

Confirming the reference interval of several biochemical testing value

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Summary

Objective: To assess the applicability of the reference interval of glucose, cholesterol, triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), urea, creatinine values in use at the Department of Clinical Biochemistry - 108 Military Central Hospital. **Subject and method:** 40 healthy individuals getting medical checkups at the 108 Military Central Hospital. **Result and conclusion:** The diagnosis values of glucose, urea, creatinine, AST, ALT and GGT of 20 healthy individuals were all within the reference intervals of the manufacturer. The diagnosis values of triglyceride, cholesterol: For the first analysis, the results of 17 out of 20 participants were within the manufacturer's reference intervals; for the second analysis, the results of 20 out of 20 healthy individuals were within the manufacturer's reference intervals. The reference intervals provided by the manufacturer for the diagnosis value of glucose, urea, creatinine, triglyceride, cholesterol, AST, ALT and GGT were suitable for the Department of Biochemistry - 108 Military Central Hospital.

Keywords: Value, reference interval, 108 Military Central Hospital.

1. Background

Reference interval is the typical distribution range of values in a biological reference population. According to international seminars and the guideline of the International Federation of Clinical Chemistry (IFCC), nowadays, terms such as "reference value" or "reference interval" are more commonly used than term "normal value". This is the case as using these terms drastically reduce the complications encountered when using the term "normal". It is important to understand that if the testing value of an individual lies outside the range of the reference interval, it does not necessarily mean the individual is sick. Furthermore, reference interval is merely a statistical concept that covers 95% of the reference population. This simply means

that, according to the definition, 5% of the completely healthy population will have results outside the reference interval, of which, 2.5% will yield higher results and in contrast, 2.5% will yield lower results. These individuals are not necessarily sick, only different from the majority [6].

Reference interval, for testing value in general and for biochemical testing value in particular for Vietnamese, has been developed since the 90s [4]. Nevertheless, the changing living conditions of modern time have had noticeable effect on the physical condition of Vietnamese. Moreover, modern, automatic testing methods have also gradually replaced conventional, semi-automatic methods. Because of this, many laboratories nowadays are using different sources of reference interval. There are many different sources and documents regarding reference intervals available for consultation (Instructions of the Ministry of Health, domestic and international documents, manufacturers of chemicals

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and equipment...). However, these biological reference interval were researched on European, hence, they are not applicable to the physical condition of Asian in general and of Vietnamese in particular. Therefore, laboratories need to confirm the accuracy of reference intervals before apply them. In order to deal with the aforementioned problems, and to meet the requirement of the ISO 15189 : 2012 standard, we have conducted the research "Confirming the reference interval of several biochemical testing value" with an aim to: *To assess the applicability of the reference interval of glucose, cholesterol, triglyceride, aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, urea, creatinine values on AU5800 Chemistry Analyzers in use at the Department of Clinical Biochemistry - 108 Military Central Hospital.*

2. Subject and method

2.1. Subject

Study group: 40 healthy individuals who came in for medical checkups at the 108 Military Central Hospital in September 2018.

Inclusion criteria: The patients were concluded to be healthy, not suffering from chronic diseases, have been clinically examined, have a good medical records and have been through screening tests (ultrasound, CT, X-ray...), ECG, willing to participate in the research.

Exclusion criteria: The patient were not healthy or unwilling to participate in the research.

2.2. Method

Study design: Conduct cross-sectional descriptive research and comparing the results with the reference values in use at the Department of Clinical Biochemistry.

Method to define and determine Reference Intervals according to the guidelines of the Clinical and Laboratory Standards Institute (NCCLS document C28-A2) [6]:

After analyzing 20 samples with the method in used at the laboratory, the results recorded were processed as follow:

If 18 out of 20 samples analyzed yielded results within the chosen reference interval, that reference interval could be used.

If there are 3 or more samples analyzed that yielded results outside the chosen reference interval, collect and conduct analysis on 20 more samples.

If 36 out of 40 samples analyzed yielded results within the chosen reference interval, that reference interval could be used. If there are 5 or more samples that yielded results outside the chosen reference interval, collect and conduct analysis on 20 more samples.

If 54 out of 60 samples analyzed yielded results within the chosen reference interval, that interval could be used. If there are 7 or more samples that yielded results outside the chosen reference interval, that interval could not be used. The laboratory must therefore, considers using a different reference interval or establish its own reference interval.

