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Reference interval of serum bilirubin panel in healthy individuals of attending tertiary care hospital - a cross sectional study

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Abstract

Introduction: Reference Intervals are the most common decision making tools for interpretation of various biochemical reports. Age, sex, ethnicity, diet, physical and socio-economic conditions, affect the physiology of population. Hence it is necessary to setup reference values that are applicable to specific population rather than to borrow them from other population based studies or from literature from reagent kit manufacturers. So we intend to establish the reference intervals of serum bilirubin profile in southern coastal Karnataka population.

Methodology: Hospital based cross sectional study was done involving 200 subjects. The subjects were randomly selected from the population, which included medical students, health professionals, and those who attended different OPDs and health camps organized by the K S Hegde hospital for general check-ups under health plan scheme. Total bilirubin and direct bilirubin was estimated by dichlorodiphenyl diazonium method. Indirect bilirubin (Total bilirubin-Direct bilirubin) was calculated.

Results: The reference intervals observed for men and women are as follows: Total bilirubin (0.1-1.3)mg/dl and (0.1-1.38)mg/dl, serum direct bilirubin (0-0.5)mg/dl and (0-0.3)g/dl, and serum indirect bilirubin (0.1-0.9)mg/dl and (0.1-1.3)mg/dl respectively. With the advancement of age, a difference in the reference interval was observed among both the sex.

Conclusion: The present study showed a difference in the reference intervals between males and females and also a categorical age wise difference was obtained.

Keywords: Bilirubin, Gender, Reference interval, Healthy population, Age

Introduction

One of the most important tool used in medical decision-making is the clinical laboratory report.[1] This is required for screening of the disease, diagnosis, monitoring disease progression and prognosis of the treatment. Accurate interpretation of laboratory tests requires accurate reference intervals from an appropriate population.[2] Reference intervals are typically established by assaying specimens from a sample group of people who meet carefully defined criteria.[3] The reference interval is usually defined as the values encompassing the central 95% of specimens; equating to 2 standard deviations on either side of the mean.[4] Generating reference intervals for a general population is a major challenge, as it requires selecting the appropriate reference population and recruiting individuals who represent relevant demographic groups that meet the inclusion criteria; collecting, processing and testing specimens; and finally, calculating reference values with possible stratification of the data into subgroups.

In the present scenario, Indian population depend on the values given from the reagent kit manufacturers, text books (Sulthana Furruqh, 2004).[5] Hence there is necessary to set up reference interval for each laboratory.

Bilirubin, catabolized product of haem part of hemoglobin, formed in the liver. It helps to assess the excretory function of liver, an important biochemical parameter to assess the hepatic function. There is not much study done in Indian population regarding the reference interval of bilirubin profile. The aim of this study to establish reference interval of bilirubin in Dakshina Kannada population.

Materials and Methods

This prospective hospital based cross sectional study was done at K S Hegde Charitable Hospital, Mangalore for a period of 2 years (2014-2016) involving 200 healthy subjects (CLSI guidelines).[6] After obtaining institutional ethical clearance, the subjects were randomly selected based on the clinical history and clinical examination which includes medical students, health professionals, and those who attended different OPDs and health camps organized by the hospital for general check-up under health plan scheme.

Age group between 20 to 60 years and who were willing to participate in the study were included in the present study. Subjects below 20yrs and above 60yrs, with history of hepatic or renal diseases, diabetes mellitus, hypertension, cardiovascular diseases, intake of OCPs, Smokers and alcoholics, pregnancy, obesity, malabsorption syndromes and nutritional anaemia were excluded from the study.

Blood samples were collected randomly, after giving 15mins of physical rest,[7] in sitting posture. 2ml of blood sample was collected from antecubital vein in a plain red-topped vacutainer tubes containing clot activator. The samples were left undisturbed for 30 mins following which they were centrifuged for 5 mins at 3000rpm. Sera was separated and analysed in Roche Hitachi c311 autoanalyzer.

All the samples were performed in duplicate. The precision of the instrument was checked on many occasions. All the analytical procedures are standardized, the reagent was calibrated to the instrument before sample analysis was done.[8,9] Serum total and direct bilirubin were estimated using dichlorodiphenyl diazonium method. Indirect bilirubin (Total bilirubin-Direct bilirubin) was calculated.

Statistical Analysis: It was done using SPSS version 16. Descriptive analysis was done using mean and standard deviation. 2.5^{th} and 97.5^{th} percentile was used to obtain reference interval.

Results

Of the 200 participants, 104 (52%) were males while 96 (48%) were females. 81 of the 200 participants (40.5%) were 20–29years old; 42 (21%) were 30–39 years old, 55 (27.5%) were 40–49 years old and 22 (11%) were 50-59 years old. The results of reference interval of Bilirubin based on gender obtained are tabulated in Table 1 and the reference interval based on categorical age and gender are tabulated in Table 2.

