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Establishment of reference intervals in biochemistry parameters from check-up records: AMS Medical Laboratory, Khon Kaen

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Abstract

Reference intervals are used as a decision-making tool to distinguish healthy individuals from those with specific diseases. Due to the variations among laboratories, reagent manufacturers, ethnic backgrounds, gender, and age, laboratories were recommended to develop their own reference intervals. Moreover, under the international standard requirement, laboratories should review their own reference intervals periodically. This study aims to re-establish the reference intervals of 10 common biochemistry parameters including glucose, BUN, creatinine, total cholesterol, triglyceride, HDL-c, LDL-c, AST, ALT, and ALP. The study was based on the retrospective dataset retrieved from AMS Medical Laboratory, Khon Kaen University. The 1,543 records of each parameter were tested for variation among age and gender subgroups prior to calculation of the reference intervals. The data showed no variation against male and female subgroups, while slightly increased values were observed in the aging subgroup. Age-dependent reference intervals were established in 6 parameters with >95% acceptance, while ranges of lipid profiles still rely on the NCEP guidelines. The findings emphasized the importance of age-affected reference intervals for populations. Moreover, the flexibility of new reference intervals was appropriate for an aging society and may motivate greater healthcare awareness.

Keywords: Reference interval, Biochemistry parameters, Khon Kaen, Age-affected reference interval, Aging society, Health promotion

1. Introduction

Reference intervals or reference ranges of laboratory parameters were widely used as a decision-making tool to distinguish the healthy individual from those with specific diseases. According to the Hoffman method, if healthy population data ideally showed a Gaussian distribution, reference intervals are generally calculated as the central 95% of the population data while the 5% of outliers will be flagged as abnormal [1]. However, there are two major variations affecting the reference interval determination: laboratory methods and biological variation among different populations. Most clinical laboratories usually apply reference intervals from manufacturers disregarding population variations. This may result in an inaccurate interpretation and diagnosis [2]. The guideline for standard laboratory practice recommends the laboratory to create their own reference interval from healthy subjects in accordance with their control circumstances of population race and ethnic background. Moreover, the reference interval should be updated and verified as frequently as the change of measurement equipment or methods. The reference interval can be calculated either from the new set of healthy populations or the retrospective data from healthy individual records. The latter approach seems to be convenient and affordable while obtaining a dataset of sufficient size [3].

The AMS Medical Laboratory under the Faculty of Associated Medical Sciences (AMS), Khon Kaen University, has complied with ISO 15189: 2012 since 2012. We provide the healthcare service and check-ups for the community in Khon Kaen province and nearby. The current reference interval used in our laboratory was obtained from Srinagarind Hospital, Faculty of Medicine, and has not been reviewed for several years. Regarding the international standard requirements for clinical laboratories (ISO 15189), biological reference intervals shall be reviewed periodically and established on their own population [4] due to the changes in lifestyle behaviour, immigration and/or genetic background. Many studies have reported the reference intervals of different ethnic groups and some demonstrated variation among gender and age [5-7]. Chanin *et al.* also reported the reference intervals of clinical biochemistry parameters in the Nong Khai population and indicated the variation among gender and age which should be of concern for the interpretation [8]. Therefore, the verification and revision of biological reference intervals will be required.

This study aimed to re-establish our own reference intervals of 10 common biochemistry parameters from retrospective healthy data from 5 years of records. Due to the influence of age and gender in some biochemistry parameters, gender- or age-dependent reference intervals were also investigated.

2. Materials and methods

2.1 Data collection and data management

This study was a part of "Health Index of the Elderly in Northeastern Thailand for the Purpose of Lifestyle Modification and Health Monitoring before Disease Occurrence" project. According to the GCP guidelines [9], confidentiality of data and individuals was respected. The 4,316 completed laboratory records (including gender, age, glucose, BUN, creatinine, total cholesterol, HDL-c, LDL-c, triglyceride, AST, ALT, and ALP parameters) were retrieved from the AMS Medical Laboratory, Faculty of Associated Medical Sciences, Khon Kaen University for the period January 2016-June 2021. Biochemistry parameters were tested by Beckman Coulter LX20 Pro autoanalyzer (Beckman Coulter, CA, USA) while the principle of each test was stated in Table 1.

