ORIGINAL ARTICLE

# Chitinase-3-like protein 1: reference interval of a healthy population and its diagnostic value for liver fibrosis in Pakistan

Faryal Husnain[1]*, Muhammad Dilawar Khan[1], Omar Rasheed Chughtai[1], Akhtar Rasheed Chughtai[1], Shakeel Ashraf[1], Muhammad Usman[1]

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## ABSTRACT

**Background and Objective**: Chitinase-3-like protein 1 (CHI3L1) is an upcoming biomarker for the diagnosis of liver fibrosis. The reference intervals (RIs) for CHI3L1 have not been established in the Pakistani population. Thus, this study aimed to determine the RIs in our population and the cut-off value for diagnosis of hepatic fibrosis.

**Methods:** This is a cross-sectional study. A total of 408 participants (202 healthy and 206 diagnosed liver fibrosis cases) were recruited. Serum CHI3L1 level was measured on CHI3L1 kits (Proprium Biotech Co. Ltd) by manual enzyme-linked immunosorbent assay. The RIs were estimated by percentile and working normal method.

**Results:** The distribution of CHI3L1 values showed no remarkable variation with gender and age. The 95% RI of CHI3L1 was 12.80-81.80 ng/ml in healthy Pakistani subjects and the cut-off for the diagnosis was 102.12 ng/ml in hepatic fibrosis cases.

**Conclusion:** The RIs in healthy adults and the cut-off for the diagnosis of hepatic fibrosis of serum CHI3L1 were determined in a selective adult Pakistani population. This will be a useful reference for further local and international studies.

**Keywords:** Chitinase-3-like protein 1, cirrhosis, hepatic fibrosis, reference interval, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), hepatocellular carcinoma.

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**Correspondence to:** Faryal Husnain
*Department of Chemical Pathology and Immunology, Chughtai Institute of Pathology, Lahore, Pakistan.
**Email:** drfaryalhusnain.5712@cll.edu.pk
*Full list of author information is available at the end of the article.*

## Introduction

Hepatic fibrosis is a healing response of hepatocytes to chronic and repeated damage caused by multiple agents like viruses, toxins, alcohol abuse, and other causes. Liver fibrosis entails a continuous, synchronous, and repetitive cycle of damage and healing of functional hepatic tissue caused by insults. It can lead to serious complications like hepatic insufficiency (which may ultimately lead to liver failure) and portal hypertension (which causes jaundice, variceal bleeding, ascites, and portosystemic encephalopathy). Liver fibrosis progresses to advanced fibrosis or cirrhosis, involving most of the liver parenchyma.[1]

Chitinase-3-like protein 1 (CHI3L1), also known as YKL-40 and cartilage glycoprotein 39, is cited as a novel biomarker for the diagnosis of hepatic fibrosis. Prior studies have implicated CHI3LI involvement in hepatic fibrosis, particularly in areas of active scar tissue formation due to various causes.[2,3] It is a glycoprotein composed of 383 amino acids and weighs 42.6 kd. In other species, it hydrolyzes the β-(1, 4)-linkage between the adjacent N-acetyl glucosamine residues of chitin; in humans, it has high affinity for chitin but no chitinase activity because of a mutation in its active site.[4]

For any marker to be used in clinical settings, a reference interval (RI) should be available for the relevant population. RIs allow clinicians to interpret laboratory results with a certain level of confidence while making an accurate and early diagnosis. RIs are utilized for diagnosis, monitoring, and prognosis of diseases, as well as medical or surgical interventions and decisions.[5,6] An RI is a numerical range or interval that helps determine whether or not an individual is healthy. An RI for an analyte provides a range of values for healthy groups of people based on age, gender, race,

pregnancy status, etc., which are considered acceptable. For example, if a patient's analyte concentration after laboratory analysis falls outside this interval, then it is considered a possible sign of disease, is and the patient referred for further evaluation. In this sense, the RI is a comparative measurement and a basic medical decision-making tool.[7,8] It is also important for the laboratory to carefully establish RIs according to standard protocols, for example, the Clinical and Laboratory Standards Institute Guidelines.[9]

However, to our knowledge, the RIs of CHI3L1 in diagnosing liver fibrosis has not been analyzed in our population. Our laboratory seeks to validate and verify novel biochemical markers for the diagnosis of liver fibrosis in our population. Therefore, we sought to determine the RIs of CHI3L1 for diagnosing liver fibrosis in Pakistan.