Table1: Reference interval of Bilirubin based on gender

Parameters	Male (n=104)		Female (n=94)	
	Median	Reference Interval	Median	Reference Interval
Total Bilirubin (mg/dl)	0.4	0.1-1.3	0.3	0.1-1.38
Direct Bilirubin (mg/dl)	0.2	0-0.5	0.1	0-0.3
Indirect Bilirubin (mg/dl)	0.3	0.1-0.9	0.2	0-1.03

Serum Direct and indirect bilirubin concentration were found to be at higher range in males when compared to females in the present study.

Table 2: Reference interval of Bilirubin based on categorical age and gender

Categorical age in yrs	Male			Female		
	Reference Interval					
	Total bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	Indirect Bilirubin (mg/dl)	Total bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	Indirect Bilirubin (mg/dl)
20-29	0.1-2.4	0-0.9	0-1.5	0.2-1.6	0-0.4	0.1-1.2
30-39	0.2-1.2	0.1-0.5	0.1-0.8	0.1-2	0-0.6	0-1.4
40-49	0.2-1.2	0-0.3	0-0.9	0.1-1.1	0-0.3	0-0.8
50-59	0.2-0.8	0.1-0.3	0.1-0.5	0.1-0.8	0-0.3	0-0.5

Table 2 shows that as age advances the serum bilirubin concentration decreases except in female subjects whose age group was between 30-39yrs.

Discussion

Currently, India is facing serious public health challenges like obesity, cardiovascular disease, diabetes and infectious diseases. Hence, clinical laboratory investigations play a major role for accurate diagnosis and also provide appropriate information about the health of an individual. The present study was aimed at establishing region-specific reference intervals for one of the most important biochemical marker of hepatic disease, serum bilirubin, among apparently healthy Indian population.

In our study, we found that established reference intervals of these biochemical parameters were altered from current reporting ranges. This cautions us the importance of establishing and incorporating the region-specific reference intervals in the laboratory reporting system in our population.

This study provides the first established clinical chemistry reference values for subjects aged 20-59 years for both males and females of Dakshina Kannada, India derived from healthy individuals. 200 apparently healthy subjects were involved in the study. The number of males (n=104) were almost equal to that of females (n=96).

It was observed that, there is a variation in reference intervals of our study when compared to the ones from other sources. Table 3 shows comparison of present study values, kit literature values and values which are published in various literature. The variation in reference interval of bilirubin in different populations are observed. This suggests that in addition to ethnical and ecological factors there will be various analytical methods used for measurement of biochemical parameters (Saathoff et al.2008).[10]

Table 3: Comparison of values observed in other literatures with present study

Analyte (mg/dl)	Sex	Lab values	Present study	Bangalore[5]	North Rift Kenya[11]	China[12]	Kempala, Uganda[13]
Total Bilirubin	M	0-1	0.1-1.3	0.3-1.2	0.28-1.73	0.3-1.77	0.39-2.6
	F	0-1	0.1-1.38	0.2-1	0.19-1.57	0.29-1.39	0.29-1.9
Direct Bilirubin	M	0-0.25	0-0.5	0.1-0.4	0.03-0.29		0.1-0.5
	F	0-0.25	0-0.3	0.1-0.4	0.01-0.22		0-0.4
Indirect Bilirubin	M	0-0.75	0.1-0.9				
	F	0-0.75	0.1-0.3				

Sex differences in the BIL-T and BIL-D values could be partly due to influence of sex hormones. These findings are in agreement with those of similar studies done in Uganda (Eller et al. 2008).[13]

The finding of this study exhibits that in males, there was an age dependent decrease in both BIL-T and BIL-D with age (Table 2) which could be due to the decrease in sex hormones which have been hypothesized to lower these parameters. These observations are consistent with the findings of Zucker et al. 2004.[14] However these findings were not observed among female gender in the present study.

Studies have revealed that the physiological functions vary with population due to variation in diet, genetics, physical, environmental and socioeconomic conditions (Koram et al. 2007).[15]

The reference values for this analyte determined in this study vary from the same population, indicating that there is a need for use of sex and age established reference values that are applicable to specific populations. Hence it is not advisable to take the reference values determined for one population and apply it to another population.

Conclusion

- The findings of this study showed variation in reference intervals of this study when compared to the ones from other sources
- It was observed that there is a difference in reference interval between males and female
- It also showed categorical age wise difference among sexes
- The reference values for the analytes determined in this study varied from the same population, indicating that there is a need for use of sex and age established reference values that are applicable to specific populations. Hence it is not advisable to take the reference values determined for one population and apply it to another population.

Limitations

As the small sample size and male to female ratio is less in the present study, the result generated in the study cannot be generalized for the population. Hence further study is needed with increased sample size in order to set up reference interval for the concerned population.

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