Original Article (Araştırma Makalesi)

Determination of Reference Interval of Tumour Markers in Kahramanmaraş Region, Turkey

Kahramanmaraş Bölgesine Ait Serum Örneklerinde Ölçülen Tümör Belirteçlerinin Referans Değerlerinin Belirlenmesi

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Öz

Amaç: Referans aralıkları hastalığın teşhisi, izlenmesi ve tedavisi için oldukça önemlidir. Bununla birlikte, referans aralıkları yaşa, cinsiyete, coğrafi bölgeye, diyet durumuna ve diğer faktörlere göre değişebilir. Bu çalışmanın amacı, Kahramanmaraş Bölgesine ait Alfa-fetoprotein (AFP), Karbonhidrat Antijen 15-3 (CA 15.3), Karbonhidrat Antijen 19.9 (CA 19.9), Karbonhidrat Antijen 125 (CA 125), Karsinoembriyonik Antijen (CEA), Prostat Spesifik Antijen (PSA), serbest PSA (fPSA) ve tiroglobulin (TG) için referans aralıklarını belirlemektir.

Gereç ve Yöntem: Toplamda 64,687 kişilik veriden 21,190 kişinin seçilmesiyle Klinik Biyokimya Laboratuvarından türetilen veriler kullanılmıştır. Tüm parametreler için referans aralıkları hesaplanırken indirect ve parametrik olmayan yüzde tahmin yöntemi kullanılmıştır.

Bulgular: Çalışılan parametreler için aşağıdaki gibi alt ve üst sınırlar belirlenmiştir - AFP: 0.52, 3.76 IU/ml; CA 15.3: 5.0, 37.60 IU/ml; CA 19.9: 2.46, 29.5 IU/ml; CA 125: 2.89, 22.7 IU/ml; CEA: 0.11, 3.67 ng/ml; fPSA: 0.02, 0.75 ng/ml; PSA: 0.14, 2.31 ng/ml; and TG: 0.34, 30.13 ng/ml.

Sonuç: Bu çalışma, üretici firmalar tarafından incelenen parametreler için alıntı yapılan referans aralıklarının, popülasyonumuza özgü referans aralıklarıyla tam olarak örtüşmediğini göstermektedir. Bölgesel referans aralıkları belirlenerek tarama testlerinin verimliliği artırabilir

Anahtar Kelimeler: : Tümör belirteçleri, referans aralık, Kahramanmaraş

Abstract

Objectives: Reference intervals are important for disease diagnosis, monitoring and treatment. However, reference intervals may vary by age, gender, geographical location, dietary status and other factors. The aim of this study is to determine the reference intervals for alpha-fetoprotein (AFP), CarbohydrateAntigen 15-3 (CA 15.3), Carbohydrate Antigen 19.9 (CA 19.9), Carbohydrate Antigen 125 (CA 125), Carcinoembryonic Antigen (CEA), Prostate Specific Antigen (PSA), free PSA (fPSA), and thyroglobulin (TG) in Kahramanmaraş Region, Turkey.

Material and Methods: Data derived from 21,190 individuals in 64,687 were obtained from the Clinical Biochemistry Laboratory. The reference intervals for the parameters were determined using indirect and non-parametric percentile estimation method.

Results: In the study, the following respective lower and upper limits for the studied parameters were determined- AFP: 0.52, 3.76 IU/ml; CA 15.3: 5.0, 37.60 IU/ml; CA 19.9: 2.46, 29.5 IU/ml; CA 125: 2.89, 22.7 IU/ml; CEA: 0.11, 3.67 ng/ml; fPSA: 0.02, 0.75 ng/ml; PSA: 0.14, 2.31 ng/ml; and TG: 0.34, 30.13 ng/ml.

Conclusion: This study demonstrates that the reference intervals quoted by the manufacturers for the parameters examined do not perfectly coincide with the reference intervals that are specific to our population. Establishing local reference intervals increases the utility of screening tests.

Key Words: Tumour markers, reference intervals, Kahramanmaraş region

INTRODUCTION

Tumour markers are substances produced by the tumour tissue or non-tumour tissue which can be found in the blood or bodily fluids, aiding the diagnosis of a tumour and differentiating the tumour from normal tissues (1, 2). The importance of tumour markers has increased gradually in the diagnosis and treatment of oncologic patients as well as in predicting disease progression (3). In addition to imaging methods, metastatic diseases can be also monitored by serum tumour markers. Serum tumour markers are biochemical markers found in the peripheral blood that are produced by tumour or non-tumour tissues in response to the presence of a malignancy (4). Unsatisfactory sensitivity and/or specificity of tumour markers limit their use in follow-up and early diagnosis (5-8). However, elevated serum markers in patients with a metastatic tumour can be used as optional parameters to monitor disease progression. No suggestion has been offered regarding measurement frequency (4, 9). However, some studies have indicated that different therapies based on the

This study was conducted by the first author as a master's thesis under the supervision of Ahmet Çelik.

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changes in serum biomarkers did not affect the outcomes (10, 11).

Several traditional tumour markers are used for screening, diagnosis, stating or disease monitoring of various types of cancer and Alpha-fetoprotein (AFP) is one of these markers (12, 13). AFP has been proposed to be marker for the detection of human hepatocellular carcinoma (12-14). Carcinoembryonic Antigen (CEA) is an elevated protein in adenocarcinomas of ovarian, lung, colorectal, urinary and breast origin (15). Carbohydrate Antigen 15-3 (CA 15.3) is used to monitor response to breast cancer treatment, recurrence and metastases (15). Carbohydrate Antigen 19.9 (CA 19.9) is the most commonly used serum tumor marker to diagnose pancreatic cancer and monitor treatment in patients with pancreatic adenocarcinoma (16). Carbohydrate Antigen 125 (CA 125) is a glycoprotein produced by the serous epithelium (17), which increases in ovarian cancer and is used in monitoring and evaluating patients. Prostate Specific Antigen (PSA) is a glycoprotein secreted by the epithelial cells of the prostate gland and elevated in prostate cancer. Although prostate cancer has a high mortality rate, PSA provides early diagnosis of cancer (15). Thyroglobulin (TG) is an established humoral biomarker fordisease monitoring of thyroid cancer of follicular origin (differentiated thyroid cancer) (18).

