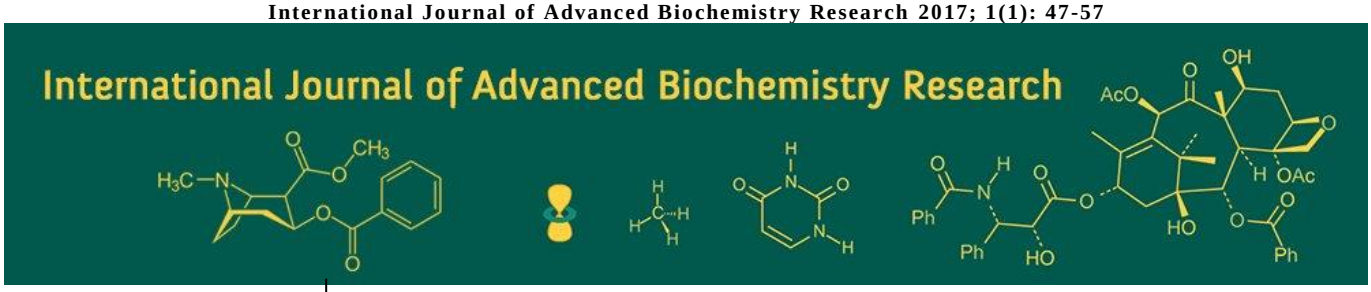

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Thyroid profile reference limits and normal values for adult and geriatric population of Taita-taveta County, Kenya

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Abstract
Reference interval limits for thyronine (TSH), thyroxine (T4) and triiodothyronine (T3) for geriatrics of the world including those of geriatrics of Taita-Taveta County, Kenya are limited. The aim of this study was to develop the 95% reference interval limits for thyronine (TSH), total thyroxine (T4), and total triiodothyronine (T3) for adults and geriatrics of Taita-Taveta County, Kenya. Two hundred and forty four referent individual randomly recruited from the four sub-county of Taita Taveta (Mwatate, Wundanyi, Voi and Taveta) County, Kenya participated in this reference interval limits development study. These referents had no history of thyroid gland diseases, were not on medication for thyroid diseases, and medications that affect the hypothalamus-pituitary-thyroid gland axis. These referents were free from HIV/ AIDS, syphilis, hepatitis B and C, and pregnancy. The serum used for the measurement of thyronin (TSH), total tetraiodothyronine (thyroxine [T4]) and triiodothyronine (T3) was obtained from blood which had been drawn from the vein of the 244 referent participants between 7-10 am after 8 to 12 hours of fasting recruited between May 2015 and December 2017. TSH, T4 and T3 were measured on a quality controlled calibrated Chemwell Auto-Analyzer machine using the principle that combines an enzyme immunoassay sandwich method with a final fluorescent detection (enzyme linked fluorescent assay (ELFA)) at the Clinical Chemistry Laboratory, Taita-Taveta Sub County Hospital, Voi, Kenya. The reference interval limits for the measured analytes was based on the median and 2.5 percentile and 97.5 percentile. The developed reference interval limits for TSH, T4 and T3 were 0-6 mU/L, 2-15 nmol/L and 0-4 nmol/L, respectively. There were significant age and sex related differences in T4. The developed reference interval limits for TSH, T4 and T3 differed from those reported in reagents manufacturer kits and literature. This study has established age and gender dependent 95% reference intervals for TSH, T4 and T3 that differ from those reported in literature. Each clinical laboratory therefore needs to develop their own reference intervals using their local healthy population for these analytes for accurate diagnosis and effective medical care for patients suffering from thyroid gland related diseases.

Keywords: tetraiodothyronine, Taita-Taveta, Wundanyi

Introduction
Clinical chemistry reference interval is the interval between, and including, two reference limits; the lower 2.5 percent and upper 97.5 percent limits. Reference intervals for thyroid hormones are developed using a minimum of 120 healthy referent individuals selected on the basis of meeting a specific exclusion and inclusion criteria and covering 95% of the healthy referent individuals. If the referent individuals are stratified by age and sex, each age category require a minimum of 120 individuals for each gender (Horowitz *et al*., 2010) [10]. Reference interval for thyroid stimulating hormone (TSH), total triiodothyronine (T3), free triiodothyronine (fT3), total thyroxine (T4), and free thyroxine (fT4) are biochemical markers that are commonly used for clinical diagnosis of thyroid function disorders. The thyroid function disorders include graves' disease, thyroid nodule (toxic autonomously functioning thyroid nodule or toxic multinodular goitre), thyroiditis, and excess iodine from some medicines which cause hyperthyroidism, and hashimoto's thyroiditis, iodine deficiency and non-functioning thyroid gland that cause hypothyroidism (Musa *et al*. 2018; Ali *et al*., 2018) [16, 1].

