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ORIGINAL RESEARCH

Preliminary Study on Reference Interval of Serum Pepsinogen in Healthy Subjects

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Objective: This study determined the reference interval of pepsinogen (PG) of healthy people in the local region to provide a basis for early screening of gastric cancer.

Methods: Among the healthy people who underwent a physical examination in our hospital from January 2020 to December 2020, 2568 subjects were selected based on the relevant screening criteria. Their serum PG I and II levels and PG I:PG II ratio were determined by chemiluminescent microparticle immunoassay (CIMA), and the results were statistically analyzed. Finally, according to document CLSI-C28-A3, the PG reference interval of the local region was determined.

Results: The PG I and II levels of the males in all age groups were higher than those of the females in the corresponding age groups, and the differences were statistically significant ($P < 0.05$). However, the differences in the PG I:PG II ratio between the genders in the different age groups were not statistically significant ($P > 0.05$). The PG I and II levels increased with age in both men and women, while the PG I:PG II ratio was not correlated with age in either gender.

Conclusion: The PG reference interval of the local region was initially determined as providing a reliable reference basis for clinical treatment.

Keywords: pepsinogen, reference interval, chemiluminescent microparticle immunoassay, CLSI-C28-A3

Introduction

Gastric cancer is one of the most common malignant tumors in China. The five-year survival rate of early gastric cancer is above 90%, but that of advanced gastric cancer is approximately 20%. Therefore, early diagnosis of gastric cancer plays a vital role in its diagnosis and treatment.[1,2] About half of all patients with early gastric cancer have no symptoms or signs, making diagnosis more difficult. At present, the diagnosis of gastritis or gastric cancer mainly depends on endoscopy and biopsy. However, these methods are invasive, consume copious human and material resources, and are not easily accepted by patients.[3,4] Pepsinogen (PG) is the precursor of pepsin, considered the "serological biopsy" of the gastric mucosal state. The serum PG can directly reflect the functional state and morphology of the gastric mucosa as well as the number of glands and cells and is an important serum marker for the diagnosis of atrophic gastritis and monitoring of early gastric cancer.[5–7] Some studies have shown that there are obvious differences in PG among healthy subjects in different countries and regions. It may be due to the different detection methods and reagents, the differences in the HP infection situation of the population in each region, gender, age composition and other factors.[8,9] At present, there are various methods for detecting PG, including

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Received: 22 July 2021
Accepted: 2 November 2021
Published: 9 December 2021

ELISA, radioimmunoassay, time-resolved fluorescence immunoassay, immunoturbidimetry, etc. However, the levels of PG detected by different laboratory testing methods are quite different. In addition, most previous studies only used normal gastric mucosa as healthy subjects, and the effects of smoking, alcohol abuse, Helicobacter pylori infection, and other infectious factors (such as inflammation, infection and other related diseases) have not been excluded, and most of the literature reports failed to refine the reference interval according to different genders and different age groups.

As the serum PG level may be affected by geographical, ethnic, and lifestyle factors, the optimal reference interval for gastric cancer screening has not yet been determined, so the clinical application value of PG is low. In view of this objective reality, it is imperative to determine the PG reference interval of the local region.

Subjects and Methods

Subjects

A total of 2568 healthy people examined in our hospital from January 2020 to December 2020 were included in this study. Their ages ranged from 17 to 90 years old, with a median age of 48 years. The ages of the 1368 male subjects ranged from 17 to 90 years old, with a median age of 46 years, while the ages of the 1200 female subjects ranged from 18 to 88 years old, with a median age of 49 years. The difference in age between the genders was not statistically significant ($P > 0.05$). According to the literature,[10] these subjects were divided into six groups: the 17–30 years old group, 31–40 years old group, 41–50 years old group, 51–60 years old group, 61–70 years old group and ≥71 years old group. None of the subjects had a history of digestive system diseases and proton pump inhibitors (PPIs) medication. The disease history of the cardiovascular, blood, endocrine, urinary systems, smoking, alcohol abuse, Helicobacter pylori infection and other infectious factors (such as inflammation, infection and other related diseases) was excluded.

