RESEARCH ARTICLE

Reference intervals of carbohydrate antigen 19-9 in the apparently healthy adult population

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Background: To establish reference intervals of carbohydrate antigen 19-9(CA 19-9) according to the CLSI CA28-A3 guideline and to evaluate age- and gender-related variations.

Methods: Serum CA 19-9 values of 10 149 healthy subjects (from 20 years old to 60 years old) were measured from location health checkups. The relationship between CA 19-9 and age was analyzed using Spearman's approach. The reference intervals of CA19-9 were established using $Q_{2.5}$ and $Q_{97.5}$, and the 90% confidence intervals of upper limits were calculated.

Results: The reference intervals of CA 19-9 were 1.98-25.12 U/mL for males (1.97-25.06 U/mL for 20-50 years old and 2.31-26.13 U/mL for 50-60 years old) and 2.36-29.29 U/mL for adult (20-60 years old) females. The upper limit of reference intervals for all individuals was 26.45 U/mL; the level of CA 19-9 is higher in females than males. Carbohydrate antigen (CA) 19-9 is significantly associated with aging in adult males($r = .0930$, $P < .0001$), but not in females ($P = .4734$).

Conclusions: Establishing reference intervals for CA19-9 and giving age-related reference intervals of CA19-9 using a big data of healthy adult, we first discovered that CA19-9 tends to increase with age in adult males but not in females.

KEYWORDS

cancer antigen 19-9, carbohydrate antigen 19-9, reference intervals

1 | INTRODUCTION

Carbohydrate antigen 19-9(CA 19-9) is called cancer antigen 19-9, cancer antigen-GI (CA-GI), or salivated Lewis an antigen. CA 19-9 was discovered by Koprowski H et al in 1979,¹ and then, it has been shown to be produced by pancreatic carcinoma.² Serum CA 19-9 is most widely used to assess the progress and prognosis of several kinds of cancers in adults, such as pancreatic cancer, gallbladder cancer, bile duct cancer, and gastric cancer.³ For example, CA 19-9 is used to help evaluate and monitor with which type of tumor (eg, bile duct cancer, some malignant germ cell tumors and immature teratomas)⁴ and evaluate and diagnosis pancreatic cancer⁵; in combination with CEA, for diagnosis and monitor with colorectal and gastrointestinal cancer, patients are undergoing treatment.⁶

However, CA 19-9 is also secreted by normal biliary epithelia. It could be markedly elevated in the case of biliary obstruction, biliary infection, and inflammatory processes caused by benign biliary stricture extra-pancreatic malignancies.^{7,8} Moreover, the tested level of serum CA 19-9 will be affected by the detection system, geographical location, genotype, living standard, ethnicity, and many other factors. Thus, the reference intervals of serum CA 19-9 could be variable in different population. However, most Chinese laboratories use the CA 19-9 reference intervals either provided by abroad (Europe and the United States) laboratories or the reagent manual. These reference intervals may not be accurate for Chinese population in clinical application. To set up a more specific and reliable reference intervals of CA 19-9 for Chinese population, we measured serum CA 19-9 in a large number of healthy adult subjects and established the reference intervals of CA 19-9 according to CLSI CA28-A3.⁹

Shu-Mei Bai and Guo-Ming Zhang authors are co-first authors and contributed equally to this work.

FIGURE 1 Establishing reference intervals of CA 19-9 data analysis protocol on the basis of CLSI guidelines document CA28-A3

2 | MATERIAL AND METHODS

2.1 | Materials and methods

From December 2013 to December 2016, 10 149 healthy populations were chosen from the center of health checkups of Shuyang People's Hospital, focusing on the exclusion of digestive system (such as complete blood counts, serum total serum bilirubin, positive HBV surface antigen, serum ALT, serum creatinine), ultrasonography for liver, biliary system, spleen, pancreas, prostate, breast and the uterus and accessory organs, and data from questionnaires). Specimens of serum were fasting venous blood from elbow vein of anterior. CA 19-9 values were measured by chemiluminescence immunoassay on Beckman DXI system 800 immunoassay analyzers (Beckman Co., Ltd., USA). The detection of lower and upper limits of the assays for CA 19-9 is 0.8 U/mL and 1950 U/mL. Regular quality control procedures are operated every day in the laboratory medicine of Shuyang People's Hospital, and the external quality assessment scheme of Jiangsu center for clinical laboratories is performed twice a year to validate the measurement quality of CA 19-9 results.

The Ethics Committee of Shuyang People's Hospital approved this study. Reference samples were chosen according to CLSI CA28-A3 guideline and a previous report.^{9,10}

2.2 | Statistical analysis

CA 19-9 data were pooled and showed, using nonparametric percentile method (95% double-sided, Tucker test for outliers, Box-Cox transformation, lambda shift parameter came from data) reference intervals according to CLSI CA28-A3 guidelines in Figure 1.¹¹ And all data analyses were performed using EXCEL (Armonk, NY, USA) and MedCalc Version 17.0.4 (MedCalc Software, Ostend, Belgium). We calculated 90% confidence intervals for the lower limit and upper limit and age-related reference intervals (a reference interval varies with the subjects' age.) The Mann-Whitney *U* test was used to determine CA 19-9 of a significant gender group. One-way analysis of variance (ANOVA) test was used to determine CA 19-9 for trending age-groups. Correlation between variables was assessed by spearman's correlation.

