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Research Article

Reference interval with age-gender variation for 4 liver function parameters in an adult segment of the Indian population

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Abstract

Objectives: The proper interpretation of the laboratory values seen in individuals depends on the validity of the reference intervals. These intervals may differ from one population to another due to variation in factors such as genetic profile, physical characteristics, and dietary intake. Reference intervals for most parameters are not available for the Indian population. This study presents reference intervals for 4 liver function parameters: total protein, albumin, globulin, and the albumin-globulin ratio for a segment of the Indian population, as well as a study of age trend and gender differences.

Methods: The results were based on a minimum of 12.264 values for each parameter from individuals aged 0 to 98 years. These values were extracted from a large database of the laboratory of a tertiary care hospital after careful filtration of the appropriate reference values. The age-gender variation in the mean and median was examined and the reference intervals were obtained as 2.5th to 97.5th percentile.

Results: There was a sufficient number of values in the various age groups and by gender except for the group aged 0-14 years. Therefore, this age group was excluded. The age trend was studied for males and females separately, as gender differences were substantial and consistent across age groups. Total protein and albumin levels declined with age, but the decline in albumin level was more rapid. Specific age-gender reference intervals were obtained for adults.

Conclusion: The effect of age and gender on all of the parameters examined was substantial. The obtained reference intervals are slightly different from those previously reported in the literature and currently used.

Keywords: Age-gender variation, albumin, albumin-globulin ratio, globulin, liver function tests, reference interval, total protein

Laboratory parameters have acquired a central role in medical practice because they provide an objective assessment of specific aspects of the health condition of an individual. The reference intervals of these parameters serve as a comparator to establish the diagnosis, interpret the severity of the condition, calibrate the treatment, and assess the prognosis, particularly when a patient is seen for the first time at a particular clinic. The appropriate intervals are necessary for the correct interpretation of the values of individuals seen in clinical practice. Preventive health check-ups can use intervals to flag a warning sign. Most clinicians and laboratories in India use the reference intervals reported in books or literature provided by diagnostic kit manufacturers [1]. These are generally based on an American or European population, which in India are usually identified as Western [2]. However, as with almost all biological parameters, laboratory parameters, too, are likely to be affected by local factors such as genetic profile, ethnicity, physical characteristics, dietary habits, and environment. Thus, it is likely that they are different for the Indian population. There is a need to establish more specific reference intervals and examine if they are different from the norms currently in use. If they are different, the use of Western intervals may be causing unknown errors of misdiagnosis and missed diagnosis. If they are nearly the same, we can be confident of the validity in our practice.

The Clinical and Laboratory Standards Institute (CLSI) mandates that the exercise of establishing norms should be based

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on the measurement of apparently healthy subjects, or reference individuals, and that these norms should be verified separately for each laboratory [3]. The challenges in establishing reference intervals for the Indian population are two-fold: First, there is broad ethnic diversity, and each group may have its own norms, and secondly, it is extremely difficult to identify and investigate a large number of healthy subjects in our clinical environment because not many healthy individuals request examination. In India, investigations are generally ordered when complaints occur.

Liver function tests are widely performed to assess the health of the liver and the results are interpreted for the diagnosis and monitoring of liver condition. Several parameters are generally evaluated, such as proteins and aminotransferase, but this report is restricted to 4 parameters, namely, the serum level of total protein, albumin, and globulin, and the albumin-globulin ratio (A/G ratio). This study is the initial result of an intensive analysis of data, including analysis of variance (ANOVA) for age differences and age trend, and investigation of age-sex differences, to reach to the appropriate reference intervals. In order to preserve the focus and length of this report, the analysis and findings will be released in stages. Other parameters will also be studied and reported separately over time.

Due to the wide ethnic diversity in India, there is no claim that the results presented in this article would apply to the entire Indian population. Only a section of the population was studied and the expectation is that the results would be valid for at least this specific segment.