Instruments and Reagents

An Abbott ARCHITECT i2000 automatic chemical analyzer and matching reagents, standards, and quality controls were used to detect the PG. This study had stable internal quality control and good external quality assessment as a guarantee of accuracy.

Methods

The subjects lived regularly for three days without performing strenuous exercise. After the subjects rested for 30 minutes, the fasting (>12 h) serum was collected, placed at room temperature for 30 min, and centrifuged at 1200 g for 10 min; 1 mL was reserved and detected by the automatic chemical analyzer.

Statistical Analysis

The statistical analysis was conducted using statistical software SPSS 19.0. Normally distributed measurement data were compared between two groups using the *t*-test and compared among multiple groups using the analysis of variance. Pairwise comparison was conducted using the SNK-q test. Non-normally distributed measurement data were compared among multiple groups using the Kruskal–Wallis *H*-test and compared between two groups using the Mann–Whitney *U*-test. A P-value <0.05 was considered statistically significant. The 95% reference interval was determined according to CLSI-C28-A3.[11]

Results

Test of Normality of PG I and II Levels and PG I:PG II Ratio and Comparison Between Genders

The subjects' PG I and II levels and PG I:PG II ratio were tested by the Kolmogorov–Smirnov test. The results revealed that the PG I and II levels were normally distributed and that the PG I:PG II ratio was non-normally distributed. The PG I and II levels of the males in all age groups were higher than those of the females in the corresponding age groups, and the differences were statistically significant ($P < 0.05$). Meanwhile, the differences in the PG I:PG II ratio between the genders in the different age groups were not statistically significant ($P > 0.05$, Table 1).

Correlation Between PG I and PG II Levels and PG I:PG II Ratio and Age

According to the statistical analysis of the PG I and II levels and PG I:PG II ratio in the different male age groups, the differences in the PG I and II levels between the 17–30 years old group and 31–40 years old group were not statistically significant ($P > 0.05$). The differences among the 41–50, 51–60 and 61–70 years old groups were also not statistically significant ($P > 0.05$). However, the levels of any of the 41–50, 51–60 and 61–70 years old groups were higher than those of the 17–

Table 1 Results of PGI, PGII, PGI:PGII Ratio in Each Age Group

Group (Year)	PGI (μg/L)			PGII (μg/L)			PGI:PGII Ratio		
	Male	Female	$P^{\&}$ value	Male	Female	P^{*} value	Male	Female	$P^{\#}$ value
17 ~ 30	45.63±18.31[a]	40.56±16.25[a]	<0.05	7.12±2.05[a]	6.94±2.01[a]	<0.05	7.02 (5.21,9.16)[c]	7.02 (5.32,8.97)[d]	>0.05
31 ~ 40	46.74±20.03[a]	42.78±18.54[a]	<0.05	7.37±2.91[a]	7.08±2.48[a]	<0.05	7.05 (5.32,9.87)[c]	7.04 (5.51,9.98) [d]	>0.05
41 ~50	57.81±23.29[b]	53.75±23.12[b]	<0.05	9.45±3.76[b]	9.04±3.57[b]	<0.05	7.10 (5.31,9.89) [c]	7.09 (5.50,10.11) [d]	>0.05
51 ~ 60	59.48±24.91[b]	54.83±24.08[b]	<0.05	9.93±3.82[b]	9.38±3.83[b]	<0.05	6.95 (5.29,9.93) [c]	7.02 (5.47,10.07) [d]	>0.05
61 ~ 70	62.67±26.12[b]	56.62±26.15 [b]	<0.05	10.12±4.76[b]	9.65±4.75[b]	<0.05	7.06 (5.23,10.95) [c]	7.01 (5.42,10.21) [d]	>0.05
≥71	69.72±30.39	62.87±29.04	<0.05	11.19±5.06	10.67±4.89	<0.05	7.08 (5.29,11.89) [c]	7.09 (5.50,11.11) [d]	>0.05
Total	59.93±24.08	53.28±23.60	<0.05	10.25±4.34	9.53±4.29	<0.05	7.05 (5.21,11.89)	7.04 (5.32,11.11)	>0.05