FIGURE 2 The histograms of CA 19-9 and age for females and males

3 | RESULTS

In total, 9436 CA 19-9 tests were drawn and analyzed, including 3284 females and 6152 males. The results of CA 19-9 in our study were not a normal distribution, and the reference intervals of CA 19-9 according to CLSI CA28-A3 guideline are shown in Figure 1. The values of CA 19-9 for females were significantly higher than males ($P < .0001$). Figure 2 shows a histogram of CA 19-9 and age distributions in adult subjects. CA 19-9 data after Box-Cox transformation in females and males are normality distribution ($P = .7049$ and $P = .2447$, respectively). The reference intervals of CA 19-9 are listed in Table 1.

As shown in Figure 3, the values of CA 19-9 were significantly positively correlated with age ($P < .001$) for males. However, the levels of CA 19-9 were not correlated with age ($P = .4734$) for females. Table 2 shows median value of CA 19-9 consistently increased across different age-groups $P < .001$ for trend in males, but not in females.

Figure 4 shows age-related reference intervals of CA 19-9 concentration for gender groups.

In the end, the reference intervals of CA 19-9: 1.97-25.06 U/mL for 20-50 years old and 2.31-26.13 U/mL for 20-50 years old for males, 2.36-29.29 U/mL for adult females.

TABLE 1 Reference intervals of CA 19-9 for apparently healthy adult population

Gender	n	Median	Lower limit	Upper limit
Female	3284	7.45	2.36 90% CI:2.30-2.43	29.29 90% CI:28.1-30.70
Male	6152	6.60	1.98 90% CI:1.94-2.03	25.12 90% CI:24.53-25.81
Total	9436	6.90	2.09 90% CI:2.05-2.13	26.45 90% CI:25.90-27.00

CI, confidence interval.
The unit of CA19-9 is U/mL.

FIGURE 3 Spearman's correlation between CA 19-9 and age and scatter plots of CA 19-9 for females and males

FIGURE 4 Age-related reference intervals of carbohydrate antigen 19-9 (CA19-9). The red line represents the 50th percentile; the lower and upper green lines represent the 2.5th and 97.5th percentiles. The unit of CA19-9 is U/mL

TABLE 2 Reference intervals of CA 19-9 for the different age-group and gender

Age-group (y)	Female			Male			P-values ^a
	n	Median	Lower limit	n	Median	Lower limit	
20-30 ^b	1193	7.7	2.28 90% CI:2.10-2.50	2150	6.4	1.87 90% CI:1.80-1.94	<.001
31-40 ^b	905	7.4	2.40 90% CI:2.27-2.53	1551	6.1	1.88 90% CI:1.80-1.96	<.001
41-50 ^b	806	7	2.30 90% CI:2.19-2.43	1449	6.8	2.15 90% CI:2.06-2.25	.006
20-50	2904	7.4	2.36 90% CI:2.30-2.43	5150	6.4	1.94 90% CI:1.89-1.98	<.001
51-60	380	7.8	2.35 90% CI:2.15-2.56	1002	7.5	2.32 90% CI:2.20-2.45	.157
P-values ^b for trend	.400			<.001 (Different ^b (P<.05) from comparing with 51-60)			

CI, confidence interval.

^aMann-Whitney U test was used to determine CA 19-9 of a significant gender group.

^bOne-way analysis of variance (ANOVA) test was used to determine CA 19-9 (Median and interquartile range) for trending age-groups.

The unit of CA19-9 is U/mL.

4 | DISCUSSION

The values of CA 19-9 and the different gender and age were not normal in this study, and then, nonparametric method using 95% (double-sided) reference intervals according to CLSI EP28-A3 guidelines were used to establish the reference intervals of CA 19-9 for apparently healthy adult individuals. We found that the upper reference interval limit (URIL) is 26.45 U/mL and the median (and interquartile range) of CA19-9 for females was higher than males, which is consistent with a previous report.¹² To the best of our knowledge, the present study is the first discovery that the serum CA 19-9 was significantly positively correlated with age in adult males, but not for females. This difference may be associated with the uterus, and ovaries of women are releasing CA125 and CA153. Therefore, CA 19-9 reference intervals of different gender and ages should be established.

In the individualized medical care for the person in the current era, providing their past test reports or baseline value (or healthy value) is especially important for assessing the current test results. If these test reports were first tested, the current report should be marked with detection system and the reference intervals of test. In the present study, we also found that CA 19-9 of the males was positively correlated with age (Figure 3), a tendency of increase with age (Table 2). As shown in Table 2 and Figure 4, different reference intervals for male subjects under 50 and over 50 years of age should be established, respectively. However, the CA 19-9 of females was not correlated with age (Figure 3). So, the reference intervals of CA 19-9 for females have not established different reference intervals. In a word, establishing age-related reference intervals of CA 19-9 was necessary.¹³

There are three limitations in this study. First, this was used a single detection system for Han and Shuyang regions study. Second, the individuals were apparently healthy adult subjects in this study. Third, smoking and genotype were not considered.

In summary, establishing reference intervals for CA19-9 and giving age-related reference intervals of CA19-9 from using a big data of adult healthy, we first discovered that CA19-9 tends to increase with age in adult males but not in females. If a test report was first tested, the report should be marked with detection system and the reference intervals and age-related reference intervals of test.

AUTHORS' CONTRIBUTION

Guo-Ming Zhang designed this study. All authors collected data, performed analysis, and drafted the manuscript. All authors read and approve the final manuscript.

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