Thyroid stimulating hormone is the most sensitive biomarker used for assessment of thyroid function diseases in addition to diagnosing subclinical hyperthyroidism and hypothyroidism, and detecting recurrence of thyroid tumors. The factors that influence the reference interval for thyroid stimulating hormone (TSH) and thyroid hormones (T4, T3) include age, sex (pregnancy), dietary habits (eating goitrogenic foods containing goitrogenic chemicals), health status, some medications (Amiodarone, lugol's solution, some cough syrups), environment, race, ethnicity, genetics, laboratory methods and reagents used for detection, and iodine nutritional status (Musa *et al*. 2018) [16]. About 2.2 billion people from 130 countries of the world (including Kenya) are at risk of developing iodine deficiency disorders (IDD), despite the introduction of IDD programs such as the use of iodized salt formula in mid 1970s (Musa *et al*. 2018) [16]. The reference interval commonly used to assess thyroid function tests for geriatric populations in African countries, including Kenya are those for the adult western (mainly from Europe and northern America) population from commercial assay manufacturers reagents test kits even though it is known that age affects the levels of thyroid hormones. These reference interval limits are therefore inappropriate for geriatric populations of Kenya. This is despite the International Federation of Clinical Chemistry recommending that each clinical medicine laboratory develops its own local reference intervals for analytes using the local geriatric population for accurate clinical diagnosis, treatment, and monitoring of the performance of therapeutic treatment regimen for thyroid hormone diseases (Horowitz *et al*., 2010) [10]. Further, different countries of the world have previously reported different age and sex specific reference interval limits for thyroid hormones (Biondi and Cooper, 2008) [4] which are mostly different. There are no age and sex specific reference interval limits for geriatric population of Kenya including that for Taita-Taveta County. The aim of this study therefore was to develop age and sex specific 95% reference interval limits for thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya and compare them with those previously reported in medical literature for other populations of the world.

Materials and Methods

Study site

This study was carried out at Taita-Taveta Moi sub-district Hospital, Voi, Kenya between May 2015 and December 2017.

Study population

The study population involved 254 were recruited, of which 10 samples haemolysed, and 2 were HIV positive. The reminder of 244 voluntarily recruited healthy adults and geriatrics including 120 males and 124 females from Taita-Taveta County, Kenya were subjected to full anaylis.

Study design

This was a cross-sectional prospective study design involving 244 referent individuals of 50-95 years randomly recruited from the four sub-counties (Mwatate, Wundanyi, Voi and Taveta) of Taita Taveta County, Kenya. These referents had no history of thyroid gland diseases, were not on medication for thyroid diseases, and medications that affect the hypothalamus-pituitary-thyroid gland axis. These referents were free from HIV/ AIDS, syphilis, hepatitis B and C, and pregnancy.

Blood collection and sample preparation

The serum used for the measurement of thyronin (TSH), total tetraiodothyronine (thyroxine [T4]) and triiodothyronine (T3) was obtained from blood which had been drawn from the vein of the 244 referent participatants between 7-10 am after 8 to 12 hours of fasting recruited between May 2015 and December 2017. The 5 millitres of blood drawn from each referent individual was with a syringe was transferred to a labeled plain vacutainer tube and allowed to clot. The tubes containing clotted blood were placed in cooll boxed and transported to the Laboratory for analysis where the specimen were centriguged at 3000 g for five minutes. Serum was separated and transfered into bar coded vials for identification purposes. These vial specimen were either immediately analysed for the levels of TSH, T4, and T3 or stored at -20^{O}C to be analysed later. Analysis was carried out with a quality controlled calibrated Chemwell Auto-Analyzer machine whose working principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (enzyme linked fluorescent assay (ELFA)).

Ethical Approval

This study was approved by Kenyatta University Ethical Committee Ref Number I84/31987/15/ NACOSTI Ref number 16/22096/14531, and Taita-Taveta County medical director.

Laboratory Analysis

TSH, T4 and T3 in the serum samples and the quality control material were measured on a quality controlled calibrated Chemwell Auto-Analyzer machine using the principle that combines an enzyme immunoassay sandwich method with a final fluorescent detection (enzyme linked fluorescent assay (ELFA)) at the Department of Clinical Chemistry, Taita-Taveta Moi sub-district Hospital, Voi, Kenya. Reference interval limits for each of the measured analytes was calculated as median and 2.5 percentile and 97.5 percentile.

Data management and Statistical Analysis

Results from the 244 healthy referents were exported from the excel spreadsheet to SPSS software where the descriptive statistics mean, variance, standard deviation, standard error of mean, median, mode, skewness, standard error of skewness, kurtosis, standard eorror of kurtosis, minimum, maximum, range, and 2.5 percentile and 97.5 percentile were genenerated. These wre used to define the normality of the dataset. Results were expressed as median and 2.5 percentile and 97.5 percentile as recommended by CLSI (2010). Comparison of statistical differences between the values of each of the measured biochemical analytes for males and females was carried out using Mann-Whitney U test. Comparison of statistical differences within and between the values of each of the measured analytes for each gender for the three age categories (50-60, 60-70, 70-95 years) was carried out using Kruskal-Wallis H test followed by Mann-Whitney U test with an adjusted significant ρ-value of less than 0.0167. Results were presented as tables.

Results

Results for quality control material for thyroid hormones

The results of quality control material for thyroid hormones are presented in Table 1. It is evident that these results were within the assigned quality control values indicating that the analytical process was performing within expectations. Therefore, the measured thyroid stimulating hormone (TSH) and total thyroid hormone values of the study participants are accurate and reliable.