Definite diagnosis was done by physicians, which requires history taking without medicinal treatment, physical examinations, and laboratory results. For the inclusion/exclusion criteria, the first visit laboratory records of healthy individuals who were diagnosed by the physicians were included. Other visiting records, diagnosis with any disease, lack of diagnostic information, no disease conditions, or incomplete data were excluded from the study, leaving 1,543 records after data ruled out (Figure 1).

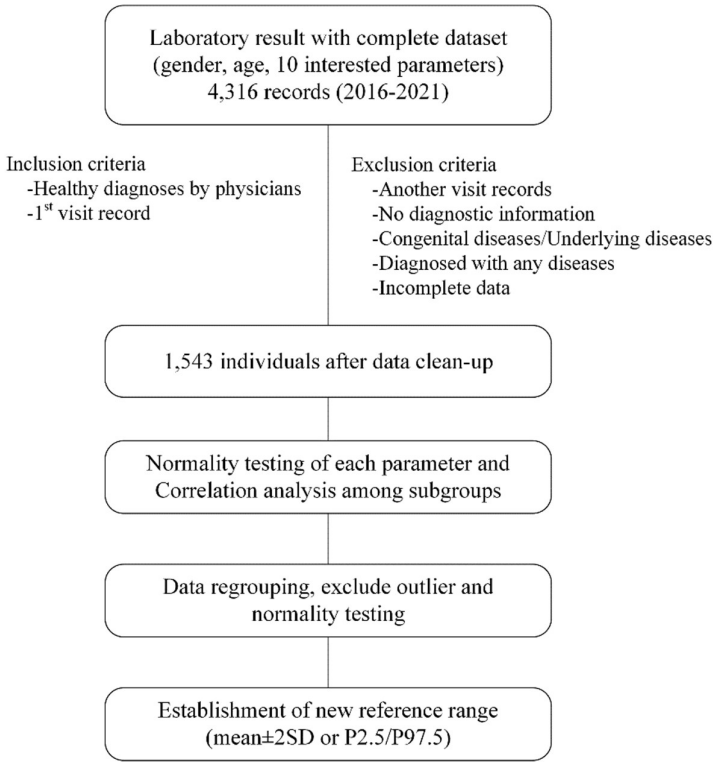

Figure 1 Experimental design of the study including data collection process, inclusion and exclusion criteria, and data processing.

2.2 Data processing and statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics v.28 under Khon Kaen University license (IBM Corporation, IL, USA). Firstly, Kolmogorov-Smirnov was used for the normality testing in each parameter. The regression method was used to investigate the correlation as well as to compare the medians between groups. This method supported multivariable analysis where more than one factor (age and gender) must be controlled at the same time. A regression method was used for parametric parameters, while quantile regression used for nonparametric parameters. Statistical significance was determined with the *p*-values ≤0.05.

Subgroups were defined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines with the minimum size of population in each subgroup (n=120). Two subgroups consisting of males and females were categorized for the gender parameter. Three subgroups of ≤40, 41-50 and >50 years old were categorized for age ranges according to active working, pre-elderly, and elderly groups, respectively.

2.3 Establishment of the reference intervals

After the correlation analysis, the subgroups were divided and treated according to variation effects. Outliers were removed according to Tukey's (1977) technique. In brief, data were arranged from minima to maxima, while Q1, Q3, and interquartile range (IQR) were defined. The values which were less than Q1-1.5 (IQR) and greater than Q3+1.5(IQR) were removed as outlier values. The outlier elimination was then repeated until no outlier could be found. Normality testing of the final dataset was performed again before defining the new reference interval. The reference interval of datasets with a normal distribution was determined using standard deviation (mean±2SD), while the 2.5th and 97.5th percentiles were used for skewed distributions instead [2,10-12].