Determining reference intervals in a healthy population is particularly important for clinical diagnosis and treatment. Lack of reference intervals for a specific population may result in inappropriate intervention by the clinicians due to wrong diagnosis and treatment. Predetermined reference intervals are used for all tests performed in the laboratory and the values are evaluated as per the reference intervals set by the manufacturing companies. However, these reference intervals may vary across different regions and populations, and according to gender, age, and diet status. Therefore, reference intervals may not reflect the actual situation of the addressed population. Considering these factors, the most ideal approach is that locally appropriate reference intervals be determined (19).

The present study investigated the calculability of reference intervals using the data of our population. Using indirect calculation method, the present study aimed to establish the reference intervals for eight tumour markers, studied at our laboratory, that are commonly used by the clinicians.

MATHERIAL AND METHODS

Ethical Considerations:

Approval for the study was granted by the Local Research Ethics Committee of Kahramanmaraş Sütçü İmam University School of Medicine (Reference number: 2015/03).

Study Population:

The study groups were composed of patients who were admitted to the outpatient clinics only once during the study period for the analysis of serum tumour markers at the Biochemistry Laboratory of Training and Research Hospital of Kahramanmaraş Sütçü İmam University between January 2007 and November 2015. Patient results from oncology clinics were not included in the study. The data of inpatients were excluded.

Analysis of Serum samples:

Results of serum AFP, CA 15.3, CA 19.9, CA 125, CEA, PSA, fPSA, and TG were collected from existing laboratory data for 64 687 serum samples from the Training and Research Hospital of Kahramanmaraş Sütçü İmam University. All assays were performed on the Advia Centaur XP Immunoassay System (Siemens, Erlangen, Germany). Reagents and calibrators provided by Siemens Healthcare Diagnostics were used. Internal control was performed with serum supplied by Bio-Rad Laboratories for AFP, CA 15.3, CA 19.9, CA 125, CEA, PSA, and fPSA. Internal control serum provided by Siemens Healthcare Diagnostics was used for TG. Regular internal quality control procedures and external quality assessment scheme were performed to validate the quality of all parameters. Eight serum tumour markers that might vary across different populations were evaluated. The data were divided into subgroups according to gender and age. No gender-specific classification was made for fPSA and PSA, as all individuals were males; only age-specific reference intervals were determined for age subgroups. In consideration of age, the patient group was separated into four subgroups (1–20 years, 21–40 years, 41–60 years, and 60 years and older). According to Clinical & Laboratory Standards Institute (CLSI) EP28-A3 guidelines[20], groups with less than 120 individuals in age subgroups were combined with another group.

Statistical methods:

This was a retrospective study and the data were collected using indirect methods. Non-parametric percentile estimation method was used to determine reference intervals, because the distribution of differences was not normal in all parameters.

The histogram of each test dataset was drawn using SPSS software before analysis. Outliers were removed after data screening and the Dixon's rule was applied to ensure that all outliers have been removed. Kolmogorov–Simirnov and Shapiro–Wilks tests were used to test whether age groups and subgroups are normally distributed. Non-parametric percentile estimation method was used to determine reference intervals within 95% confidence interval, because all data were not normally distributed. Differences between age subgroups and gender were evaluated based on the Kruskal–Wallis test. Statistically significant subgroup differences were defined as $p < 0.05$. Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA, version 15.0) software package was used in the statistical analysis. Descriptive statistics included arithmetic mean, median, standard deviation, and 2.5 and 97.5 percentile values.