Currently, scant data are available for the RIs of CHI3L1 levels in Pakistani populations, in both health and disease. Therefore, this study was designed to determine the "reference range" of serum CHI3L1 in healthy people and cut-off in patients with hepatic fibrosis. This may subsequently be helpful for the clinicians as a reference value for the diagnoses of hepatic fibrosis in the local population.

## Methods

This cross-sectional study was undertaken at the Department of Chemical Pathology Chughtai Institute of Pathology, Lahore, Pakistan. It was carried out from 15th January 2021 to 31st March 2021 after getting approval from the Institutional Review Board, letter no. CIP/IRB/1060. Blood samples were collected from the 202 healthy subjects and 206 diagnosed hepatic fibrosis patients.

Four ml venous blood sample was drawn from each participant to obtain at least 1.2 ml serum. The sample was centrifuged for 15 minutes at 3,000 rpm. Healthy subjects' ($n$ = 202) samples that were screened for routine liver function tests were included and cases of hepatic fibrosis ($n$ = 206) were selected from among chronic hepatitis B and C patients via history and laboratory reports. Those serum samples were considered ineligible for analyses which were contaminated with fibrin, red blood cells, or other particulate matter. Moreover, lipemic, hemolytic, or icteric samples were also discarded.

These tests were carried out on CHI3L1 kits (Hangzhou Proprium Biotech Co. Ltd, Hangzhou, Zhejiang, China) by manual enzyme-linked immunosorbent assay. This assay is a sandwich immunoassay for immunochromatography.[10] Data were processed by NCSS software and the RIs and cut-off values for diagnosis of liver fibrosis were established.

### *Statistical analysis*

Mean, median, standard deviation (SD), and interquartile ranges (IQR) were determined for both healthy subjects and hepatic fibrosis cases. Skewness and Shapiro-Wilk normality were also determined for both sets of samples by the NCCS software. RIs in healthy individuals ($n$ = 202) were calculated by two-sided percentile method. The following formulation for the percentile method was given by Horn et al.[11] In this case, the lower and upper limits of the RIs are defined as 100 (α/2) and 100 (1 - α/2) percentiles of the sorted data values. The methods for percentile estimation used are Method A: $p$ ($n$ + 1) Method A, which calculates an index $p$ ($n$ + 1).

RIs in hepatic fibrosis cases ($n$ = 206) were calculated after a Box-Cox transformation as the data was not normally distributed by normal theory method. The formula for this method is as follows:

The lower and upper limits of the RIs are defined as RL = $\bar{x}$ + ta/2, $n$-1 ^$s$ √1+1/$n$

Ru = $\bar{x}$+ $t$1 -$a$/2, $n$-1^$s$ √1+1/$n$, where $\bar{x}$ is the sample mean and $s$ is the sample SD.

The data were analyzed using NCSS version 2021 computer software.

## Results

A total of 408 participants (mean age = 39.6 ± 12.6 years; 277 male and 131 female) were included in this analysis. The 95% RI of chitinase-like protein 1 was 12.80-81.80 ng/ml in healthy Pakistani subjects and the cut-off for the diagnosis was 102.12 ng/ml in hepatic fibrosis cases. Our RIs in healthy and diseased patients were calculated by the percentile method and working normal method, respectively. We determined the RIs by using the interval covering the 2.5 to 97.5 percentile of cases in healthy individuals and the lower 5th percentile cut-off for the diagnosis of hepatic fibrosis via the traditional normal theory formula in hepatic fibrosis cases. As the data in hepatic fibrosis cases was not normally distributed, the Box-Cox transformation procedure was carried out to bring the distribution closer to normal, before calculating the cut-off (Tables 1 and 2). Figures 1 and 2 show the distribution of CHI3L1 values in healthy individuals and hepatic fibrosis cases, respectively.