3. Results

3.1 Demographic information of the study

A total of 1,543 healthy individuals who received physical and biochemistry examinations at AMS Medical Laboratory and for whom complete data including gender, age, glucose, BUN, creatinine, total cholesterol, HDL-c, LDL-c, triglyceride, AST, ALT, and ALP values were obtained (Figure 1). The dataset included 526 males and 1,017 females covering the age from 20 to 78 years old. The age parameter was divided into 3 subgroups including (a) ≤40 years old (n=406), (b) 41-50 years old (n=470) and (c) >50 years old (n=667). Median and range (minimum and maximum values) of biochemistry parameters were summarized in Table 1.

Normality testing using Kolmogorov-Smirnov test was performed to justify the statistical method for correlation analysis as described in 2.2. All data of the 10 biochemistry parameters represented skewed distributions, so quantile regression was further analyzed.

3.2 Statistical analysis for gender and age variation

Subgroups of gender and age were tested with the quantile regression method. To evaluate the variation among genders, males were compared with females, whereby the subgroup with age >50 years was defined as the main variable compared with another age subgroups.

Box plots of all 10 parameters were generated to represent the median, IQR, 95th percentile, and range in accordance with different gender and age subgroups as shown in Figure 2. Quantile regression analysis results in Figure 2 also showed no variation between males and females (*p*-value >0.05) indicating no gender variation, while all parameters showed variation with age dependence. Significant increases of the median in the >50 years subgroup was found in glucose, BUN, creatinine, total cholesterol, triglyceride, LDL-c, AST, ALT, and ALP ($p \leq 0.001$) although there was a moderate decrease of HDL-c ($p \leq 0.05$); hence the new reference intervals should be established with different age subgroups.

Table 1 Characteristics of biological data and biochemistry parameters.

Parameters	Measurement principle [13]	Chemical principle [13]	Median*	Range (min-max)
Sex				
Male (n=526)				
Female (n=1,017)				
Age			47.77	(20-78)
≤40 (n=406)				
41-50 (n=470)				
>50 (n=667)				
Clinical chemical parameters				
Fasting plasma glucose	Conductivity	Glucose oxidase	86	(60-171)
(FPG)	Conductivity	Urease	13	(5-27)
BUN	Colorimetry	Alkaline picrate	0.8	(0.4-1.7)
Creatinine	Colorimetry	Cholesterol oxidase	218	(101-382)
Total cholesterol	Colorimetry	CHE, CHO, Peroxidase	51	(22-146)
HDL-c	Colorimetry	CHE, CHO, Peroxidase	143	(30-286)
LDL-c	Colorimetry	Glycerokinase	96	(19-449)
Triglyceride	Colorimetry	Transamination of L-	24	(7-115)
AST	Colorimetry	aspartate	19	(3-119)
ALT	Colorimetry	Transamination of L-alanine	52	(18-187)
ALP		*p*-nitrophenyl-phosphate		

*All data show skewed distributions ($p \leq 0.001$).