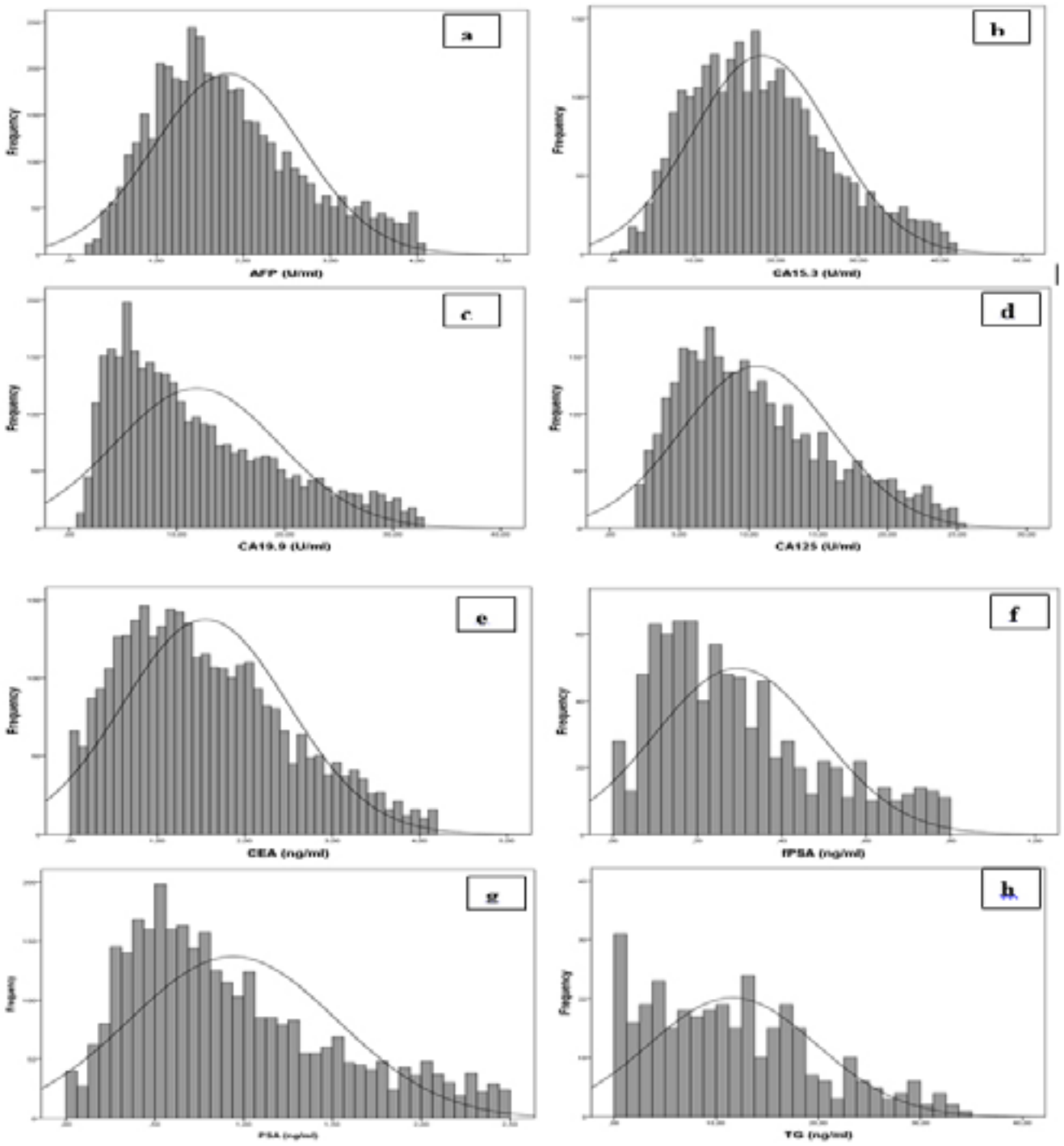
RESULTS

Reference intervals in our population were calculated using non-parametric percentile estimation method, because the study's data did not show normal distribution ($p< 0.05$).

A total of 64,687 serum results for healthy

outpatients were identified for which the reference intervals for 8 tumour markers were to be determined. According to the normality test, the data had a non-normal distribution (Figure 1) hence outliers were removed reducing the total included data to 21,190.

Figure 1. Distribution (frequency) of all parameters in the studied population.



In the study, the following lower and upper limits for the analyzed parameters were determined: for AFP, lower limit: 0.52, upper limit: 3.76 IU/ml; for CA 15.3, lower limit: 5.0, upper limit: 37.60 IU/ml; for CA 19.9, lower limit: 2.46, and upper limit: 29.5 IU/ml; for CA 125, lower limit: 2.89, upper limit: 22.7 IU/ml; for CEA, lower limit: 0.11, upper limit: 3.67 ng/mL; for fPSA, lower limit: 0.02, upper limit 0.75 ng/mL; for PSA, lower limit: 0.14, upper limit: 2.31 ng/mL; for TG, lower limit: 0.34, upper limit: 30.13 ng/mL (Table 1).

The reference intervals were calculated separately for male and female for all parameters that were expected to be distributed differently between age subgroups. The differences may be clinically significant. (Table 2 and Table 3).

Values for all serum tumour marker parameters for age subgroups are presented as boxplots (Figure 2). Generally, the patient group was separated into four subgroups however groups with less than 120 individuals in age subgroups were combined with another group. Therefore, in some parameters there were less than four subgroups (Figure 2b, c, e, f, g, h).

Table 1. Reference intervals, mean, median, and SD for all parameters and reference intervals quoted by the manufacturer.

Parameter	n	Mean	Median	SD	Reference intervals		Reference intervals quoted by the manufacturer	
					Lower limit	Upper limit	Lower limit	Upper limit
AFP (IU/ml)	4509	1.82	1.68	0.84	0.52	3.76	0.5	5.5
CA 15.3 (IU/ml)	2695	18.28	17.40	8.51	5.00	37.60	6.4	38.4
CA 19.9 (IU/ml)	3257	11.84	9.82	7.57	2.46	29.50	0	37
CA 125 (IU/ml)	3061	10.56	9.50	5.37	2.89	22.70	0	35
CEA (ng/ml)	3270	1.56	1.40	0.96	0.11	3.67	0	5
fPSA (ng/ml)	852	0.29	0.25	0.19	0.02	0.75	0.05	0.42
PSA (ng/ml)	3213	0.94	0.80	0.58	0.14	2.31	0.04	4
TG (ng/ml)	333	11.60	10.70	8.23	0.34	30.13	1.6	59.9

AFP, alpha-fetoprotein, CA, carbohydrate antigen CEA, carcinoembryonic antigen; PSA, prostate specific antigen; fPSA, free PSA; TG, thyroglobulin.

Table 2. Reference intervals, mean, median, and SD according to age in different age groups for males.

			Reference intervals				
Parameter (Unit)	Groups of ages	n	Lower limit	Upper limit	Mean	Median	SD
AFP (IU/ml)	1–20	100	0.24	3.35	1.32	1.19	0.72
	21–40	557	0.46	3.80	1.69	1.52	0.85
	41–60	621	0.52	3.80	1.91	1.83	0.83
	60≤	827	0.54	3.76	1.92	1.81	0.84
	Total	2105	0.50	3.78	1.83	1.70	0.85
CA 15.3 (IU/ml)	1–60	210	4.45	37.50	17.53	15.75	8.70
	60≤	300	3.75	38.19	18.21	17.00	9.21
	Total	510	4.17	38.02	17.93	16.20	9.01
CA 19.9 (IU/ml)	1–40	123	1.86	26.36	9.45	7.87	6.31
	41–60	330	1.67	28.87	11.33	9.18	7.58
	60≤	590	2.54	30.04	12.67	10.78	7.99
	Total	1043	2.12	29.67	11.86	9.53	7.75
CA 125 (IU/ml)	1–60	134	2.42	22.58	9.21	8.00	5.04
	60≤	155	2.48	22.99	9.50	7.50	5.81
	Total	289	2.53	22.70	9.36	7.60	5.46
CEA (ng/mL)	1–40	125	0.10	3.59	1.23	1.02	0.89
	41–60	366	0.21	3.97	1.78	1.66	0.98
	60≤	678	0.37	4.02	1.99	1.90	0.98
	Total	1169	0.24	3.97	1.84	1.76	1.00
fPSA (ng/mL)	1–60	296	0.01	0.72	0.24	0.22	0.16
	60≤	556	0.02	0.75	0.32	0.28	0.20
	Total	852	0.02	0.75	0.29	0.25	0.19
PSA (ng/mL)	1–40	164	0.02	1.87	0.59	0.51	0.41
	41–60	1448	0.19	2.11	0.86	0.75	0.51
	60≤	1601	0.12	2.38	1.04	0.90	0.63
	Total	3253	0.11	3.67	1.54	1.40	0.94
TG (ng/mL)	Total	57	0.35	29.14	11.87	11.90	8.27