Table 1: Results for quality control material for thyroid hormones

Analyte (unit)	Assigned QC report			Study QC report		
	Mean	SD	% CV	Mean	SD	% CV
TSH (mU/L)	4.2`	0.12	2.9	4.3	0.07	1.6
T3 (mU/L)	2.9	0.08	2.6	2.9	0.08	2.3
T4 (nmol/L)	12.9	0.31	2.4	12.8	0.30	2.5

Results of the normality statistics of thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya

The results of the normality statistics of thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya are depicted in Table 1.2. For the whole sample of 244 combined male and female, separate 120 male and 124 female referents of 50-95 years, the degree of skewness of the measured analytes values is 77.78% (7/9), while the kurtosis level is 55.56% (5/9). For the age category 50-60 years, the skewness level of the measured analytes values for the combined 68 male and female, separate 32 male and 36 female referents is 100% (9/9) while the kurtosis level is 22.22% (2/9). The three measured analytes for referent males were normally distributed in addition to TSH and T4 of combined male and female, and separate female referents were normally distributed. For the age category 60-70 years, the level of skewness for the measured analytes values if 66.67% (6/9) while that for kurtosis is 55.56% (5/9). For age category 70-95 years, the level of kurtosis for the measured analytes values is 55.56% (5/9) while that for kurtosis is 44.44% (4/9).

Table 2: Results of the normality statistics of thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya

Parameter	**Gender**	**Normality statistics for thyroid hormones**			
≥50-95 years		**N**	**TSH (mU/L)**	**T4 (nmol/L)**	**T3 (nmol/L)**
Mean	M&F	244	2.16±1.72	5.91±3.64	1.73±0.87
	F	124	2.24±1.87	5.87±3.49	1.66±0.79
	M	120	2.08±1.54	5.94±3.84	1.81±0.96
SES	M&F	244	0.110	0.233	0.056
	F	124	0.168	0.313	0.070
	M	120	0.141	0.348	0.087
Median	M&F	244	2 (0-6)	4 (2-15)	2 (0-4)
	F	124	2 (0-6)	4 (3-15)	2 (0-3)
	M	120	2 (0-5.97)	4 (1-15.98)	2 (0-5.95)
Mode	M&F	244	2	4	2
	F	124	2	4	2
	M	120	2	4	2
SD	M&F	244	1.716	3.643	0.874
	F	124	1.871	3.485	0.785
	M	120	1.543	3.814	0.955
Variance	M&F	244	2.944	13.271	0.764
	F	124	3.502	12.146	0.616
	M	120	2.380	14.543	0.913
Skewness	M&F	244	1.162	1.355	1.516
	F	124	1.321	1.430	0.578
	M	120	0.780	1.300	1.983
SES	M&F	244	0.156	0.156	0.156
	F	124	0.217	0.217	0.217
	M	120	0.221	0.221	0.221
Kurtosis	M&F	244	3.813	0.894	7.356
	F	124	4.722	1.073	4.439
	M	120	1.032	0.778	8.046
SEK	M&F	244	0.310	0.310	0.310
	F	124	0.413	0.413	0.423
	M	120	0.438	0.438	0.438
Range	M&F	244	12	17	6
	F	124	12	15	5
	M	120	8	17	6
Minimum	M&F	244	0	0	0
	F	124	0	1	0
	M	120	0	0	0
Maximum	M&F	244	12	17	6
	F	124	12	16	5
	M	120	8	17	6
Percentiles					
2.5	M&F	244	0	2	0
	F	124	0	3	0
	M	120	0	1	0

97.5	M&F	244	6	15	4
	F	124	6	15	3
	M	120	5.97	15.98	5.95
≥50-60 years	Gender	N	TSH (mU/L)	T4 (nmol/L)	T3 (mU/L)
Mean	M&F	68	2.09±1.69	6.47±4.04	1.69±0.78
	F	36	2.25±1.84	6.06±4.09	1.67±0.93
	M	32	1.91±1.51	6.94±4.00	1.72±0.58
SEM	M&F	68	0.205	0.490	0.094
	F	36	0.307	0.682	0.154
	M	32	0.267	0.707	0.103
Median	M&F	68	2 (0-6.55)	4 (1.73-16.28)	2 (0-3.82)
	F	36	2 (0-3)	4 (1-9.5)	2 (0-2.03)
	M	32	2 (0-3)	5 (2-9.75)	2 (0-2.00)
Mode	M&F	68	2	4	2
	F	36	1	4	2
	M	32	0	4	2
SD	M&F	68	1.690	4.043	0.778
	F	36	1.842	4.091	0.926
	M	32	1.510	3.999	0.581
Variance	M&F	68	2.858	16.342	0.605
	F	36	3.393	16.740	0.857
	M	32	2.281	15.996	0.338
Skewness	M&F	68	0.832	1.084	2.176
	F	36	1.120	1.286	2.796
	M	32	0.109	0.959	-0.956
SES	M&F	68	0.291	0.291	0.291
	F	36	0.393	0.393	0.393
	M	32	0.414	0.414	0.414
Kurtosis	M&F	68	1.000	0.027	13.351
	F	36	1.430	0.596	13.499
	M	32	-1.061	-0.244	1.388
SEK	M&F	68	0.574	0.574	0.574
	F	36	0.768	0.768	0.768
	M	32	0.809	0.809	0.809
Range	M&F	68	8	16	6
	F	36	8	16	8
	M	32	5	14	3
Minimum	M&F	68	0	1	0
	F	36	0	1	0
	M	32	0	2	0
Maximum	M&F	68	8	17	6
	F	36	8	17	6
	M	32	5	16	3
Percentiles					
2.5	M&F	68	0	1.73	0
	F	36	0	1	0
	M	32	0	2	0
97.5	M&F	68	6.55	16.28	3.82
	F	36	3	9.50	2.03
	M	32	3	9.75	2.00
≥60-70 years	Gender	N	TSH (mU/L)	T4 (nmol/L)	T3 (mU/L)
Mean	M&F	103	2.17±1.82	5.83±3.60	1.75±0.79
	F	41	1.95±1.41	5.54±3.81	1.78±0.65
	M	62	2.32±2.05	6.02±3.47	1.73±0.87
SEM	M&F	103	0.180	0.355	0.078
	F	41	0.221	0.595	0.102
	M	62	0.260	0.441	0.111
Median	M&F	103	2 (0-6)	4 (1-15.40)	2 (0-4.40)
	F	41	2 (0-5)	4 (0.05-15.95)	2 (0.05-3.95)
	M	62	2 (0-8.55)	4 (2.15-15.43)	2 (0-5)
Mode	M&F	103	2	4	2
	F	41	2	4	2
	M	62	2	4	2
SD	M&F	103	1.823	3.599	0.789
	F	41	1.413	3.809	0.652
	M	62	2.047	3.471	0.872
Variance	M&F	103	3.322	12.950	0.622
	F	41	1.998	14.505	0.425