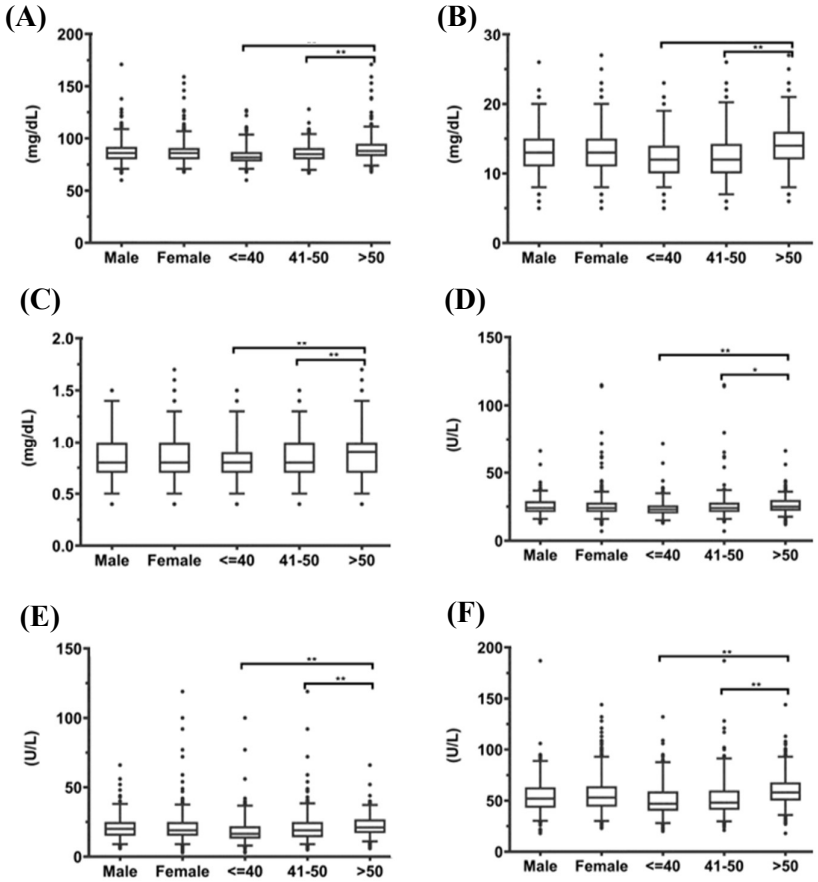

Figure 2 Plots indicate range (circle; ●), P2.5 and P97.5 (whisker), and IQR (box plot) of 5 subgroups (male, female, ≤40, 41 – 50, and >50 subgroups) in different biochemistry parameters including (A) glucose, (B) BUN, (C) creatinine, (D) AST, (E) ALT and (F) ALP. Statistical significance of quantile regression can be observed in age subgroups (*; *p*-value ≤0.05 and **; *p*-value ≤0.00

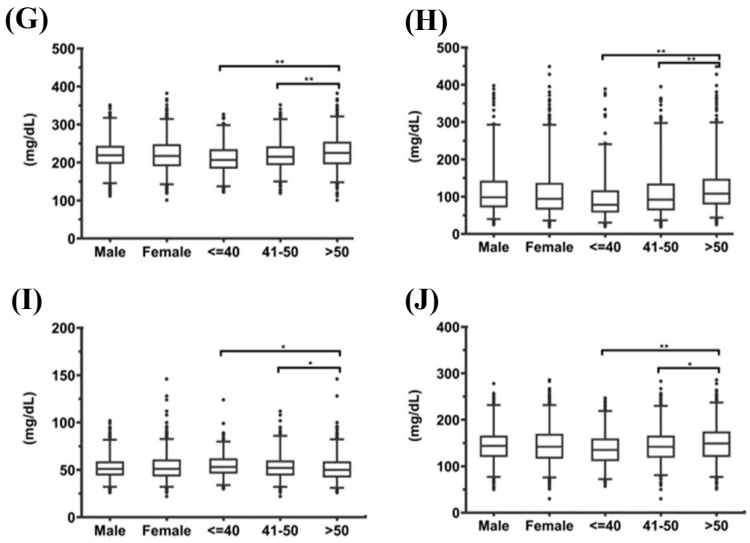

Figure 2 (continued) Plots indicate range (circle; ●), P2.5 and P97.5 (whisker), and IQR (box plot) of 5 subgroups (male, female, ≤40, 41 – 50, and >50 subgroups) in different biochemistry parameters including (G) total cholesterol, (H) triglyceride, (I) HDL-c and (J) LDL-c. Statistical significance of quantile regression can be observed in age subgroups (*; p-value ≤0.05 and **; p-value ≤0.001).

3.3 Establishment of new reference intervals with age dependence

To define the new reference intervals according to age group, the outliers were first removed and subsequent testing for normality was conducted. Most of the datasets provided a skewed distribution except for total cholesterol at >50 and LDL-c at ≤40 and >50 years old as shown in Table 2. Reference intervals were determined by mean±2SD for datasets with a normal distribution and P2.5 – P97.5 percentiles for a skewed distribution [10-12]. Because no variation among genders was observed in this study, the calculation of new reference intervals with and without age effect consideration was also tabulated. The obtained intervals without age effect of glucose, BUN, creatinine, AST, and ALT show similar intervals compared to our current reference intervals except for the ALP and lipid parameters (total cholesterol, triglycerides, HDL-c and LDL-c). In accordance with age variation, the higher upper values in the elderly were observed in all parameters including glucose, BUN, creatinine, total cholesterol, triglyceride, LDL-c, AST, ALT, and ALP, with the exception of HDL-c, suggesting the new reference intervals with 3 different subgroups. For the creatinine and ALT parameters, the 41-50 and >50 years old subgroups were combined according to an overlapping upper range.