AFP, alpha-fetoprotein, CA, carbohydrate antigen CEA, carcinoembryonic antigen; PSA, prostate specific antigen; fPSA, free PSA; TG, thyroglobulin.

The statistical differences between the groups were calculated, as well as the reference intervals for all age subgroups. A statistically significant difference ($p<0.05$) was found between all age subgroups except 41–60/60≤ ($p=0.99$) in AFP subgroups. We found no significant change in mean CA 1.53 amongst subgroups in the analyzed population. When we compared the mean values of CA 19.9, we found a statistically significant difference ($p<0.05$) for 1–40/60≤ and 41–60/60≤ subgroups. We found a significant difference between CA 125 age subgroups except 1–20/21–40 ($p=1.00$) and 1–20/41–60 ($p=0.07$). Also, statistically significant difference ($p<0.05$) was found in CEA, fPSA and PSA all age subgroups (Table 4).

Similarly, the statistical difference between the genders was calculated. A statistically significant difference ($p<0.05$) was found between male and female mean values only for CA 125 and CEA parameters (Table 5).

Table 3. Reference intervals, mean, median, and SD according to age in different age groups for females.

			Reference intervals				
Parameter (Unit)	*Groups of ages*	*n*	*Lower limit*	*Upper limit*	*Mean*	*Median*	*SD*
AFP (IU/ml)	*1–20*	*140*	*0.35*	*3.58*	*1.30*	*1.17*	*0.74*
	21–40	*615*	*0.53*	*3.68*	*1.70*	*1.51*	*0.80*
	41–60	*947*	*0.62*	*3.83*	*1.93*	*1.79*	*0.83*
	60 ≤	*702*	*0.62*	*3.70*	*1.84*	*1.74*	*0.82*
	Total	*2404*	*0.55*	*3.73*	*1.81*	*1.66*	*0.83*
CA 15.3 (IU/ml)	*1–40*	*646*	*5.60*	*36.64*	*17.61*	*16.75*	*7.93*
	41–60	*916*	*4.80*	*37.30*	*18.51*	*17.80*	*8.31*
	60 ≤	*623*	*4.98*	*38.84*	*18.92*	*17.70*	*8.93*
	Total	*2185*	*5.10*	*37.53*	*18.36*	*17.50*	*8.39*
CA 19.9 (IU/ml)	*1–40*	*638*	*2.47*	*29.92*	*11.87*	*9.76*	*7.76*
	41–60	*914*	*2.54*	*28.61*	*11.39*	*9.84*	*7.11*
	60 ≤	*662*	*2.57*	*30.13*	*12.39*	*10.30*	*7.68*
	Total	*2214*	*2.56*	*29.50*	*11.83*	*9.92*	*7.48*
CA 125 (IU/ml)	*1–20*	*120*	*3.60*	*22.88*	*11.50*	*10.55*	*4.91*
	21–40	*993*	*3.19*	*23.43*	*11.41*	*10.50*	*5.41*
	41–60	*1151*	*2.94*	*22.60*	*10.56*	*9.49*	*5.34*
	60 ≤	*508*	*2.59*	*21.82*	*9.36*	*8.08*	*5.07*
	Total	*2772*	*2.97*	*22.76*	*10.68*	*9.60*	*5.35*
CEA (ng/mL)	*1–40*	*546*	*0.02*	*2.84*	*1.06*	*0.90*	*0.73*
	41–60	*839*	*0.08*	*3.35*	*1.32*	*1.18*	*0.83*
	60 ≤	*699*	*0.18*	*3.47*	*1.69*	*1.67*	*0.89*
	Total	*2084*	*0.08*	*3.33*	*1.38*	*1.24*	*0.86*
TG (ng/mL)	*Total*	*276*	*0.33*	*31.43*	*11.55*	*10.40*	*8.24*

AFP, alpha-fetoprotein, CA, carbohydrate antigen; CEA, carcinoembryonic antigen; TG, thyroglobulin.

DISCUSSION

Reference intervals of tumour marker parameters are crucial for evaluation of the medical condition. Reference values are also necessary for evaluation of cancer diagnosis and progression. However, there is no reference interval for tumour marker for outpatient adults. Because of the lack of locally derived reference intervals for all tumour marker parameters, clinicians use reference intervals obtained from other populations (manufacturer). This is an indication that it is essential to locally derive population-based reference interval for use in medical practice. Table 1 summarized the tumour markers parameters reference intervals in Kahramanmaraş region in the current study. In our study, eight tumour markers commonly tested at our Biochemistry Laboratory were evaluated and the reference intervals determined for all parameters except CA 1.53, fPSA, and TG were narrower than quoted by the manufacturer (Table 1).