Method of testing the values of glucose, cholesterol, triglyceride, AST, ALT, GGT, urea, creatinine and reference interval [5]:

2ml of blood was taken from the patients in early morning after fasting for 8 hours before the blood was taken.

The blood samples, after collected and stored in Li heparin antifreeze tube, were centrifuged at 3600rpm for 5 minutes before being analyzed on the Beckman Coulter (USA) AU5800 Chemistry Analyzers at the Department of Clinical Biochemistry - 108 Military Central Hospital. The reference intervals used were provided by the manufacturer.

Glucose test: Quantifying glucose by using the Hexokinase method. Reference interval: 3.9 - 6.4mmol/l.

Cholesterol test: Quantifying cholesterol using colorimetric method. Reference interval: below 5.2mmol/l.

Triglyceride test: Quantifying triglyceride using Enzymatic colorimetric method. Reference interval: below 1.7mmol/l.

AST test: Quantifying AST by using the enzyme kinetics method. Reference interval: below 50U/l.

ALT test: Quantifying ALT by using the enzyme kinetics method. Reference interval: Below 50U/l.

Urea test: Quantifying urea by using the enzyme kinetics method. Reference interval: 2.8 - 7.2mmol/l.

Creatinine test: Quantifying creatinine by using the Jaffe method. Reference interval: 64 - 104μmol/l.

Gamma glutamyl transferase test: Quantifying GGT by using the enzyme kinetics method. The reference interval is below 55U/l.

2.3. Data processing

The data is analyzed and processed using the medical statistics method on the program SPSS 20.0.

3. Result

3.1. General characteristics of the study group

3.1.1. Age distribution

Table 1. The age of the study group

	Age of the study group (Years)			p
	($\bar{x} \pm SD$)	Mean	Max	
Male (1)	53.2 ± 14.5	15	79	p ₁₋₂ >0.05
Female (2)	49.4 ± 17.8	21	74	
Average	51.3 ± 17.4	15	79	

Comment: The average age of the research group was 51.3 ± 17.4 (the youngest was 15, the oldest was 79). There was no difference between the average age of the two groups of male and female participants with p>0.05.

3.1.2. Gender distribution

Table 2. Gender distribution of the study group

Gender	Number of study group	Percentage (%)	p
Male (1)	20	50	p ₁₋₂ >0.05
Female (2)	20	50	
Average	40	100	

Comment: There was no difference in the male to female ratio with p>0.05.

3.2. The results confirmed the reference interval of the values of glucose, urea, creatinine, triglyceride, cholesterol, AST, ALT and GGT

Table 3. The first testing results of glucose, urea, creatinine, triglyceride, cholesterol, AST, ALT and GGT

TT	Glucose (mmol/l)	Urea (mmol/l)	Creatinine (μmol/l)	Triglyceride (mmol/l)	Cholesterol (mmol/l)	AST (U/l)	ALT (U/l)	GGT (U/l)
1	4.51	5.49	75	0.82	4.56	22	14	11
2	4.58	4.52	72	0.83	3.63	17	14	22
3	6.12	7.18	103	1.01	4.76	25	36	26
4	4.98	3.51	67	4.04	6.42	33	9	28

5	4.5	5.1	48	2.12	4.65	17	17	15
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Table 3. The first testing results of glucose, urea, creatinine, triglyceride, cholesterol, AST, ALT and GGT (Next)

TT	Glucose (mmol/l)	Urea (mmol/l)	Creatinine (μmol/l)	Triglyceride (mmol/l)	Cholesterol (mmol/l)	AST (U/l)	ALT (U/l)	GGT (U/l)
6	4.84	5.73	47	0.93	4.77	19	10	18
7	5.95	4.72	55	1.9	3.29	20	11	12
8	4.69	5.24	62	1.36	6.63	35	23	27
9	4.23	5.25	79	6.5	5.17	30	30	23
10	4.69	5.7	86	1.88	4.31	24	19	50
11	4.86	3.37	56	0.82	3.78	16	8	20
12	5.42	6.02	55	2.29	5.1	31	21	14
13	4.69	4.03	61	1.51	5.18	24	13	12
14	4.1	4.97	51	1.5	2.9	15	8	11
15	4.95	5.17	53	1.58	4.37	25	28	14
16	5.27	4.58	50	1.09	4.46	14	11	37
17	6.18	3.57	51	1.04	6.58	27	31	25
18	6.08	6.38	66	1.28	5.12	24	11	10
19	5.27	4.48	98	1.29	4.04	27	17	14
20	5.06	3.79	88	6.17	4.9	30	16	19