	M	62	4.189	12.049	0.760
Skewness	M&F	103	1.707	1.368	0.972
	F	41	0.258	1.570	0.250
	M	62	1.870	1.279	1.187
SES	M&F	103	0.238	0.238	0.238
	F	41	0.369	0.369	0.369
	M	62	0.304	0.304	0.304
Kurtosis	M&F	103	7.063	0.926	5.229
	F	41	-0.616	1.580	3.225
	M	62	6.972	0.686	5.392
SEK	M&F	103	0.472	0.472	0.472
	F	41	0.724	0.724	0.724
	M	62	0.599	0.599	0.599
Range	M&F	103	12	16	5
	F	41	5	16	4
	M	62	12	15	5
Minimum	M&F	103	0	0	0
	F	41	0	0	0
	M	62	0	1	0
Maximum	M&F	103	12	16	5
	F	41	5	16	4
	M	62	12	16	5
Percentiles					
2.5	M&F	103	0	1	0
	F	41	0	0.05	0.05
	M	62	0	2.15	0
97.5	M&F	103	6	15.40	4.40
	F	41	5	15.95	3.95
	M	62	8.55	15.43	5
≥70-95 years	Gender	N	TSH (mU/L)	T4 (nmol/L)	T3 (mU/L)
Mean	M&F	73	2.22±1.60	5.49±3.28	1.75±1.06
	F	30	2.43±1.85	4.43±2.37	1.47±0.78
	M	43	2.07±1.40	6.23±3.63	1.95±1.19
SEM	M&F	73	0.187	0.384	0.125
	F	30	0.338	0.435	0.142
	M	43	0.214	0.554	0.182
Median	M&F	73	2 (0-6)	4 (1.85-15.30)	2 (0-6)
	F	30	2.5 (0-4)	4 (3-5.4)	2 (0-2.2)
	M	43	2 (0-5.90)	4 (1.10-16.80)	2 (0-6)
Mode	M&F	73	2	4	2
	F	30	0	4	2
	M	43	2	4	2
SD	M&F	73	1.601	3.279	1.064
	F	30	1.851	2.373	0.776
	M	43	1.404	3.631	1.194
Variance	M&F	73	2.562	10.753	1.133
	F	30	3.426	5.633	0.602
	M	43	1.971	13.183	1.426
Skewness	M&F	73	0.424	1.690	1.509
	F	30	0.253	3.405	-0.593
	M	43	0.466	1.174	1.676
SES	M&F	73	0.281	0.281	0.281
	F	30	0.427	0.427	0.427
	M	43	0.361	0.361	0.361
Kurtosis	M&F	73	-0.306	2.491	5.784
	F	30	-0.906	13.724	-0.325
	M	43	0.380	0.864	4.989
SEK	M&F	73	0.555	0.555	0.555
	F	30	0.833	0.833	0.833
	M	43	0.709	0.709	0.709
Range	M&F	73	6	16	6
	F	30	6	12	3
	M	43	6	16	6
Minimum	M&F	73	0	1	0
	F	30	0	3	0
	M	43	0	1	0
Maximum	M&F	73	6	17	6
	F	30	6	15	3

	M	43	6	17	6
Percentiles					
2.5	M&F	73	0	1.85	0
	F	30	0	3	0
	M	43	0	1.10	0
97.5	M&F	73	6	15.30	6
	F	30	4	5.4	2.2
	M	43	5.90	16.80	6

Reference interval limits for thyroid hormones for adults and geriatrics of Taita-Taveta County, Kenya

The established reference intervals for thyroid stimulating hormone (TSH), total triiodothyronine (T3), and total thyroxine (T4) for male population of Taita-Taveta County, Kenya was statistically similar to that of the female population of the same age range ($\rho > 0.05$). Therefore combined reference interval limits of these parameters for this population were established. The established reference interval limits for thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya is 2 (0-6) mU/L for TSH, 2 (0-4) mU/L for T3, and 4 (2-15) nmol/L for T4 (Table 1.3).