Many studies have been conducted worldwide in an attempt to determine reference intervals and cut-off values for the tumour markers. In a previous research, Qin et al. reported an upper limit of 4.76 IU/ml for AFP in male individuals residing in a defined area in China (21). Zhang et al. reported an upper limit of 7.51 IU/ml for AFP (22). The authors have attributed varying upper limits to the different methodologies used in the studies. Furthermore, Qin et al. suggested that smoking could also affect the results (21). Yan et al. reported that the upper limit for the Chinese Han population was 7.07 ng/ml

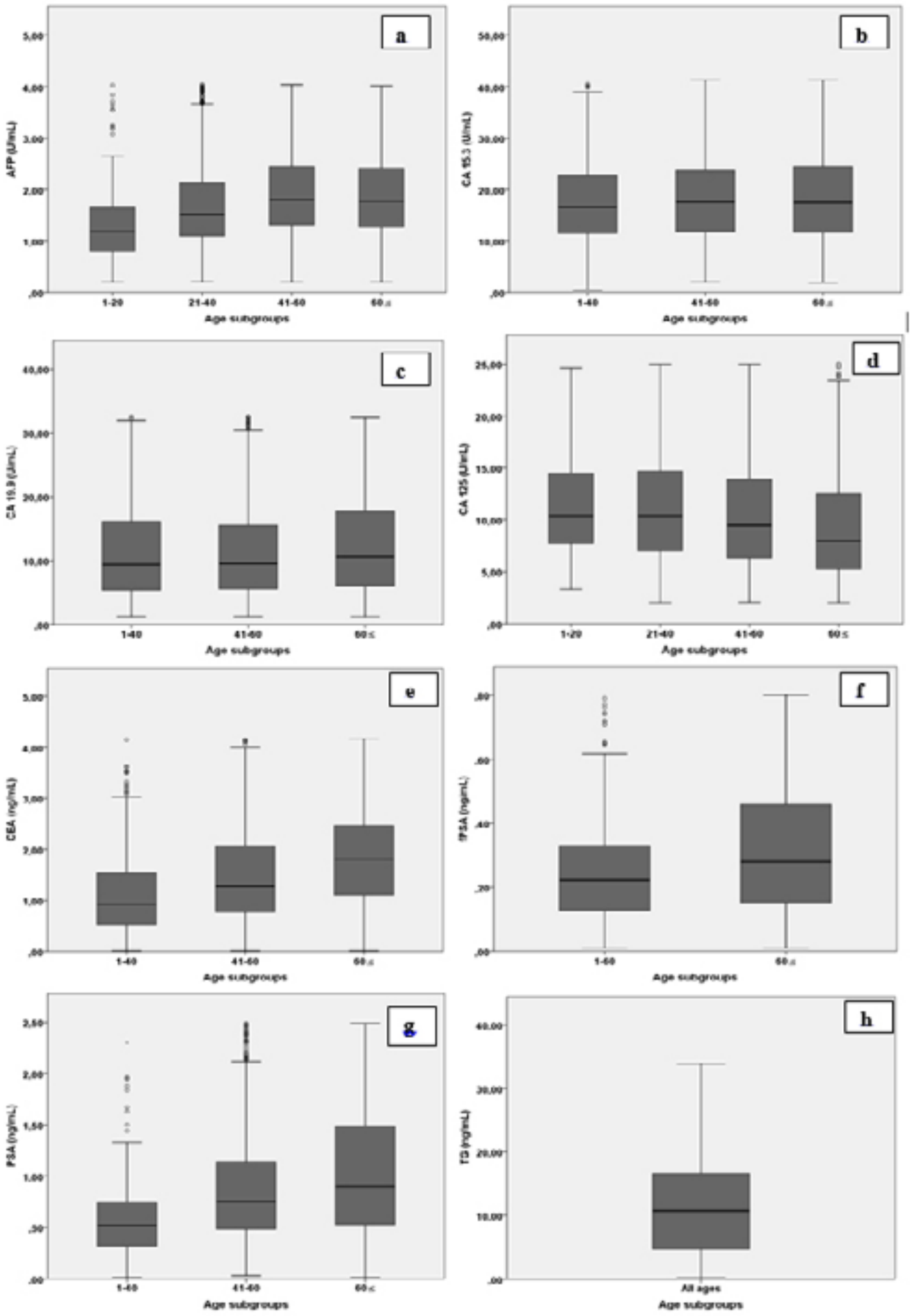


Figure 2. Boxplot illustrating differences in age subgroups for all parameters.

Table 4. Reference intervals, mean, and statistical differences between age subgroups.

Parameter (Unit)	Subgroup	Groups of ages			Significant differences between groups*		
		n	Mean	Lower-upper Limit	Groups	Differences between means	p
AFP (IU/ml)	1–20	240	1.31	0.35–3.53	1–20/21–40	0.38	0.00
	21–40	1172	1.69	0.51–3.71	1–20/41–60	0.62	0.00
	41–60	1568	1.93	0.61–3.80	1–20/60≤	0.57	0.00
	60≤	1529	1.88	0.59–3.73	21–40/41–60	0.24	0.00
	Total	4509	1.82	0.52–3.76	21–40/60≤	0.19	0.00
	-	-	-	-	41–60/60≤	0.05	0.99
CA 15.3 (IU/ml)	1–40	699	17.51	5.45–36.55	1–40/41–60	0.92	0.09
	41–60	1073	18.43	4.68–37.41	1–40/60≤	1.18	0.06
	60≤	923	18.69	4.53–38.68	41–60/60≤	0.26	1.00
	Total	2695	18.28	5.00–37.60	-	-	-
CA 19.9 (IU/ml)	1–40	761	11.48	2.30–29.70	1–40/41–60	0.10	1.00
	41–60	1244	11.38	2.33–28.70	1–40/60≤	1.04	0.00
	60≤	1252	12.52	2.57–30.09	41–60/60≤	1.14	0.00
	Total	3257	11.84	2.46–29.50	-	-	
CA 125 (IU/ml)	1–20	124	11.53	3.62–22.88	1–20/21–40	0.26	1.00
	21–40	1022	11.27	3.07–23.40	1–20/41–60	1.03	0.07
	41–60	1252	10.50	2.90–22.56	1–20/60≤	2.14	0.00
	60≤	663	9.39	2.59–22.04	21–40/41–60	0.77	0.00
	Total	3061	10.56	2.89–22.70	21–40/60≤	1.88	0.00
	-	-	-	-	41–60/60≤	1.11	0.00
CEA (ng/mL)	1–40	671	1.10	0.04–2.97	1–40/41–60	0.36	0.00
	41–60	1205	1.46	0.10–3.55	1–40/60≤	0.74	0.00
	60≤	1377	1.84	0.24–3.84	41–60/60≤	0.38	0.00
	Total	3253	1.54	0.11–3.67	-	-	-
fPSA (ng/mL)	1–60	296	0.24	0.01–0.72	1–60/60≤	0.08	0.00
	60≤	556	0.32	0.02–0.75	-	-	-
	Total	852	0.29	0.02–0.75	-	-	-
PSA (ng/mL)	1–40	164	0.59	0.02–1.87	1–40/41–60	0.27	0.00
	41–60	1448	0.86	0.19–2.11	1–40/60≤	0.45	0.00
	60≤	1601	1.04	0.12–2.38	41–60/60≤	0.18	0.00
	Total	3213	0.94	0.14–2.31	-	-	-
TG (ng/mL)	Total	333	11.60	0.34–30.13	-	-	-