Materials and Methods

The database used in the present study belongs to the laboratory of a large tertiary care hospital in the Delhi-National Capital Region. This laboratory performs hundreds of thousands of analyses every year. The study data were extracted from the laboratory records of a 6-month period from January 2019 to June 2019. A total of 18.422 investigations of liver function were performed during this period for individuals aged 0 to 98 years, and 45.1% of the patients were female. Although data on various liver function indicators, such as total protein, albumin, globulin, A/G ratio, alkaline phosphatase (ALP), bilirubin (direct, indirect, and total), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGT), were available for a large population, this study is restricted to the examination of serum levels of total protein, albumin, globulin, and the A/G ratio. Two large scale studies are cited, 1 in India and the other in the UK, which also are based on retrospective data.

The hospital studied in this research is in the private sector and generally caters to a prosperous community around it, including the staff and executives of companies with which the hospital is empaneled. Such a population may be more suitable for establishing reference values because it is expected to have a better level of nutrition and better health relative to the population served by public hospitals. Officials of the empaneled companies also come for preventive health checkups, as permitted under their healthcare package.

This study was approved by the institutional ethics committee (no: RS/MSSH/VSH/CRL/IEC/PATH/19-19).

Laboratory analytical methods

The venous sample was collected during the day. The time of day should not affect our reference interval as no major diurnal variation is seen in liver function parameters [4]. All of the samples were collected with the patient in a sitting position after 2 minutes' rest with a single venepuncture using a BD Vacutainer SST II Advance Serum Separator (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with a standard tube holder, and the tubes were filled to the predefined mark. A 22G BD Vacutainer Eclipse Blood Collection Needle (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was used in all cases. The blood was allowed to clot for 30 minutes and then centrifuged at 3000 rpm for 10 minutes. The analysis was performed within 3 hours. Hemolyzed samples were rejected and not included.

All of the assays were performed using a Cobas C501 autoanalyzer (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's specifications. Total protein was analyzed with the Roche Total Protein Gen.2 reagent (Roche Diagnostics, Basel, Switzerland) using a biuret colorimetric assay and the endpoint method. Albumin was analyzed using the Roche Albumin Gen.2 reagent (Roche Diagnostics, Basel, Switzerland), a bromocresol green colorimetric assay, and the endpoint method. These 2 analytes were assayed directly on the analyzer.

Intra-assay analytical coefficients of variation were determined using 2 levels of control material: Roche PreciControl ClinChem Multi-1 and Roche PreciControl ClinChem Multi-2 (Roche Diagnostics, Basel, Switzerland). The results were interpreted according to the Westgard multirule algorithm and plotted as a Levey-Jennings chart. A quality control check was performed every day. All of the quality control sample values for total protein and albumin were within ±2SD of the respective target mean throughout the entire investigation. Reagents, calibrations, methodologies, and quality control were unchanged during this period. The laboratory used in this study is accredited by the National Accreditation Board for Testing and Calibration Laboratories, in compliance with International Organisation for Standardization 15189 requirements. The laboratory also participates in the regular Bio-Rad External Quality Assurance Service program (Bio-Rad Laboratories, Inc., Hercules, CA, USA)

Globulin was obtained by subtraction (total protein–albumin), and the A/G ratio as the ratio of albumin to globulin for each individual. These 2 parameters are calculated and not measured, but a reference interval for them cannot be obtained using this calculation because abnormal values of total protein and albumin can result in a normal-looking value of globulin and A/G ratio. Therefore, the reference interval was also obtained using the same method used for total protein and albumin level. The laboratory uses non-SI units.

Data filtration

The guidelines of the CLSI state that the reference population for this exercise should be apparently healthy people [3]. It was a challenge to filter the right values for our study from the dataset, as it was anonymized and did not contain any information on the clinical condition of the person at the time of sampling. The outliers and abnormal values of those likely to be pathological or not normal for a healthy person were filtered out using the procedure subsequently described. The final values obtained after filtration may not be perfect for establishing norms, but perfect should not be the enemy of good because that is a guarantee to achieve little [5].

A 2-stage process was used to extract reference values from the dataset. Since the dataset was from a hospital laboratory, many of the values were for repeat investigations of the same person. Repeat investigations could be identified by the patient ID. In all, 5272 (28.6%) values from a total of 18.422 results were excluded in the first stage, and 13150 remained with a unique ID. The age-gender distribution of the individuals with values in the initial sample and the filtered sample after excluding repeat investigations is displayed in Table 1. Age is divided into 15-year intervals. The age group of 60-74 years and 75+ had the most repeat investigations (37.4% each) and the age group of 30-44 years had the fewest (18.4%).