Notes: [a]Comparison of the same sex, there were no significant differences in PGI and PGII between 17~30 years group and 31~40 years group (P>0.05). [b]Comparison of the same sex, there were no significant differences in PGI andPGII among the 41~50 years group, 51~60 years group and 61~70 years group(P>0.05). The PGI and PGII of 41~50 years group, 51~60 years group and 61~70 years group were higher than those of 17~30 years group and 31~40 years group (P<0.05). And The PGI and PGII of ≥71 years group were also higher than the above five age groups (P<0.05). [c]There was no significant difference in PGI:PGII ratio among the males of different age groups(P>0.05). [d]There was no significant difference in PGI:PGII ratio among the females of different age groups (P>0.05). [*,&,#]Comparison between male and female in the same age group (year). [c,d] Comparison of different age groups in the same sex.

Abbreviations: PGI, pepsinogen I; PGII, pepsinogen II.

30 and 31–40 years old groups ($P < 0.05$). The levels of the ≥71 years old group were also higher than those of the other five age groups ($P < 0.05$). There was no difference in the PG I:PG II ratio among the different age groups of either gender ($P > 0.05$, Table 1). The results of the PG I and II levels and PG I:PG II ratio in the different female age groups were the same as those in the corresponding male age groups.

Reference Interval

According to the results in Table 1, the age groups without differences were combined, and the male and female subjects were respectively divided into the 17-40 years old group, 41–70 years old group and ≥71 years old group according to their PG I and II levels. Since the differences in the PG I:PG II ratio between the genders and among the age groups were not statistically significant, the subjects were assigned to one group according to the PG I:PG II ratio. According to CLSI-C28-A3, the 95% distribution range of the PG I and II levels was calculated with ±1.96 standard deviation, and the 95% distribution range of the PG I:PG II ratio was calculated with the percentile. The 95th percentile (P95) was regarded as the upper limit of the reference interval, and the P5 was regarded as the lower limit of the reference interval. The results are presented in Table 2.

Discussion

At present, PG remains a useful serological marker for the diagnosis of atrophic gastritis, with the advantages of high sensitivity, non-invasiveness, and low cost. Many countries have listed it as one of the indicators for monitoring gastric cancer. To accurately evaluate test results, the PG reference interval should be determined scientifically.[12,13] However, the detection method used and gender, age, race, and eating habits of the subjects directly affect the PG level, resulting in a certain deviation in the PG evaluation of gastric mucosal function. Currently, there are few authoritative reports on the reference interval of PG detection by chemiluminescent microparticle immunoassay (CMIA) in healthy Chinese people.[9] Although CMIA detection of PG has been proven to be reliable, due to the lack of systematic large-sample studies, there is no credible PG reference interval, which limits the clinical application of PG. Therefore, it is necessary to determine the PG reference interval in CMIA detection. Moreover, the grading reference interval should be established according to gender and age to provide evaluation criteria for the diagnosis, treatment, and prevention of atrophic gastritis, gastric ulcer, and gastric cancer as well as accumulate abundant data for large-sample studies in China.

Table 2 Reference Interval of PGI, PGII and PGI/PGII Ratio in Healthy Subjects

Project	Gender	Age (Year)	$\bar{x}\pm s$ or P_5~ P_{95}	Reference Interval
PGI (μg/L)	Male	17~40 41 ~ 70 ≥71	45.26±21.47 59.86±28.54 69.72±36.39	35.62 ~ 75.39 41.67 ~ 109.51 48.62 ~ 129.35
	Female	17~40 41 ~ 70 ≥71	41.73±20.85 55.04±27.15 62.87±35.04	32.69 ~ 73.08 39.05 ~ 99.59 45.79 ~ 118.64
PGII (μg/L)	Male	17~40 41 ~ 70 ≥71	7.35±2.86 9.84±3.96 11.19±6.06	3.89 ~ 11.98 4.21 ~ 16.16 4.59 ~ 20.68
	Female	17~40 41 ~ 70 ≥71	6.98±2.48 9.41±3.75 10.67±6.89	3.51 ~ 11.20 4.05~ 15.14 4.39 ~ 18.90
PGI/PGII	Male and Female	≥17	1.26 ~ 12.98	≥7.04

Abbreviations: PGI, pepsinogen I; PGII, pepsinogen II.