Comment: The diagnosis values of glucose, AST, ALT, GGT, urea, creatinine for 20 healthy individuals were all within the reference intervals of the manufacturer. The diagnosis values of cholesterol, triglyceride for 3 out of 20 healthy individuals yield results (numbers that are underline and in bold) outside the reference intervals of the manufacturer.

Table 4. The second testing results of triglyceride and cholesterol

TT	Triglycerid (mmol/l)	Cholesterol (mmol/l)
1	1.35	5.02
2	0.9	3.22
3	1.25	5.09
4	1.41	4.58
5	0.93	4.85
6	1.31	4.81
7	0.9	4.49
8	1.07	4.67
9	1.7	4.03
10	1.46	4.59
11	1.63	5
12	1.03	4.86
13	1.35	4.21
14	1.1	5
15	1.58	4.9
16	1.23	5.08
17	0.97	4.28
18	1.41	3.79
19	1.20	3.03

20	1.06	4.08
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Comment: The value analysis of cholesterol and triglyceride of 20 healthy individuals were within the reference interval of the manufacturer.

4. Discussion

4.1. Common findings among the research subjects

Our research was carried out on 40 patients with the average age of 51.3 ± 17.4 , of which the youngest was 15 years old and the oldest was 79 years old.

This result is consistent with the average age documented in the researches of Le Ngoc Trong when he examined the biological values of ordinary Vietnamese in the 90s.

Madhumita Das establishes her reference interval with the sample size of 1470 people with age averaging from 35 - 86. In 2017, [Xuejiao L. Hu](#) conduct his research on reference interval of several biochemical test in Arab with group, whom age ranging from 2 to 87 [4], [8], [9].

The ratio between male and female participants is 1/1, consistent with the researches of Dang Thi Ha et al in 2018 when establishing the reference values for various basic coagulation tests in children. The research was conducted on 1620 healthy children with the male to female ratio of 1 to 1. According to the 2016 United Nations Population Fund, the world's ratio of male to female is 97.3 to 100. In 2017, [Xuejiao L. Hu](#) researched the reference interval of several biochemical tests in Arab with the ratio of 316 (50.6%) female and 309 (49.4%) male [2], [3], [9]. In terms of male to female ratio, our study is different from KS Chua et al (350 male and 50 female) [7].

4.2. The results confirmed the reference interval of the value of glucose, urea, creatinine, triglyceride, cholesterol, AST, ALT and GGT

When using reference interval provided by the manufacturer, laboratories need to confirm the

accuracy of the reference intervals using the guideline of the Clinical and Laboratory Standards Institute (NCCLS document C28-A2). In our research, the values of cholesterol and triglyceride must be confirmed on 40 patients following the guidelines. This may be due to errors in the pre-test period (the condition of the patients, sampling technique...) or the consistency of the testing method, the employees' skills, the condition of the equipment and the analyzing environment. However, according to the standards, the laboratory's usage of the reference intervals for these 8 testing values is appropriate. In addition, these results were also similar to the reference intervals in the Ministry of Health's guideline 2014 for the implementation of specialized biochemical process [1], [6].

5. Conclusion

The diagnosis values of glucose, urea, creatinine, AST, ALT and GGT of 20 healthy individuals were all within the reference interval of the manufacturer.

The diagnosis values of triglyceride, cholesterol: The first analysis showed that there were 17/20 healthy individuals; the second analysis showed that there were 20/20 healthy individuals within the manufacturer's reference interval.

The reference intervals provided by the manufacturer for the diagnosis values of glucose, urea, creatinine, triglyceride, cholesterol, AST, ALT and GGT are applicable for the Department of Clinical Biochemistry - 108 Military Central Hospital.

Suggestion

Popularizing the reference intervals provided by the manufacturer at the Department of Clinical Biochemistry - 108 Military Central Hospital.

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