In the second stage, an ingenious statistical method of double filtration [6] was used in which the first filter was for outliers and the second for abnormal values. In both of these filtrations, values less than Q_1–1.5*IQR and more than Q_3+1.5*IQR were excluded, where Q_1 is the first quartile, Q_3 is the third quartile, and IQR is the interquartile range [7]. The second filtration was performed with recomputed quartile values after the exclusion of outliers in the first filtration. These filtrations were done separately for each age group and gender. The number of values remaining for analysis for each age group and gender are shown in Table 2 for total protein, albumin, globulin, and the A/G ratio. These varied from parameter to parameter, depending on how many were excluded in the second stage of filtration. A total of a minimum of 12.264 values for albumin and a maximum of 12.784 for the A/G ratio were available for final analysis. Thus, nearly 5% of the values were excluded at this stage. Those remaining were the reference values for this study. Other than the age-gender group of females 0-14 years, the available values in all of the age-gender groups were larger than the 120 individuals recommended by the CLSI for obtaining reference intervals.

The data analysis was completed separately in each age group for males and females to determine if age and gender had any effect on the average. The gender difference in the mean was statistically examined using an unpaired Student's t-test and the age differences with an ANOVA F-test and a pairwise Tukey test, although this amounts to multiple tests on the same data. We comment on this later in this report, particularly in view of the recent controversy on the utility of p values due to overuse [8]. These statistical tests were valid in our case, as the number of values in each age-gender group was large enough to be able to disregard the shape of the distribution (Gaussian or otherwise) [9]. IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) was used for all of the calculations and statistical tests.

Calculations of reference intervals

At least 4 different methods are available to delineate reference intervals [10]. The relative merits and demerits have been described elsewhere [6]. We used the most common method of considering the 2.5th to 97.5th percentile of the reference values as the reference interval (3), which includes the central 95% of values and excludes the 2.5% of extreme values on either side. This exclusion is the norm, as some individuals may have extreme values despite being absolutely healthy. The use of such percentiles obviated the need to consider the shape of the statistical distribution of these values because these limits are the same as (mean±2SD) range for Gaussian distribution.

Results

The mean and SD of total protein, albumin, globulin, and the A/G ratio are provided in Table 2 by age group and gender. Most p values for the difference between age groups and

Table 1. Age-gender distribution of the study subjects

Age group (years)	Male		Female		Total	
	Initial n	After excluding repeat investigations n	Initial n	After excluding repeat investigations n	Initial n	After excluding repeat investigations n
0–14	242	170	129	109	371	279
15–29	908	658	799	625	1707	1283
30–44	1742	1397	1546	1286	3288	2683
45–59	2839	2210	2601	1934	5440	4144
60–74	3286	2007	2588	1671	5874	3678
75+	1094	672	648	411	1742	1083
Total	10111	7114	8311	6036	18422	13150

between males and females were <0.001, but they are of academic interest only in this case, as the statistical significance was a foregone conclusion due to the large number in each group in our dataset. Moreover, these p values were for the null hypothesis of no difference, since it would be naive to expect no difference across age groups or gender for such biological parameters. In addition, p values have to be interpreted cautiously, as multiple p values have been obtained from the same dataset, and thus the conclusion must also consider other corroborative evidence [8]. A pairwise Tukey test for the mean difference between age groups (results not shown) revealed a p value <0.02 each time, barring a few exceptions. However, the p value was >0.05 for the mean difference between the age groups of 15-29 and 30-44 years for all of these parameters in both males and females. All of these p values were used only to consider if the differences were worthy of further exploration [11] and the conclusions were not based on p values alone, as advised by Wasserstein et al. [8]. For the record, the SD values indicated that the variance was generally higher for all 4 parameters in the age groups of 0-14 and 75+ years, and the lowest in the 30-44 years age group.