In order to compare the interpretation of our new reference intervals with different reference intervals from various sources, all data of healthy subjects for the 10 parameters were calculated for the incorrect interpretation as % out of healthy range as shown in Table 3. For the suggested new reference intervals, less than 5% out of range were observed for glucose, BUN, creatinine, AST, ALT, and ALP. Creatinine, AST, and ALT were 9.61%, 8.63%, and 10.35% out of healthy range from the reference intervals from the study of Chanin *et al.* and Srinagarind Hospital. This indicates an acceptance interpretation of our suggested reference with >95% compared to 90% using other reference values. Unfortunately, the % out of healthy range were markedly higher (from 3.84 up to 42.47%) among lipid profiles using reference values from Srinagarind Hospital, implying that the presence of dyslipidemia in the healthy dataset was included in this study.

Table 2 New reference intervals of the 10 biochemistry parameters created by age subgroup compared to current reference intervals.

Biochemistry parameters (Current reference interval)	New reference interval without gender and age effect		New reference interval with age effect				Suggested reference interval
	n[$]	Lower-Upper	Age	n[$]	Normality testing	Lower-Upper	
Glucose (mg/dL)	1499	71-103	≤40	387	S	71-96	71-96
(70-110 mg/dL)			41-50	460	S	70-101	70-101
			>50	656	S	74-108	74-108
BUN (mg/dL)	1526	8-19	≤40	401	S	8-18	8-18
(7.8-20.5 mg/dL)			41-50	459	S	7-19	7-19
			>50	662	S	8-20	8-20
Creatinine (mg/dL)	1529	0.5-1.3	≤40	394	S	0.5-1.2	<40 = 0.5-1.2
(0.6-1.1 mg/dL)			41-50	468	S	0.5-1.3	>40 = 0.5-1.3
			>50	658	S	0.5-1.3	
Total cholesterol (mg/dL)	1519	145-299	≤40	400	S	139-286	<200*
(<200* mg/dL)			41-50	457	S	151-294	
			>50	660	N	141-309	
Triglyceride (mg/dL)	1457	37-212	≤40	373	S	31-166	<150*
(<150* mg/dL)			41-50	425	S	37-187	
			>50	626	S	44-215	
HDL-c (mg/dL)	1514	32-77	≤40	401	S	34-78	>40*
(>40* mg/dL)			41-50	458	S	32-76	
			>50	651	S	31-76	
LDL-c (mg/dL)	1521	77-219	≤40	400	N	66-204	<100*
(<100* mg/dL)			41-50	460	S	84-216	
			>50	663	N	71-227	
AST (U/L)	1518	16-35	≤40	398	S	15-33	15-33
(12 – 32 U/L)			41-50	454	S	16-35	16-35
			>50	662	S	18-36	18-36
ALT (U/L)	1526	9-36	≤40	357	S	8-27	≤40 = 8-27
(4 – 36 U/L)			41-50	461	S	9-37	>40 = 9-37
ALP (U/L)			>50	662	S	11-37	
(37 – 147 U/L)	1514	30-86	≤40	388	S	28-73	28-73
			41-50	453	S	30-78	30-78
			>50	652	S	36-87	36-87

"N" defines a normal distribution (p-value >0.05 and "S" defines a skewed distribution (p-value ≤0.05). The "$" defines the remaining number of the population after outlier removal and "*" defines the values suggested by NCEP guidelines.

