, and 4.83 ng/mL for 70–79 years). The difference between the developed PSA reference intervals of this study and those reported in medical literature for people of different races could be explained by differences in changes in prostate gland volume and prostate gland PSA density. Khezri et al. (2009) [9] reported different prostate gland volume and prostate gland PSA density in USA whites, Japanese, and Arab males of similar age, respectively. The developed age independence of the CA 125 (ovarian cancer biomarker) reference interval for the female population of Taita-Taveta County agrees with the findings reported by Park et al. (2012) [10], who demonstrated an age independent reference interval of CA 125 of 27.8 [22.1-39.1] U/mL for Asian female populations of 50–65 years. The reported age-independent upper reference interval limit for CA 125 is 35 U/mL. Bjerner et al. (2008) [4] also reported an age-independent upper reference interval limit of CA 125 of 35.8 U/mL. The difference in this study's CA 125 reference interval from the previously reported reference intervals for this parameter could be attributed to the genetic differences between the races, lifestyles, and geographical locations of the referent individuals. Pauler et al. (2001) [19] demonstrated a race-dependent CA 125 reference interval in postmenopausal women: 9.0 (4.0-26.0) U/mL for African women, 13.0 (5.9-33.3) U/mL for Asian women, and 14.2 (6.0-41.0) U/mL for Caucasian women.

The limitations of this study are that these developed breast, ovarian, cervical, prostate, and pancreatic cancer biomarker reference intervals may not be suitable for the adult and geriatric healthy male and female populations of

other counties other than those of Taita-Taveta County, Kenya. Secondly, the other limitation was that the developed reference intervals for the five selected tumor markers based on age have sample sizes that are less than the 120 referent individuals recommended by the CLSI EP28-A3c guideline [1]. Thirdly, the healthy status of the referent individuals was based on self-reporting by the referent individuals, who were not confirmed medically. Fourthly, the life-style status of individuals such as smokers or non-smokers and alcoholics or non-alcoholics was also based on self-reporting by the referent individuals.

In conclusion, this study has developed age- and gender-specific 95% (double-sided) reference interval limits for breast, ovarian, cervical, prostate, and pancreatic cancer biomarkers that are different from those previously reported in medical literature using other communities from different parts of the world. These reference intervals can be adopted and used as cutoff points to allow more accurate diagnosis of various forms of cancer and rule out benign diseases for the Taita-Taveta County population. This confirms the need for each community in the world to develop their own reference interval limits for breast, ovarian, cervical, prostate, and pancreatic cancer biomarkers appropriate for use in the accurate diagnosis and management of various types of cancer.

5. Declarations

5.1. Author Contributions

Conceptualization, R.G. and E.N.; methodology, R.G.; software, R.G.; validation, S.W., J.G., and E.N.; formal analysis, R.G. and E.N.; investigation, R.G.; resources, R.G.; data curation, R.G.; writing—original draft preparation, R.G.; writing—review and editing, E.N., S.W., and J.G.; visualization, S.W.; supervision, E.N., S.W., and J.G.; project administration, R.G.; funding acquisition, R.G. All authors have read and agreed to the published version of the manuscript.

5.2. Data Availability Statement

The data presented in this study are available in the article.

5.3. Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

5.4. Institutional Review Board Statement

The ethical approval to carry out this study was given by Kenyatta University Ethical Review Committee (KU-ERC) Ref Number I84/31987/15/ PKU/ 22096/1661.

5.5. Informed Consent Statement

Not applicable.

5.6. Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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