AFP, alpha-fetoprotein, CA, carbohydrateantigen; CEA, carcinoembryonic antigen; PSA, prostate specific antigen; fPSA, free PSA; TG, thyroglobulin.

**Multiple group comparisons in age subgroups were performed using the Kruskal–Wallis test. The significance level is 0.05.*

(23). Also, this study did not show significant association between AFP and age. The upper limits determined in the present study were lower than those adopted from the developed nations as well as those reported by other researchers, and lower upper limit might allow higher sensitivity in diagnosis. There is variation of reference value based on environment, gender, diet and smoking status etc.

Reference values for CA 15.3 were found in a range between 5.0 IU/ml and 37.6 IU/ml in our study. Bjerner et al. reported an upper reference limit of 31.7 IU/ml in cases aged 40s and an upper reference limit of 37.5 IU/ml in cases aged 70s (24). While lower and upper reference limits change according to gender and age, reference intervals may also be affected by the ethnicity. Ri et al. reported higher upper reference limits in males than in females (25). Where normal reference values in the present study did not change according to gender.Sagi-Dain et al. determined reference intervals of serum CA 15.3 levels in the triage of adnexal masses (26). In this

Table 5. Reference intervals, mean, and statistical differences between genders.

Parameter (unit)	Gender	Groups of gender			Significant differences between groups*		
		n	Mean	Lower-upper limit	Groups	Differences between means	p
AFP (IU/ml)	Male	2105	1.83	0.50–3.78	Male/Female	0.02	0.33
	Female	2404	1.81	0.55–3.73			
	Total	4509	1.82	0.52–3.76			
CA 15.3 (IU/ml)	Male	510	17.93	4.17–38.02	Male/Female	0.43	0.11
	Female	2185	18.36	5.10–37.53			
	Total	2695	18.28	5.00–37.60			
CA 19.9 (IU/ml)	Male	1043	11.86	2.12–29.67	Male/Female	0.03	0.74
	Female	2214	11.83	2.56–29.50			
	Total	3257	11.84	2.46–29.50			
CA 125 (IU/ml)	Male	289	9.36	2.53–22.70	Male/Female	1.32	0.00
	Female	2772	10.68	2.97–22.76			
	Total	3061	10.56	2.89–22.70			
CEA (ng/mL)	Male	1169	1.84	0.24–3.97	Male/Female	0.46	0.00
	Female	2084	1.38	0.08–3.33			
	Total	3253	1.54	0.11–3.67			
fPSA (ng/mL)	Male	852	0.29	0.02–0.75	-	-	-
	Female	-	-	-			
	Total	852	0.29	0.02–0.75			
PSA (ng/mL)	Male	3213	0.94	0.14–2.31	-	-	-
	Female	-	-	-			
	Total	3213	0.94	0.14–2.31			
TG (ng/mL)	Male	57	11.87	0.35–29.14	Male/Female	0.32	0.71
	Female	276	11.55	0.33–31.43			
	Total	333	11.60	0.34–30.13			

AFP, alpha-fetoprotein, CA, carbohydrateantigen; CEA, carcinoembryonic antigen; PSA, prostate specific antigen; fPSA, free PSA; TG, thyroglobulin.

**Multiple group comparisons in age subgroups were performed using the Kruskal–Wallis test. The significance level is 0.05.*

retrospective study, CA15.3 level of 21 IU/mL was shown to be the optimal reference interval for malignancy detection. Our study and other studies have several important limitations. One of those is the retrospective method of data acquisition. Inconsistent cutoff levels may obviously affect the sensitivity and specificity of this test. Zhang et al. reported non-parametric reference intervals (upper limit) for Han and Shuyang regions were 26.45IU/mL for CA 19.9 (27). The researchers have shown that the reference intervals increases with age. Upper reference limit for CA 19.9 was found to be 29.50 IU/ml in the present study. Although this value was below the upper reference limit quoted by the manufacturer, this value was quite close to the upper reference limits reported by Woo et al. and Bjerner et al. for CA 19.9 (24, 28).

Woo et al. suggested that CEA levels are elevated with smoking. They also suggested variability in other parameters in association with age, gender, smoking, and menopausal status (28). Behbehani et al. reported higher upper reference limits for CEA in males than in females and the same in smokers than in nonsmokers (29). Consistent with the findings of Benbehani et al., the present study found higher upper reference limits in males than in females. Lao et al. reported non-parametric

reference intervals (upper limit) for Zhuang ethnic males was 5.12 ng/mL for CEA (30). Zhang et al. reported an upper limit of 4.53ng/ml for CEA (22). Researchers show that the reference interval is changed by gender, smoking, ethnicity and different detection systems.