4. Discussion

The verification of the reference interval is widely accepted as an essential activity in clinical laboratories because of its impact for clinical usage including diagnosis, treatment monitoring, and health check-up purposes. In accordance with different ethnic backgrounds, races, and lifestyle behaviours, the international guidelines recommend each laboratory to set their own reference intervals instead of using the values from manufacturers. However, the limitation for reference interval estimation is concerned with the sufficiency of data of healthy subjects as well as the costs involved. This study, therefore, demonstrates a model of big data retrieval from retrospective records of clinical laboratories, which helps to solve this problem. We also attempt to demonstrate that age and gender are the key important variations affecting the reference intervals of common biochemistry parameters in healthy populations in Khon Kaen province. Our findings from the total of 1,543 records show age-dependent effects upon all parameters including glucose, BUN, creatinine, AST, ALT, ALP, and lipid profiles, hence resulting in the recommended reference intervals according to age group, except for lipid profiles. Unequal sample sizes from males and females in our study resulted in no significant difference for each of the blood parameters in males and females, which may cause the contradictory evidence to other reports [14,15]. The result of no gender effect using regression analysis at the first step helps in generating subgroups divided by age for further steps.

Most medical laboratories usually estimate reference intervals without gender and age consideration. Accordingly, new reference intervals calculated without gender and age effects were also included (Table 3) which show similarity to our previous reference intervals, except for ALP and lipid profiles. AMS Medical Laboratory followed the recommended National Cholesterol Education Program (NCEP) guidelines as the cut-off values for the lipid profiles instead of using the calculated intervals which are significantly higher in all age subgroups.

Notably, the exclusion criteria did not remove the abnormal lipid profiles, resulting in dyslipidemia being included in the healthy population which is then related to the consumption behaviour and lifestyle of the population. Age-related trends in lipid levels were also be reported in Chinese populations [16]. According to the context of disease prevention purposes for obesity and cardiovascular disease, the NCEP guidelines which presented one-side cut-off values or threshold values are strongly recommended, while urging the awareness of dyslipidemia which indicates potential concerns of coronary artery disease [17].

A slight increase in the upper range in creatinine was observed in the elderly. This may or may not be correlated with the filtration rate. Moreover, it can be hypothesized that age may increase the incidence of degenerative diseases and risk factors for vascular and kidney diseases [18]. Structural changes of the kidney affecting functional glomeruli from increased prevalence of nephrosclerosis, decrease in kidney volume, etc., can be observed in those of older age [19]. Besides, the biochemistry parameter directly explains the functional and metabolism mechanism of individuals. Alteration of an upper border or lower border in aging may represent the organism state which was previously reported by Pyrkov et al [20].

Finally, the result of verification in using new reference intervals showed >95% acceptance interpretation compared to >90% acceptance with the different reference intervals from other sources. The difference might come from different inclusion criteria in each study such as health criteria, ethnic backgrounds, time of the study, and social transformations resulting in the different reference intervals. However, all 3 different sources except for the manufacturer in Table 3 were obtained from the same ethnic populations in Khon Kaen and Nong Khai provinces, which are located in the Northeast of Thailand. The larger the dataset, the more accurate the estimated value obtained. The reference values from this study might be representative of the population of Khon Kaen province but not the wider Northeastern population.

Table 3 The comparison of healthy interpretations between the new reference intervals with various other reference interval values.

Parameters	Reference interval (% out-of-reference interval)			
	Suggested reference interval	Chanin et al study† [8]	Srinagarind Hospital	Manufacturer's recommendation
Glucose (mg/dL)	≤40: 71-96 (>96 = 1.81%) 41 – 50: 70-101 (>101 = 1.96%) >50: 74-108 (>108 = 1.83%)	N/A	70-110 (>110 = 0%)	74-106 (>106 = 0.47%)
BUN (mg/dL)	≤40: 8.0-18.0 (>18.0 = 1.5%) 41-50: 7.0-19.0 (>19.0 = 0.65%) >50: 8.0-20.0 (>20.0 = 2.11%)	N/A	5.8-19.1 (>19.1 = 2.49%)	6-20 (>20 = 1.18%)
Creatinine (mg/dL)	≤40: 0.5-1.2 (>1.2 = 0%) 41-50: 0.5-1.3 (>1.3 = 0.85%) >50: 0.5-1.3 (>1.3 = 1.98%)	0.53-1.17 (>1.17 = 9.61%)	0.5-1.5 (>1.5 = 0%)	0.51-1.17 (>1.17 = 9.61%)
Total cholesterol (mg/dL)	NCEP: <200* (≥200 = 66.62%)	N/A	127-262 (>262 = 14.88%)	<200* (≥200 = 66.62%)
Triglyceride (mg/dL)	NCEP: <150* (≥150 = 15.58%)	N/A	<200 (≥200 = 3.84%)	<150* (≥150 = 15.58%)
HDL-c (mg/dL)	NCEP: >40* (≤40 = 15.72%)	41-85 (<41 = 15.72%)	>35 (≤35 = 5.75%)	>40* (≤40 = 15.72%)
LDL-c (mg/dL)	NCEP: <100* (≥100 = 88.23%)	48-99 (>99 = 88.23%)	<150 (≥150 = 42.47%)	<100* (≥100 = 88.23%)