## Discussion

CHI3L1 is not expressed under physiological conditions; its levels increase in patients with inflammatory diseases like rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, sarcoidosis, chronic obstructive pulmonary disease, asthma, atherosclerosis, type 1 and type 2 diabetes, liver fibrosis, and a variety of solid carcinomas.[12] Its role is still unclear; however, it is expressed and secreted by a diverse kind of cells such as hepatic stellate cells, vascular smooth muscle cells, chondrocytes, fibroblast-like synovial cells, and macrophages. It is also expressed in inflammation, tissue remodeling, and fibrosis.[13] Prior research has shown CHI3L1

***Table 1.*** *Descriptive statistics for cut-off for hepatic fibrosis (n = 206).*

| Descriptive statistics of healthy individuals (*n* = 202) | | | | | | |
|---|---|---|---|---|---|---|
| **Count** | **Mean** | **Median** | **SD** | **IQR** | **Minimum** | **Maximum** |
| 202 | 54.895 | 59.900 | 18.3437 | 24.300 | 0.001 | 85.900 |
| **Normality report of healthy individuals (*n* = 202)** | | | | | | |
| **Mean** | **SD** | **Coefficient of variation (COV)** | **Skewness (normal = 0)** | **Kurtosis (normal = 3)** | **Anderson-Darling normality *p*-value** | **Shapiro-Wilk normality *p*-value** |
| 54.895 | 18.3437 | 0.3342 | -0.7300 | 2.9331 | 0.000 | 0.000 |
| **Quantile report of healthy individuals (*n* = 202)** | | | | | | |
| **5th percentile** | **10th percentile** | **25th percentile** | **50th percentile** | **75th percentile** | **90th percentile** | **95th percentile** |
| 20.000 | 27.000 | 44.300 | 59.900 | 68.600 | 76.000 | 78.700 |
| **Two-sided 95th percentile RIs of healthy individuals (*n* = 202)** | | | | | | |
| **2.5% lower reference limit (90% confidence interval)** | | | **97.5% upper reference limit(90% confidence interval)** | | | |
| **Value** | **Lower** | **Upper** | **Value** | **Lower** | **Upper** | |
| 12.800 | 0.001 | 20.000 | 81.800 | 79.300 | 85.900 | |

***Table 2.*** *Descriptive statistics of CHI3L1 in healthy individuals (n = 202).*

| Descriptive statistics of hepatic fibrosis cases (*n* = 206) | | | | | | |
|---|---|---|---|---|---|---|
| **Count** | **Mean** | **Median** | **SD** | **IQR** | **Minimum** | **Maximum** |
| 206 | 240.479 | 150.000 | 206.8317 | 199.00 | 54.400 | 800.000 |
| **Normality report of hepatic fibrosis (*n* = 206)** | | | | | | |
| **Mean** | **SD** | **COV** | **Skewness (normal = 0)** | **Kurtosis (normal = 3)** | **Anderson-Darling normality *p*-value** | **Shapiro-Wilk normality *p*-value** |
| 240.479 | 206.8317 | 0.8601 | 1.6931 | 4.8742 | 0.0000 | 0.000 |
| **Quantile report of hepatic fibrosis cases (*n* = 206)** | | | | | | |
| **5th percentile** | **10th percentile** | **25th percentile** | **50th percentile** | **75th percentile** | **90th percentile** | **95th percentile** |
| 67.400 | 86.200 | 104.000 | 150.000 | 303.000 | 552.000 | 800.000 |
| **Lower 5% normal theory-reference bound of hepatic fibrosis cases (*n* = 206)** | | | | | | |
| **Count** | **Value** | **Lower** | **Upper** | | | |
| 206 | 102.102 | 138.460 | 65.477 | | | |

activity in areas with fibrosis, particularly areas with active scar tissue formation on immunohistochemical analysis. Many research articles have concluded that CHI3L1 is a novel biomarker for hepatic fibrosis arising from viral and alcohol damage.[14,15]

The identification of appropriate RIs for a biomarker is one of the essential steps for its clinical application. It is necessary to define a reference range for a biomarker, and particularly an upper limit of normal in the clinical setting.[16] The absence of information about age, sex, race, and region of origin for assay-specific RIs can directly affect medical interventions and clinical therapeutic strategies.[16,17] CHI3L1 has become a useful biomarker in the diagnosis of hepatic fibrosis, but only limited information and few formal large-scale studies have been conducted to define its RIs in healthy subjects.