Some studies have shown variability in reference intervals of CA 125 due to interaction with some variables (24, 28, 31). Barcelo et al. has drawn attention to changes in reference intervals in association with age and menopausal status (31). Bonfrer et al. reported an upper reference limit of 31 IU/ml in cases aged below 45 years and an upper reference limit of 21 IU/ml in cases aged above 55 years (32). The reference upper limit determined in the present study was considerably lower than quoted by the manufacturer. Some studies have reported similar, whereas others have reported highly different upper reference limits (24, 28, 31). The upper limit depends on age and other variables.

Many international studies have been conducted to determine reference intervals for fPSA and PSA. Researches from Asian countries showed that Asians have lower serum PSA levels than other ethnic groups (33, 34). For this reason, the PSA reference intervals provided by the manufacturer may not be applicable to Kahramanmaraş region. Liu et al. reported reference intervals that were different than those quoted by the manufacturer. They suggested that their reference intervals were more appropriate for Chinese males (33). Yang et al. highlighted variability in reference limits according to the diversity in the population, geographic characteristics, living conditions, measurement systems, and analytical methods (35). Upper limits determined in the present study are different than those reported in other studies. Although our data is not reflecting the whole Kahramanmaraş population, because the study was conducted in a well-defined area, it can serve as a basis for the region's reference intervals to be determined.

In a research on males and females, Nakamura et al. reported no significant change according to gender or age in reference intervals of TG (36). Giovanella et al. reported higher upper reference limits in females (37). Upper reference limit determined in the present study was considerably lower than quoted by the manufacturer; however, no gender-specific difference was observed. The paucity of data was the most important limitation while determining reference intervals for thyroglobuline. Despite limited number of data, the present study found reference interval that was close to that reported by other researchers (36, 37). Considering the very different TG reference intervals determined in the present study and those reported by other studies, we recommend avoiding the use of reference intervals quoted by the manufacturer.

The present study determined the reference intervals for AFP, CA 15.3, CA 19.9, CA 125, CEA, fPSA, PSA, and TG screening tests considering demographic characteristics and traditional dietary habits of the local population in the province of Kahramanmaraş, and the authors suggest that these results would provide more reliable alternative reference intervals and guide cancer specialists in screening of local population for tumours, diagnosis, postoperative follow-up, recognition of recurrences, and disease follow-up. Lowering of the upper limits of these tumour screening tests would increase test sensitivity and reduce the number of cases with missed malignancy. This approach may slightly reduce the specificity of screening tests. Nonetheless, missed diagnosis of malignancy is far more a severe medical problem than lower specificity of tumour markers.

This study has some limitations. The first limitation is its retrospective study, and for this reason our results may be vulnerable to various errors. Second, this study has small sample size particularly for some parameters. Thus, it may not have had enough statistical data. For more precise data, a prospective study based on a larger population is essential. And other one, these data for a long time may be affected by several factors such as manufacturers, devices, kits, and technician who performed the analysis.Lot-to-lot variability in reagents may cause effect on upper limits.Considering the findings pertaining to the parameters discussed above, we suggest that establishing reference intervals for screening tests by considering factors such as regional characteristics, ethnic origin, age, gender, and smoking status would substantially increase the efficiency of screening tests.

Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

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REFERENCES

1. Diamandis E, Fritche H, Lilja H, Chan D, Schwartz M. Tumor Markers: Physiology, Pathobiology, Technology and Clinical Applications. Washington, DC, USA: AACC Press; 2002.
2. Sturgeon CM, Lai LC, Duffy MJ. Serum tumour markers: how to order and interpret them. Brit Med J. 2009;339: b3527
3. Schilsky RL. Personalizing cancer care: American Society of Clinical Oncology presidential address 2009. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009; 27: 3725-30.
4. Virji MA, Mercer DW, Herberman RB. Tumor markers in cancer diagnosis and prognosis. CA: a cancer journal for clinicians. 1988; 38: 104-26.
5. Perkins GL, Slater ED, Sanders GK, Prichard JG. Serum tumor markers. American family physician. 2003; 68:1075-82.
6. Cheung KL, Graves CR, Robertson JF. Tumour marker measurements in the diagnosis and monitoring of breast cancer. Cancer treatment reviews. 2000; 26: 91-102.
7. Farinati F, Marino D, De Giorgio M, Baldan A, Cantarini M, Cursaro C, et al. Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? Am J Gastroenterol 2006; 101:

524-32.

8. 8. Strom BL, Maislin G, West SL, Atkinson B, Herlyn M, Saul S, et al. Serum CEA and CA 19-9: potential future diagnostic or screening tests for gallbladder cancer? International Journal of Cancer. 1990; 45: 821-4.

9. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, et al. American society of clinical oncology 2007 update of recommendations for the use of tumor markers in breast cancer. Journal of Clinical Oncology. 2007; 25: 5287-312.

10. Rustin GJ, van der Burg ME, Griffin CL, Guthrie D, Lamont A, Jayson GC, et al. Early versus delayed treatment of relapsed ovarian cancer (MRC OV05/EORTC 55955): a randomised trial. Lancet. 2010;376: 1155-63.

11. 11. Smerage JB, Barlow WE, Hortobagyi GN, Winer EP, Leyland-Jones B, Srkalovic G, et al. Circulating tumor cells and response to chemotherapy in metastatic breast cancer: SWOG S0500. Journal of clinical oncology : J Clin Oncol 2014; 32: 3483-9.

12. Tatarinov YS. Content of embryo-specific alpha-globulin in fetal and neonatal sera and sera from adult humans with primary carcinoma of the liver. Fed Proc Transl Suppl. 1966; 25: 344-6.

13. Abelev GI. Alpha-fetoprotein in ontogenesis and its association with malignant tumors. Adv Cancer Res. 1971; 14: 295-358.

14. Nishi S, Watabe H, Hirai H. Production of antibody to homologous -fetoprotein in rabbits, rats and horses by immunization with human -fetoprotein. J Immunol. 1972; 109: 957-60.