Gender differences

The values in Tables 2 show that the mean albumin level was generally higher in males than in females in most age groups, the mean globulin value was generally lower, and the mean A/G ratio generally higher, but the mean total protein value

Table 2. Mean and SD of 4 liver function parameters in males and females of different age groups and the significance of the differences: total protein, albumin, globulin and the A/G ratio

Parameter	Age group	Male		Female		
	(years)	n	Mean (SD) (g/L)	n	Mean (SD) (g/L)	p
Total protein	0-14	160	69.8 (7.5)	97	70.7 (6.4)	0.326
	15-29	616	75.1 (5.2)	611	74.0 (0.6)	<0.001
	30-44	1296	75.0 (4.7)	1243	73.7 (5.1)	<0.001
	45-59	2092	73.4 (5.0)	1843	74.2 (5.2)	<0.001
	60-74	1873	71.9 (6.3)	1579	72.6 (5.9)	<0.001
	75+	661	68.0 (7.6)	402	69.1 (0.7)	<0.001
	Combined	6698	72.9 (6.0)	5775	73.2 (5.8)	0.004
	p value*	<0.001		<0.001		
Albumin	0-14	156	43.0 (5.0)	106	42.5 (6.10)	0.467
	15-29	617	46.8 (4.4)	603	43.8 (4.60)	<0.001
	30-44	1264	46.4 (3.2)	1224	43.5 (3.70)	<0.001
	45-59	1997	44.7 (3.7)	1809	43.3 (0.36)	<0.001
	60-74	1868	41.3 (5.3)	1554	41.1 (0.45)	0.241
	75+	662	37.4 (6.5)	404	37.4 (5.80)	>0.999
	Combined	6564	43.5 (5.4)	5700	42.3 (4.50)	<0.001
	p value*	<0.001		<0.001		
Globulin	0-14	166	26.4 (5.7)	106	26.8 (5.9)	0.577
	15-29	637	27.9 (3.8)	613	30.1 (4.1)	<0.001
	30-44	1328	28.7 (3.9)	1270	30.1 (4.0)	<0.001
	45-59	2142	28.9 (4.2)	1895	31.0 (4.3)	<0.001
	60-74	1944	30.3 (4.9)	1636	31.4 (5.0)	<0.001
	75+	646	30.6 (4.8)	403	31.2 (5.3)	0.059
	Combined	6863	29.3 (4.5)	5923	30.8 (4.6)	<0.001
	p value*	<0.001		<0.001		
A/G ratio**	0-14	160	1.63 (0.36)	105	1.61 (0.46)	0.692
	15-29	637	1.66 (0.29)	611	1.46 (0.27)	<0.001
	30-44	1329	1.61 (0.27)	1257	1.44 (0.24)	<0.001
	45-59	2106	1.54 (0.29)	1883	1.40 (0.25)	<0.001
	60-74	1984	1.35 (0.34)	1635	1.30 (0.29)	<0.001
	75+	666	1.24 (0.32)	411	1.21 (0.31)	0.311
	Combined	6882	1.48 (0.33)	5902	1.37 (0.28)	<0.001
	p value*	<0.001		<0.001		

*P value for difference between age groups. **Ratio has no unit.

did not follow any consistent pattern across age groups. As reported later, the reference intervals based on 2.5th and 97.5th percentiles were not much different for males and females when all age groups were combined.

Is there any age trend?

Figure 1 indicates that there was a general trend of a slight decrease in the median values of total protein, and a pronounced decrease in albumin and the A/G ratio with age in both male and female adults (age 15+ years) but the globulin level slightly increased with age in both genders. Values in the age group of 0-14 years in both genders were an exception to this trend. The median was used here because that is consistent with reference intervals based on percentiles. These percentiles are also shown in the figure. The mean values in Table 2 corroborate this trend finding. The mean values indicated that total protein and albumin values declined more rapidly after the age of 60 years in both males and females.

Although measurement uncertainties in total protein and albumin are generally <5% [12] and the optimal imprecision is 3% [13], if an arbitrarily expanded threshold of 10% of the highest mean is considered the margin of error due to intra-individual sampling fluctuations and measurement uncertainties, the difference between the highest mean and the lowest mean across age groups was greater than this threshold for most parameters in both males and females. If this is considered evidence of the presence of age-trend and p values are disregarded, it seems that, on average, age did affect these values.