Table 3: Reference interval limits for thyroid hormones 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
TSH (mU/L)	M&F	244	2.16±1.72	0	6	0-6	6	-0.519	ρ = 0.604
	M	120	2.08±1.54	0	5.97	0-5.97	5.97		
	F	124	2.24±1.87	0	6	0-6	6		
T3 (mU/L)	M&F	244	1.73±0.87	0	4	0-4	4	-0.174	ρ = 0.862
	M	120	1.81±0.96	0	5.95	0-5.95	5.95		
	F	124	1.66±0.79	0	3	0-3	3		
T4 (nmol/L)	M&F	244	5.19±3.64	2	15	2-15	13	-0.835	ρ = 0.403
	M	120	5.94±3.84	1	15.98	1-15.98	14.98		
	F	124	5.87±3.49	3	15	3-15	12		

Results are expressed as Mean ± standard deviation (SD) and Median and 95% 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 $\rho < 0.05$

Effects of age on the reference interval for thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya

The effects of age on the reference interval limits for thyroid hormone for adult and geriatric population of Taita-Taveta County, Kenya are presented in Table 2. This effect was investigated by dividing the study population into three age categories as follows: (a) age category 1 (≥ 50-60 years), (b) age category 2 (≥ 60-70 years), (c) age category 3 (≥ 70-95 years). Reference interval limits differences between males and females were estimated within each age category using Mann-Whitney U test. Reference interval limits differences within and between the three age categories were carried out using Kruskal-Wallis H test followed by Mann-Whitney U test with Bonferroni correction where ρ-values less than 0.0167 were considered statistically significant.

Results indicate that using Kruskal-Wallis H test, the established reference interval limits for thyroid stimulating hormone (TSH) ($\chi^2(2) = 0.390$, $\rho = 0.823$), total thyroxine ($\chi^2(2) = 1.955$, $\rho = 0.376$) and total triiodothyronine (T3) ($\chi^2(2) = 449$, $\rho = 0.799$) for combined male and female adults and geriatrics of Taita-Taveta County, Kenya, were not significantly altered by advancement in age ($\rho > 0.05$) (Table 4). There was therefore no need for a follow-up pairwise comparison using Mann-Whitney U test with adjusted significant ρ-value of 0.0167.

In addition results indicate that using Kruskal-Wallis H test, the established reference interval limits for thyroid stimulating hormone (TSH) ($\chi^2(2) = 1.137$, $\rho = 0.567$), and total triiodothyronine (T3) ($\chi^2(2) = 2.018$, $\rho = 0.365$) for female adults and geriatrics of Taita-Taveta County, Kenya were not significantly altered by advancement in age (Table 4). However, the reference interval limits for total thyroxine (T4) for female adults and geriatrics of Taita-Taveta County, Kenya were statistically significantly altered with advancement in age ($\chi^2(2) = 12.409$; $\rho = 0.002$). There was therefore need for a follow-up pairwise comparison using Mann-Whitney U test with adjusted significant ρ-value of 0.0167.

A follow-up pairwise comparison using Mann-Whitney U test indicates that the median reference interval limits for total thyroxine (T4) for female adults (5 (2-9.75) nmol/L) in the fifth decade with mean rank of 38.31 was statistically significantly higher than that of female geriatrics (4 (3-5.4) nmol/L) in the seventh decade onwards with mean rank of 24.23 ($U = 262$, $z = -3.220$, $\rho = 0.001$, $r = 0.4089$). However, this reference interval limit for total thyroxine (T4) for female adults in the fifth decade was statistically

similar to that of female geriatrics in sixth decade. Further, the median reference interval limits for total thyroxine (T4) for female geriatrics (4 (2.15-15.43) nmol/L) in the sixth decade with mean rank of 51.60 was statistically significantly higher than that of female geriatrics (4 (3-5.4) nmol/L) in the seventh decade onwards with mean rank of 35.97 (U = 614, z = -2.864, ρ = 0.004, r = 0.2986). Thus, the median reference interval limits for total thyroxine (T4) for female adults and geriatrics appear to be similar in the fifth and sixth decade but significantly drops from the seven decade onwards.

Results indicate that a Kruskal-Wallis H test for reference interval limits for thyroid stimulating hormone (TSH) ($\chi^2(2) = 0.161$; ρ = 0.923), total thyroxine (T4) ($\chi^2(2) = 1.352$; ρ = 0.509), and total triiodothyronine (T3) ($\chi^2(2) = 2.798$; ρ = 0.247) for male adults and geriatrics of Taita-Taveta County, Kenya were not significantly affected by advancement in age (ρ > 0.05). There was therefore no need for a follow-up pairwise comparison using Mann-Whitney U test with adjusted significant ρ-value of 0.0167.

Further, an investigation on the effect of gender on the reference interval limits for thyroid hormones in specific age categories using Mann-Whitney U test indicates that the total thyroxine (T4) for male geriatrics (4 (1.1-16.8) nmol/L) in the seventh decade onwards with mean rank of 42.42 was statistically significantly higher than that for their female counterparts (4 (3-5.4) nmol/L) in the seventh decade onwards with mean rank of 29.23 (U = 412, z = -2.779, ρ = 0.005, r = 0.3253).

Table 4: Effects of age on the median reference interval for thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya

Analyte (Units)	Changes in thyroid hormone concentration with age								
	Sex	**N**	**≥ 50-60 years**	**N**	**≥ 60-70 years**	**N**	**≥ 70-95 years**	**N**	**≥ 50-95 years**
TSH (mU/L)	M&F	68	2.09±1.69	103	2.17±1.82	73	2.22±1.60	244	2.16±1.72
			2 (0-6.55)		2 (0-6)		2 (0-6)		2 (0-6)
	M	36	2.25±1.84	41	1.95±1.41	43	2.07±1.40	120	2.08±1.54
			2 (0-3)		2 (0-5)		2 (0-5.90)		2 (0-5.97)
	F	32	1.91±1.51	62	2.32±2.05	30	2.43±1.85	124	2.24±1.87
			2 (0-3)		2 (0-8.55)		2.5 (0-4)		2 (0-6)
T4 (nmol/L)	M&F	68	6.47±4.04	103	5.83±3.60	73	5.49±3.28	244	5.91±3.64
			4 (1.73-16.28)		4 (1-15.40)		4 (1.85-15.30)		4 (2-15)
	M	36	6.06±4.09	41	5.54±3.81	43	6.25±3.63	120	5.94±3.84
			4 (1-9.50)		4 (0.05-15.95)		4 (1.1-16.80)↑*		4 (1-15.98)
	F	32	6.94±4.00	62	6.02±3.47	30	4.43±2.37	124	5.87±3.49
			5 (2-9.75)		4 (2.15-15.43)		4 (3-5.4)↓^{bc}		4 (3-15)
T3 (mU/L)	M&F	68	1.69±0.78	103	1.69±0.78	73	1.75±1.06	244	1.73±0.87
			2 (0-3.82)		2 (0-4.40)		2 (0-6)		2 (0-4)
	M	36	1.67±0.93	41	1.78±0.65	43	1.95±1.19	120	1.81±0.96
			2 (0-2.03)		2 (0.05-3.95)		2 (0-6)		2 (0-5.95)
	F	32	1.72±0.58	62	1.73±0.87	30	1.47±0.78	124	1.66±0.79
			2 (0-2)		2 (0-5)		2 (0-2.2)		2 (0-3)

Results are expressed as mean ± standard deviation (SD), and median and 95% range of the number of subjects indicated in the column labeled N. *ρ < 0.05 when male median reference interval limits are significantly different when compared to female median reference interval limits per each age category by Mann-Whitney U test; ^{a}ρ < 0.0167 when median reference interval limits in age range ≥ 50-60 years is significantly different when compared to median reference interval limits in age range ≥ 60-70 years, ^{b}ρ < 0.0167 when median reference interval limits in age range ≥ 50-60 years is significantly different when compared to median reference interval limits in age range ≥ 70-95 years, and ^{c}ρ < 0.0167 when median reference interval limits in age range ≥ 60-70 years is significantly different when compared to median reference interval limits in age range ≥ 70-95 years by Kruskal-Wallis H test followed by Mann-Whitney U test with Bonferroni corrections. Bracketed values are the median 2.5 and 97.5 percentiles for combined and separate gender in each of the three specific age categories.

Comparison of developed reference intervals for thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya with those reported in literature

A comparison of developed reference intervals for thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya with those reported in literature are presented in Table 1.5. These comparisons were performed by comparing the lower and upper reference interval limits of this study's reference intervals with those of other studies previously reported in medical literature.

Results indicate that for TSH, this study's gender independent lower reference interval limit is lower than that of Darfur (Sudan) (Ali *et al*., 2018), British (Kratzsch *et al*., 2005) [12], Australian (Hickman *et al*., 2017) [8], American (Hollowell *et al*., 20) [9], and Srpska Republic (Mirjani-Azaric *et al*., 2017) [15] populations, and gender dependent upper reference interval limit for Khartoum (Sudan) (Musa *et al*., 2018) [16] population. However, the gender independent upper reference interval limit for TSH in this study is higher than that of gender independent upper reference interval limits for Darfur (Sudan) (Ali *et al*., 2018), British (Kratzsch *et al*., 2005) [12], Australian (Hickman *et al*., 2017) [8], American (Hollowell *et al*., 20) [9], and Srpska Republic (Mirjani-Azaric *et al*., 2017) [15] populations, and gender dependent upper reference interval limit for Khartoum (Sudan) (Musa *et al*., 2018) [16] population.

For T3, this study's gender independent lower reference interval limit is lower than that of gender dependent British (Kratzsch *et al*., 2005) [12] population, and similar to that of Srpska Republic (Mirjani-Azaric *et al*., 2017) [15] population, while the upper reference interval limit is higher than that of British (Kratzsch *et al*., 2005) [12] population, and lower than that of the Srpska Republic (Mirjani-Azaric *et al*., 2017) [15] population.

For T4, this study' gender independent lower and upper reference interval limits are lower than that of gender independent reference limits for American (Hollowell *et al*.,

2002) [9], Darfur (Sudan) (Ali *et al*., 2018), and Srpska Republic (Mirjani-Azaric *et al*., 2017) [15] populations, and gender dependent lower and upper reference interval limits for Khartoum (Sudan) (Musa *et al*., 2018) [16], and British (Kratzsch *et al*., 2005) [12] populations. Age dependent reference interval limits for TSH were reported by Hollowell *et al*. (2002) [9] for the American population, and Ali *et al*. (2018) for western Sudan population (Table 1.5). Further, Ali *et al*. (2018) reported age dependent reference interval limits for T4 for western Sudan population (Table 1.6). These age dependent reference interval limits for TSH and T4 differ from those reported in this study.

Table 5: Effects of age on the median reference interval for thyroid hormones for adult and geriatric population of Taita-Taveta County, Kenya

Analyte (Unit)	Gender	This study RI	Hollowell *et al*., 2002 [9]	Kratzsch *et al*., 2005 [12]	Hickman *et al*., 2017 [8]	Mirjani-Azaric *et al*., 2017 [15]	Musa *et al*., 2018 [16]	Ali *et al*., 2018
TSH (mU/L)	**M&F**	**0-6**	0.45-4.12	0.40-3.77	0.43-3.28	0.65-5.39		0.05-3
	M	0-5.97					0.5-3	
	F	0-6					0.5-3.4	
T3 (mU/L)	M&F	**0-4**					0.8-2.7	0.8-2.8
	M	0-5.95		1.23-2.97		0-4.61		
	F	0-3		1.28-2.33		0-4.97		
T4 (nmol/L)	**M&F**	**2-15**	66.9-165.9			73.01-127.7		72-161.1
	M	1-15.98		71.4-166			63-165	
	F	3-15		68.4-125			62-148.2	

American population by Hollowell *et al*. (2002) [9], British population by Kratzsch *et al*. (2005) [12], Srpska Republic population by Mirjani-Azaric *et al*. (2017) [15], Australian population by Hickman *et al*. (2017) [8], Khartoum (Sudan) population by Musa *et al*. (2018) [16], Darfur (Sudan) population by Ali *et al*. (2018).