At present, most studies only use normal gastric mucosa as the standard for healthy people, which is inappropriate. The 2568 subjects investigated in this study were chosen based on reasonable exclusion criteria to eliminate the impacts of smoking, alcohol abuse, *Helicobacter pylori* (HP) infection, proton pump inhibitors (PPIs) medication, and other infectious factors, such as inflammation and other related diseases. This ensured the true reflection of the relationship between PG and several medical indicators in the normal state. This study revealed that as the age increased, the PG level also increased. Moreover, the PG level in men was higher than that in women of the same age, as confirmed in previous studies. However, the PG I:PG II ratio had no correlation with age or gender, differing from the results reported by Yiming Zhong.[19] This may have been related to the facts that the subjects in the previous studies were not healthy and different detection methods were used. This study demonstrated that PG I and PG II maintain a certain balance in the normal physiological state that is not affected by age or gender.

The incidence of gastric cancer differs between the genders and among different age groups (eg, the incidence of gastric cancer in people ≤40 years old is significantly different from that in people >40 years old). Therefore, gender and age should be considered when PG is used to detect gastric cancer. Although the decrease of the PG I:PG II ratio can better reflect the state of atrophic gastric mucosa than the PG I level, this level is also increased in some patients with non-atrophic gastritis, HP infection, and gastric ulcer infection.[14,15] Thus, the appropriate reference interval of PG I could be used for the differential diagnosis of atrophic gastritis. In this study, the PG I and PG II levels and PG I:PG II ratio of different age groups were statistically analyzed; the age groups without significant differences were combined, and, finally, the reference intervals of the different gender and age groups were established. At present, in most studies, subjects are roughly grouped according to the age standard recommended by the World Health Organization.[16] However, in this study, the subjects were grouped based on a statistical analysis of their age and gender differences, so the reference intervals were more detailed. This could improve the level of laboratory diagnosis and quality of medical care and large-scale screening. In addition, the PG reference intervals reported in different countries also differ. For example, in Japan, the reference intervals of PG detection by radioimmunoassay are PG I ≤70 ng/mL and PG I:PG II ≤3.0.[17,18] Moreover, in the Republic of Korea, the reference intervals of PG I and II in 521 HP-negative healthy subjects studied by Kim were lower than those in the present study.[9] Currently, PG I >70 ng/mL and PG I:PG II >3.0 are the most commonly used reference intervals reported in Japanese studies, but these values were far different in the present study. The reference interval determined here could alleviate patient concerns caused by false abnormal test results (not within the reference interval provided by the manufacturer), effectively improve the level of diagnosis and treatment, and reduce patients' economic and psychological burden. There were also several differences between the reference intervals determined in this study and those determined by Xin Wang, Yiming Zhong, and Meng Huang, possibly due to the different geographical locations, climatic conditions, and lifestyles of the investigated populations. In addition, the different

methodology used may have been another reason. As this is a single-center study, the statistical results may differ from those of other regions. In the next step, we plan to add more centers to enrich clinical data and develop a more scientific and reasonable PG reference interval to make up for the shortcomings of this study.

In summary, this is the first systematic study on the PG level (CMIA) of the healthy population in the local region according to the CLSI-C28-A3 standard. The reference intervals of the different genders and age groups determined in this study are useful and reliable and can be used in clinics and laboratories. It is believed that these reference intervals have significant clinical value.

Ethics Approval and Consent to Participate

This study was conducted with approval from the Ethics Committee of First People's Hospital of Linping District, Hangzhou. This study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants. And the patient guardians under 18 years of age provided informed consent.

Acknowledgments

We would like to acknowledge the hard and dedicated work of all the staff that implemented the intervention and evaluation components of the study.

Funding

There is no funding to report.

Disclosure

The authors declare that they have no competing interests.

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