A study by Saleh et al.[18] calculated the RIs in their respective populations as 10.5-190 ng/ml in healthy Egyptians. The mean age of the participants in the study was 62.7 ± 5.82 years and the male-to-female ratio was 8:2. This shows that there are ethnic differences and that every country should establish their own RIs, with more than one for different ethnic groups.

One study from Pakistan estimated the RI of CHI3L1 to be 90 ng/ml using a receiver operating curve (ROC) analysis in patients with hepatic fibrosis. The mean age of the patients was 48.6 ± 12.6 years and majority of them were male.[19]

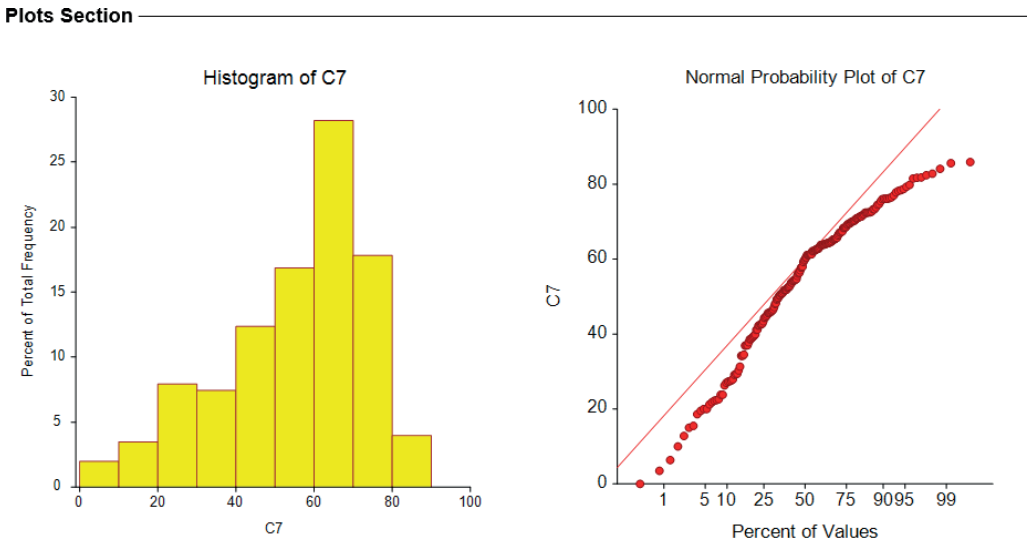


***Figure 1.*** *Distribution of CHI3L1in healthy individuals (n = 202).*

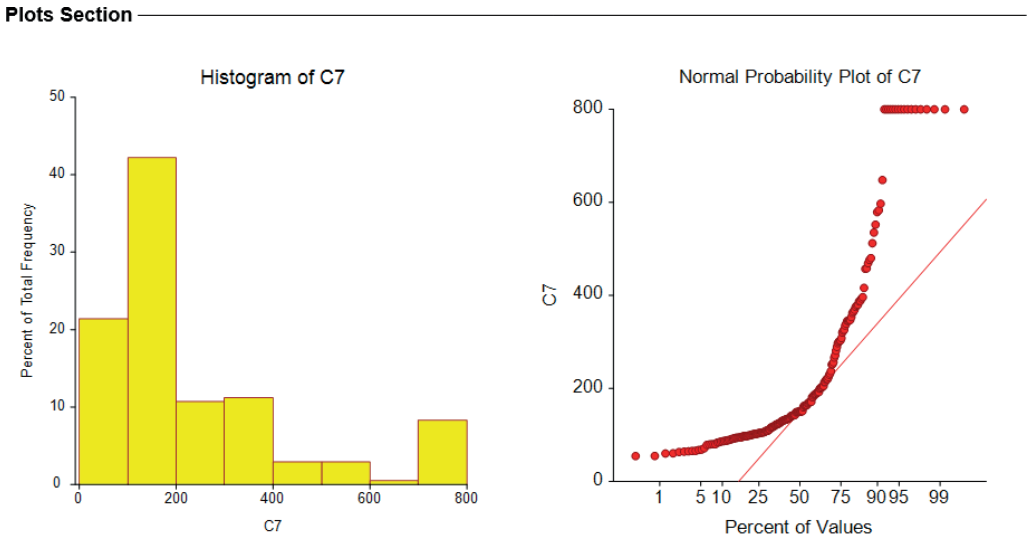


***Figure 2.*** *Distribution of CHI3L1 in hepatic fibrosis cases (n = 206).*

Another Japanese study by Kumagai et al.[20] calculated the cut-off for significant fibrosis as 165 ng/ml in cases with hepatic fibrosis.

The current study will augment the expanding database of CHI3L1 RIs. These data will facilitate the better use of CHI3L1 in the Pakistani population. This marker can have great utility in our setup where the burden of hepatitis B, hepatitis C, and diabetes mellitus is heavy, which can lead to advanced liver fibrosis and cirrhosis. It is a preventable and progressive condition which leads to hepatocellular carcinoma. We have a paucity of screening tools like radiologic exams (such as elastography), but a marker like CHI3L1 can help diagnose and monitor hepatic fibrosis and a reliable RI is a prerequisite for that.

## Conclusion

The CHI3L1 RI in healthy Pakistani subjects is 12.80–81.80 ng/ml, while the cut-off level for the diagnosis of hepatic fibrosis is 102.12 ng/ml. These data will facilitate the better use of CHI3L1 in predicting the course of disease in patients with advanced liver fibrosis in our clinical settings. Leading laboratories in the country must take the responsibility to address the gaps related to population-specific RIs.