Table 6: Comparison of age related developed reference interval limits for thyroid hormones for adults and geriatrics of Taita-Taveta County, Kenya with those reported in medical literature

Analyte							
	This study RI		≥ 50-60 years	≥ 60-70 years	≥ 70-95 years		50-95 years
TSH		M&F					**0-6**
(mU/L)		M					
		F					
	Americans[H]		≥ 50-60 years	≥ 60-70 years	70-80 years	≥ 80 years	
		M&F	0.52-4.03	0.49-4.33	0.45-5.90	0.33-7.50	
		M	0.50-4.04	0.56-4.27	0.47-6.39	0.36-6.82	
		F	0.53-4.02	0.45-4.48	0.44-5.77	0.17-7.87	
	Germany[V]		50-79 years				
		M&F	0.19-2.09				
	Sudan[A]		51-60 years	≥ 60 years			
		M&F	0.5-2.6	0.2-2.5			
	Srpska		51-60 years	61-70 years			
		M&F	0-5.82	0-5.82			
T4	This study RI	M&F	**2.38-15.63**	**2.38-15.63**			**2-15**
(nmol/L)		M				**1.1-16.8***	
		F				**3.0-5.4[bc]**	
	Sudan[A]		51-60 years	≥ 60 years			
		M&F	137-162	165-190.2			
	Srpska		51-60 years	61-70 years			
		M&F	0-286.04	0-286.04			
T3	This study RI	M&F					**0-4**
(mU/L)		M					
		F					
	Sudan[A]		51-60 years	≥ 60 years			
		M&F	1-3	1-3.1			
	Srpska		51-60 years	61-70 years			
		M&F	0-1.49	0-1.49			
		M					
		F					

American population by Hollowell *et al*. (2002) [9], Western Pomerania, northeast of Germany population by Völzke *et al*. (2005) [22], Srpska Republic population by Mirjani-Azaric *et al*. 2017 [15], Nyala, Darfur region, western Sudan population by Ali *et al*. (2018).

Discussion, Conclusions and Recommendations

Results indicating that the thyroid stimulating hormone (TSH), total thyroxine (T4) and total triiodothyronine (T3) reference interval limits for the adult and geriatric male and female population of Taita-Taveta County, Kenya were statistically similar implies that these parameters are gender independent. The developed age and gender independent reference intervals for thyroid hormones for both males and females of Taita-Taveta County, Kenya are 0-6 mU/L for TSH, 2-15 nmol/L for T4, and 0-4 mU/L for T3,

respectively. This study findings agrees with those of Hickman *et al*. (2017) [8] who reported gender and age independent reference intervals for TSH (0.43-3.28 mU/L). Wang *et al*. (2017) [23] also reported gender independent reference intervals for T4 of 73.48-138.93 nmol/L. Further, these results also agree with those reported by Kratzsch *et al*. (2005) [12] who reported gender independent reference interval for TSH of 1.36 (0.40-3.77) mU/L. However, these results contrast the gender dependent reference interval for total T4 with females (113 [71.4-166] nmol/L) having a significantly higher reference intervals than males (93.4 [68.4-125] nmol/L), and T3 with females (1.94 [1.23-2.97] mU/L) having a significantly higher reference intervals than males (1.72 [1.28-2.33] mU/L) reported by Kratzsch *et al*. (2005) [12]. These results are also in agreement with those reported by Mirjani-Azaric *et al*. (2017) [15] who reported gender independent reference interval limits for TSH of 0.75-5.32 mU/L, and T4 of 73.49-126.30 nmol/L and contrast the gender dependent reference interval limits for T3 with males (0-4.97 mU/L) having a significantly higher reference interval limits than females (0-4.61 mU/L). Wang *et al*. (2017) [23] reported gender dependent referenc interval limits for TSH of 0.66-4.95 mU/L for males and 0.72-5.84 mU/L for females with females having higher values than males, and T3 of 1.24-2.18 nmol/L for males and 1.20-2.10 nmol/L for females with males having higher values than females, respectively. Li *et al*. (2020) [13] reported gender dependent and age independent reference interval limits for TSH of 0.65-3.92 mU/L for males and 0.43-4.67 mU/L for females, T4 of 78.52-144.94 nmol/L for males and 64.49-145.20 nmol/L for females, and T3 of 1.17-2.04 nmol/L for males and 1.04-2.00 nmol/L for females with males having higher values tha females, respectively.