Reference intervals

The difference between the means of all of the study parameters in the age groups of 15-29 and 30-44 years was not only statistically not significant, but was minor and appeared to be within the limits of laboratory variation (Table 2). Thus, these 2 age groups could have been combined for the purpose of reference interval; however, we provided separate reference intervals for each age group of males and females for uniformity (Table 3). These are 2.5th and 97.5th percentiles of the reference values, as stated earlier. Nonetheless, since the adoption of separate intervals for different age groups and gender could be complicated for most practitioners, we combined age groups and also provided a consolidated reference interval for each parameter for male and female adults (Table 3). The group of 0-14 years was excluded from these tables because obtaining a reference interval for this group would have been hazardous in this study for 2 reasons: First, the values in this age group in our case did not fall in the age trend otherwise seen, and second, there were fewer than 120 female values, the minimum required to obtain a reference interval. Most of the reference intervals provided in books and websites are for adults without breakdown by age or gender (See e.g., 14).

Discussion

Age and gender are generally ignored for adults and a common reference interval is used for parameters such as albumin level [14]. However, we observed a substantial age and gender variation in the values of all the parameters under study; therefore, separate intervals may be useful.

Gender differences in the mean values were not only statistically significant, but also seem medically relevant as the mean albumin level was consistently higher in males than in females in all age groups beginning at 15 years. The mean globulin level was consistently lower and the A/G ratio consistently higher. The mean total protein level had no such consistent pattern, as it was higher in some age groups and lower in others. Sairam et al. [15] also reported a statistically significant difference between the mean in males and females for almost all of the analytes they studied, including total protein and albumin. That study had data from 4 centers across India with a total of more than 10.000 values for each parameter and used the 2.5th and 97.5th percentiles as reference limits. Furruqh et al. [1] also observed a gender difference in the mean total protein and albumin levels in a small study in Bangalore. They also determined reference limits based on 2.5th and 97.5th percentiles.

Table 3. Reference interval (g/L) for 4 liver function parameters in different age groups according to gender

Parameter	Age (years)	Male	Female
Total protein (g/L)	15-29	64–84	61–85
	30-44	65–84	63–83
	45-59	63–83	63–85
	60-74	58–83	60–84
	75+	53–82	55–82
	All adults (15+)	60–84	61–84
Albumin (g/L)	15-29	37–55	34–51
	30-44	39–52	35–50
	45-59	36–51	35–50
	60-74	29–50	31–48
	75+	22–47	24–47
	All adults (15+)	30–52	32–50
Globulin (g/L)	15-29	21–36	22–39
	30-44	22–36	23–38
	45-59	21–38	23–41
	60-74	22–41	22–42
	75+	22–41	21–43
	All adults (15+)	21–39	23–41
A/G ratio**	15-29	1.0–2.2	0.9–2.0
	30-44	1.0–2.1	1.0–1.9
	45-59	0.9–2.1	0.9–1.9
	60-74	0.7–2.0	0.7–1.8
	75+	0.6–1.8	0.6–1.8
	All adults (15+)	0.8–2.1	0.8–1.9