## Limitations of the study

There may be some constraints in the present study. Firstly, all of our samples were collected from Lahore, so it is not a good representation of all major ethnic populations of Pakistan, as our sample group included mostly Punjabis. There are significant racial differences among different populations and RIs must be verified for different populations, and if significant differences are detected then RIs must be established for each ethnic group. Secondly, this is a cross-sectional analysis, so it is was not possible to determine whether there is any time-dependent change in CHI3L1 levels in healthy subjects. Thirdly, we had chosen diagnosed cases of hepatic fibrosis, so we could not use the ROC analysis for determining the cut-off for diagnosis; instead, we calculated our cut-off using the percentile method.

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## List of Abbreviations

| | |
|---|---|
| CHI3L1 | Chitinase-3-like protein 1 |
| RI | Reference interval |

## Conflict of interest

None to declare.

## Grant support and financial disclosure

This study was funded by the Chughtai Institute of Pathology Lahore, Pakistan.

## Ethical approval

This study is approved by the Institutional Review Board of Chughtai Institute of Pathology, Lahore, Pakistan, vide letter no. CIP/IRB/1060 dated 01/01/2021.

## Authors' contribution

**FH:** Study design, drafting of manuscript, and acquisition of data.
**MDK, ORC, and ARC:** Concept of the study, data interpretation, and approval of final version of the manuscript.
**SA and MU:** Data acquisition, analysis and interpretation, and final drafting of the manuscript.
**ALL AUTHORS:** Approval of the final version of the manuscript to be published.

## Author details

Faryal Husnain[1], Muhammad Dilawar Khan[1], Omar Rasheed Chughtai[2], Akhtar Rasheed Chughtai[2], Shakeel Ashraf[1], Muhammad Usman[1]

1. Department of Chemical Pathology and Immunology, Chughtai Institute of Pathology, Lahore, Pakistan
2. Department of Histopathology, Chughtai Institute of Pathology, Lahore, Pakistan

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