The "†" represented Nong Khai population, "N/A" represented no data and "*" defined reference intervals recommended by NCEP guidelines.

Table 3 (continued) The comparison of healthy interpretations between the new reference intervals with various other reference interval values.

Parameters	Reference interval (%out-of-reference interval)			
	Suggested reference interval	Chanin et al study† [8]	Srinagarind Hospital	Manufacturer's recommendation
AST (U/L)	≤40: 15-33 (>33 = 2.51%) 41-50: 16-35 (>35 = 0.88%) >50: 18-36 (>36 = 1.51%)	15-34 (>34 = 3.56%)	12-32 (>32 = 8.63%)	0-40 (>40 = 0%)
ALT (U/L)	≤40: 8-27 (>27 = 2.52%) 41-50: 9-37 (>37 = 1.52%) >50: 11-37 (>37 = 1.66%)	12-31 (>31 = 10.35%)	4-36 (>36 = 2.42%)	0-41 (>41 = 0%)
ALP (U/L)	≤40: 28-73 (>73 = 2.52%) 41-50: 30-78 (>78 = 2.43%) >50: 36-87 (>87 = 1.99%)	40-113 (>113 = 0%)	42- 121 (>121 = 0%)	40-129 (>129 = 0%)

The "†" represented the Nong Khai population, "N/A" represented no data and "*" defined reference intervals recommended by NCEP guidelines.

The definition of "healthy" for individuals recruited in this study is the most common question for the study of reference intervals as the limitation of the study. Since the definite diagnosis was done by physicians whose decisions rely on the history screening, physical examination, and laboratory result, this could ensure that every included individual underwent preliminary screening. However, some outliers may also remain. Quantile regression is more robust against outlier data compared to ordinary least squares regression, which can be useful to analyze the relationship between variables [21]. Moreover, data treatment for outliers was statistically excluded (lower than Q1-1.5(IQR) and greater than Q3+1.5(IQR)) prior to calculation of the new reference interval. This statistical treatment helped to overcome the limitations of healthy subjects, resulting in the new reference value being comparable to the previous value in many parameters. Although this study did not provide the gender-related reference interval due to the regression analysis, these intervals needed further validation by the laboratory regarding the CLSI guidelines.

The main benefit of this study is focused on the usefulness of reference intervals from big data sets in Khon Kaen province or nearby. Also, our report demonstrates a model for laboratories to establish their own reference intervals from retrospective data. For further study, the big data analysis of clinical laboratory parameter reference intervals in Northeastern populations would be established and provide a greater impact for the larger population.

5. Conclusion

The findings of this study emphasized the importance of establishing specific reference interval values for specific populations, particularly age-affected biochemistry parameters. Age-dependent reference intervals were provided for 6 parameters including glucose, BUN, creatinine, AST, ALT, and ALP. For lipid profiles, use of the NCEP guidelines is recommended. The provision of age-related reference interval information can be applied in routine laboratories using the same analytical instrument. Moreover, the indirect benefits would include the motivation of greater healthcare awareness in the aging group.

6. Ethical approval

The study was conducted under the approval of the Ethical Committee for Human Research, Khon Kaen University (HE602172).

7. Acknowledgements

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