**Ratio has no unit.

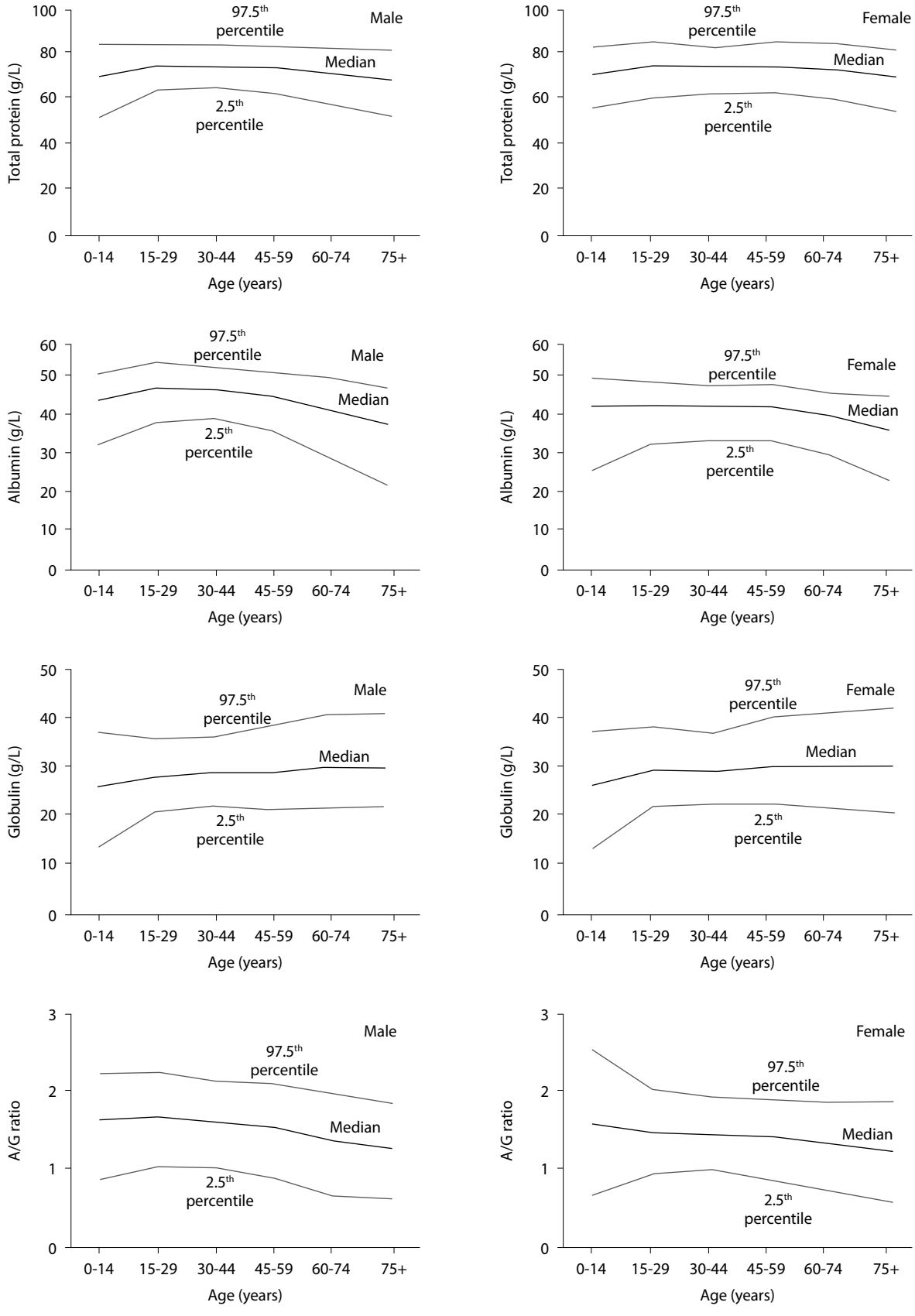

Figure 1. Age trend of 4 liver function parameters.

However, Ashavaid [16] also used 2.5th and 97.5th percentiles and reported the same reference interval for males and females in Mumbai, suggesting that that the gender differences were not significant. In England, Weaving et al. [17] reported a significant difference in the mean albumin level between males and females for almost all age groups. They did not report reference intervals. The study was based on more than a million values from the tests ordered in primary care and was records-based. The gender difference indeed could be real because the liver expresses different subsets of genes that affect the organ's ability to mobilize certain hormones [18]. Perhaps further research is required to understand the exact cause of a gender difference in liver function parameters.

For a nationwide study in Turkey of a total of 3066 healthy individuals, Ozarda et al. [19] used a complex parametric (based on Box-Cox transformation) and a nonparametric method to calculate reference intervals. There was no significant difference between the reference intervals obtained using these 2 methods, including total protein and albumin levels. Thus, the nonparametric method based on percentiles is as good, or in fact better, as it does not require consideration of the normal or any other distribution.

There was a clear trend of a slight decrease in the level of total protein with increasing age, and a substantial decrease in the albumin level and the A/G ratio in both male and female adults, but the globulin level exhibited a slight increase with age in both genders. The increase in the globulin level and the decrease in the A/G ratio may be directly due to a more rapid decline in the albumin level with age compared with total protein. Sairam et al. [15] did not study the influence of age on liver function parameters in Indian subjects but Furruqh et al. [1] studied and found a declining albumin level with age in Bangalore, but did not find any such trend with total protein. Weaving et al. [17] reported a decline in the albumin level with increasing age in men and women in England. Ozarda et al. [19] also reported declining albumin level values. This decline can occur due to deteriorating liver function with advancing age, [20] which could be related to a decline in blood flow in the liver in older subjects [21]. The effect of age on liver function also needs more in-depth study with regard to the specific cause/s and the exact measurement of the average decrease in total protein and albumin level in adults so that the decline due to the degenerative process can be segregated from pathological decline.