15. Schiffman JD, Fisher PG, Gibbs P. Early detection of cancer: past, present, and future. Am Soc Clin Oncol Educ Book. 2015:57-65.

16. Scara S, Bottoni P, Scatena R. CA 19-9: Biochemical and Clinical Aspects. Adv Exp Med Biol. 2015; 867: 247-60.

17. Kabawat SE, Bast RC, Bhan AK, Welch WR, Knapp RC, Colvin RB. Tissue distribution of a coelomic-epithelium-related antigen recognized by the monoclonal antibody OC125. Int J Gynecol Pathol. 1983; 2: 275-85.

18. Rossing RM, Jentzen W, Nagarajah J, Bockisch A, Gorges R. Serum Thyroglobulin Doubling Time in Progressive Thyroid Cancer. Thyroid. 2016; 26: 1712-8.

19. Solberg HE. International Federation of Clinical Chemistry (IFCC), Scientific Committee, Clinical Section, Expert Panel on Theory of Reference Values, and International Committee for Standardization in Haematology (ICSH), Standing Committee on Reference Values. Approved Recommendation (1986) on the theory of reference values. Part 1. The concept of reference values. Journal of clinical chemistry and clinical biochemistry Zeitschrift fur klinische Chemie und klinische Biochemie. 1987; 25: 337-42.

20. Wayne PA. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline. Clinical and Laboratory Standards Institute. 2010.

21. Qin X, Lin L, Mo Z, Lv H, Gao Y, Tan A, et al. Reference intervals for serum alpha-fetoprotein and carcinoembryonic antigen in Chinese Han ethnic males from the Fangchenggang Area Male Health and Examination Survey. The International Journal of Biological Markers. 2011; 26: 65-71.

22. Zhang GM, Guo XX, Ma XB, Zhang GM. Reference Intervals of Alpha-Fetoprotein and Carcinoembryonic Antigen in the Apparently Healthy Population. Med Sci Monit 2016;22:4875-80.

23. Yan C, Yang J, Wei L, Hu J, Song J, Wang X, et al. Serum reference interval of ARCHITECT alpha-fetoprotein in healthy Chinese Han adults: Sub-analysis of a prospective multi-center study. Clin Biochem. 2018;52:164-6.

24. Bjerner J, Hogetveit A, Wold Akselberg K, Vangsnes K, Paus E, Bjoro T, et al. Reference intervals for carcinoembryonic antigen (CEA), CA125, MUC1, Alfa-foeto-protein (AFP), neuron-specific enolase (NSE) and CA19.9 from the NORIP study. Scan J Clin Lab Invest 2008; 68: 703-13.

25. Ri G, Ohno S, Yamamoto T, Ito E, Furutani M, Furutani Y, et al. Serum Levels of CA15-3, KL-6 and BCA225 Are Positively Correlated with Each Other in the General Population. Anticancer Res. 2009; 29: 4239-42.

26. Sagi-Dain L, Lavie O, Auslander R, Sagi S. Clinical Use and Optimal Cutoff Value of Ca15-3 in Evaluation of Adnexal Mass: Retrospective Cohort Study and Review of the Literature. Am J Clin Oncol. 2018; 41: 838-44.

27. Zhang GM, Bai SM, Zhang GM, Ma XB. Reference intervals of carbohydrate antigen 19-9 in the apparently healthy adult population. J Clin Lab Anal 2018; 32: e22380.

28. Woo HY, Kim YJ, Park H. Establishment of reference intervals of tumor markers in Korean adults. Korean J Lab Med. 2008; 28: 179-84.

29. Behbehani AI, Mathew A, Farghaly M, van Dalen A. Reference levels of the tumor markers carcinoembryonic antigen, the carbohydrate antigens 19-9 and 72-4, and cytokeratin fragment 19 using the Elecsys Relecsys 1010 analyzer in a normal population in Kuwait. The importance of the determination of local reference levels. Int J Biol Markers 2002; 17: 67-70.

30. Lao X, Yang D, Mo Z, Gao Y, Deng Y, Qin X, et al. Reference Intervals for Alpha-Fetoprotein (AFP) and Carcinoembryonic Antigen (CEA) in Guangxi Zhuang Ethnic Males from the FAMHES Project. Clin Lab 2016; 62: 955-61.

31. Barcelo B, Ayllon O, Belmonte M, Barcelo A, Vidal R, Forteza-Rey J, et al. Proposed reference value of

the CA 125 tumour marker in men. Potential applications in clinical practice. Clin Biochem 2008; 41: 717-22.

32. Bonfrer JM, Korse CM, Verstraeten RA, van Kamp GJ, Hart GA, Kenemans P. Clinical evaluation of the Byk LIA-mat CA125 II assay: discussion of a reference value. Clin Chem 1997; 43: 491-7.
33. Liu ZY, Sun YH, Xu CL, Gao X, Zhang LM, Ren SC. Age-specific PSA reference ranges in Chinese men without prostate cancer. Asian J Androl. 2009; 11: 100-3.
34. Lee SE, Kwak C, Park MS, Lee CH, Kang W, Oh SJ. Ethnic differences in the age-related distribution of serum prostate-specific antigen values: a study in a healthy Korean male population. Urology. 2000; 56: 1007-10.
35. Yang J, Tang A, Zhang S, Sun X, Ming L. The age-specific reference intervals for tPSA, fPSA, and %fPSA in healthy Han ethnic male. J Clin Lab Anal 2016.
36. Nakamura S, Sakata S, Minamori Y, Komaki T, Kojima N, Kamikubo K, et al. Serum thyroglobulin (Tg) concentration in healthy subjects: absence of age- and sex-related differences. Endocrinologia japonica. 1984; 31: 93-8.
37. Giovanella L, Imperiali M, Ferrari A, Palumbo A, Furlani L, Graziani MS, et al. Serum thyroglobulin reference values according to NACB criteria in healthy subjects with normal thyroid ultrasound. Clin Chem Lab Med 2012; 50: 891-3.