These results also differ from those reported by Gesing *et al*. (2012) [7] and Barbesino (2019) [2] who reported age dependence of thyroid hormone levels. Mirjani-Azaric *et al*. (2017) [15] also demonstrated that the reference interval of TSH decreased with advancement of age from age 20-30 years upto age 50 years after which its level reverts to that of age range 20-30 years and above 50 years. Musa *et al*. (2018) [16] reported gender dependent reference interval for TSH with males (1.7 [0.5-3.4] mU/L) having a significantly higher reference interval limits than females (1.1 [0.5-3.0] mU/L), T4 with females (106.0 [63-165] nmol/L) having a significantly higher reference interval limits than males (96.5 [62.0-148.2] nmol/L), and gender independent reference interval for total T3 of 1.4 (0.8-2.7) mU/L. Musa *et al*. (2018) [16] also demonstrated age dependent reference interval for T4 with increasing reference interval with advancement of age (for age range 20-30 years to above 60 years) and age independence of reference interval for TSH and T3. Ali *et al*. (2018) reported gender independent reference interval for TSH of 1.2 (0.50-3.0) mU/L, T4 of 111.0 (72.0-161.1) nmol/L and T3 of 1.5 (0.8-2.8) mU/L, respectively. Ali *et al*. (2018) also demonstrated age dependence reference interval for T4 and T3 with increasing reference interval with advancement of age. Völzke *et al*. (2005) [22] reported a gender independent reference interval for TSH of 0.25-2.12 mU/L. Völzke *et al*. (2005) [22] also demonstrated age dependence reference intervals for TSH with decreasing reference interval with advancement of age with age 20-49 years having a reference interval of 0.27-2.15 mU/L and age 50-79 years having a reference interval of 0.19-2.09 mU/L, respectively. Hollowell *et al*. (2002) [9] reported a gender independent reference interval for TSH of 1.39 (0.45-4.12) mU/L for the American population and a gender dependent reference interval for T4 with females (111.8 [66.9-165.9] nmol/L) having significantly higher reference interval than males (107.3 [64.4-156.0] nmol/L). Hollowell *et al*. (2002) [9] also demonstrated age dependent reference interval for TSH with advancement of age from 50-80 years (1.50 [0.52-4.03] mU/L for 50-59, 1.67 [0.49-4.33] mU/L for 60-69, 1.76 [0.45-5.90] mU/L for 70-79, and 1.90 [0.33-7.50] mU/L for above 80 years, respectively); T4 reference interval was demonstrated to decrease with advancement of age.

The decrease of T4 concentration with age especially in the seventh decade onwards in females but not in males could be associated with the observed corresponding increase in TSH concentration in females relative to its unchanging concentration in males resulting in overt hypothyroidism (Bensenor *et al*., 2012; Völzke *et al*., 2005) [3, 22] in females. This could be accounted for by the decrease in thyroid gland function with advancement with age especially in the females which is associated with hypothyroidism; that is, increased TSH levels and decreased T4 levels. For males, TSH levels are reported not to vary with aging as observed in this study population but increases with aging in females as observed in this study in the seventh decade onwards (Yeap *et al*., 2017) [24]. However, this increase in TSH levels in females with age is overcome by excluding females with antithyroid antibodies (Bensenor *et al*., 2012) [3]. The presence of both increased TSH levels and antithyroid antibodies is more common in females and increases with age. Elderly females have a higher prevalence of iodine deficiency than males and increased TSH levels is a sensitive biomarker of iodine deficiency in geriatrics (Bensenor *et al*., 2012) [3].

The difference between the age independent thyroid function tests reference intervals developed in this study for the Taita-Taveta County population from the age and sex dependent thyroid function tests previously developed and reported from other parts of the world by other researchers could be due to: differences in ethnicity, iodine supply and hence iodine nutritional status of the referent individuals, the selection criteria used in defining the referent individuals, the principles and procedures of the detection methods used, and genetic factors.

The limitations of this study were: firstly, urinary iodine which would have been used to assess the association between the developed reference interval for thyroid hormones and iodine nutritional status was not measured. Secondly, the presence of thyroid antibodies, anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG) antibodies which can result in higher than appropriate reference intervals of thyroid hormones were not assessed in this study. Thirdly, ultrasound was not used to assess the thyroid volume and thyroid enlargement to further confirm the healthy status of the selected referent population. The study used a health questionnaire, drug history, and clinical assessment to define thyroid disease free referent individuals which could result in including individuals with a developing thyroid disease resulting in higher than appropriate reference intervals; recall bias may affect results when using a questionnaire. Further, the health questionnaire and clinical assessment were used to remove other factors such as smoking and obesity which influence the reference intervals of thyroid hormones. Fourthly, this

study only developed reference intervals for total thyroxine (T4) and triiodothyronine (T3) without developing the reference intervals for free T4 and free T3 which are more sensitive indicators of iodine nutritional status as in other previously reported studies. Because samples were not collected at fixed times such as 08.00am to 12.00 noon, the developed reference intervals for thyroid hormones may be influenced by effects caused by circadian rhythm. The study involved a referent population of Taita-Taveta County and thus covered a narrow scope. A larger sample size of referent population including clinical laboratories of the eight counties of Kenya is needed to generate national reference intervals for thyroid hormones. Finally, the effect of age on the reference intervals for thyroid hormones used a sample size below the recommended minimum of 120 referents for each age category which could bias the results.

In conclusions, the developed reference interval limit for TSH, and T3 for the Taita-Taveta County, Kenya population are both gender and age independent, while those of T4 are age dependent for females and gender dependent for age 70-95 years. However, these developed reference interval limits for TSH, T4 and T3 are different from those indicated in the manufacturers' inserts reagent kits, and those developed and reported previously in medical literature in their lower and upper limits using different referent individuals and analytical methods of different parts of the world. These developed reference intervals for thyroid hormones can therefore be adopted for use in the Department of Clinical Chemistry, Moi Subcounty Hospital, Voi, Taita-Taveta County, Kenya and other hospitals within the County.

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