Whereas the present effect of age and gender on some liver functions seems to be the first such finding in the Indian context, the focus of the present exercise is to obtain the reference intervals for the 4 liver function parameters under study. In view of the age and gender variation in the levels of total protein, albumin, globulin, and the A/G ratio, we provided a reference interval for each age group and gender in Table 3. Clinicians are used to working with a common interval for adults of all ages irrespective of gender for these parameters. They may now also consider the age gradient and gender differences in the evaluation of the clinical implications of the value seen in a particular individual. This is important to determine a correct assessment and should no longer be ignored in this era of exactitude, since the effect of age and gender appears to be more than mere measurement variation. These are cautious statements in view of the recent advice to be aware of uncertainties and be modest in communicating results [8].

Many laboratories and diagnostic services may find it difficult to convey age-gender specific reference intervals in their report. Furthermore, some clinicians prefer to ignore age intervals for adults. The Pathology Harmony Group of the UK has reported such intervals for adults without consideration of age or gender differences [22]. Most, including the Pathology Harmony Group, realize that the levels for children are different. Many clinicians and laboratories seem to appreciate gender differences, and, for this reason, we provided reference intervals for males and females. These are based on more than 5000 values for each gender.

A comparison of our reference intervals with those reported by Sairam et al. and those approved by Pathology Harmony Group revealed the following: (i) Our total protein reference interval was nearly the same as that determined for the UK, but lower than that reported by Sairam et al. for Indian centers, (ii) the lower limit of the albumin level for our subjects was lower than that reported by both these groups, although the upper limit was nearly the same, and (iii) our globulin levels were lower than those reported by Sairam et al. (Table 4). The Pathology

Table 4. Comparison of our reference intervals with other studies

Liver function parameter	Pathology Harmony Group Adults (21) (Age not specified)	Sairam et al. (16) (Multicentric in India Age 20–70 years)		Present study Age 15+ years	
	P	M	F	M	F
	(Same for M and F)	(n=7478)	(n=3187)	(n≥6500)	(n≥5700)
Total protein (g/L)	60–80	68–85	67–85	60–84	61–84
Albumin (g/L)	35–50	39–51	37–49	30–52	32–50
Globulin (g/L)	Not reported	24–39	25–42	21–39	23–41
A/G ratio	Not reported	Not reported	Not reported	0.8–2.1	0.8–1.9

F: Female; M: Male; P: Persons.

Harmony Group of the UK did not consider gender difference significant for separate reporting, although Weaving et al. found a significant difference for the albumin level in England. Weaving et al. did not provide reference intervals, but rather medians for different age groups by gender because their objective was to study age-gender variation and not reference intervals.

As a side note for our statistical colleagues, we also studied the statistical distribution of total protein, globulin, and the A/G ratio and found them to be symmetric, but that of the albumin level was slightly skewed to the left. This means that lower levels of albumin were more common than the higher levels. This may be typical for India as a result of nutritional factors. The second finding is that generally, the lowest SD of all these parameters in both males and females was seen in the age group of 30-44 years relative to the other age groups. This is consistent with the general perception about stability of values in this age group. In a way, this also expresses confidence regarding the validity of the reference values obtained in this study after the 2-stage filtration process.

In conclusion, as a possible limitation, it should be noted that this exercise was based on the data from the laboratory records of a hospital, although a 2-stage process was used to eliminate all repeat investigation values, outliers, and abnormal values. Weaving et al. used all of the values from primary care from records in England to study age and sex variation in albumin concentration without any filtering although for them, too, clinical information regarding the reason for the investigation was not available. Sairam et al. identified apparently healthy individuals for their study with values in 4 centers in India, although their research was retrospective based on records.

This study determined reference intervals for 4 liver function tests for a section of the Indian population. More such studies should be performed so that a meta-analysis can be undertaken to firmly establish reference intervals for the Indian population.

Conflict of interest: None declared.

Ethics Committee Approval: This study was approved by the institutional ethics committee (no: RS/MSSH/VSH/CRL/IEC/PATH/19-19).

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