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Reference Interval of Enhanced Liver Fibrosis (Elf) Score in Healthy Multi-ethnic Malaysian Population

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) is one of the commonest causes of liver fibrosis in our general population. While liver biopsy remains the gold standard method for the diagnosis and staging of liver fibrosis in MASLD, it is not without its limitations. The Enhanced Liver Fibrosis (ELF) test is a set of non-invasive direct markers of liver fibrosis composed of tissue inhibitor of metalloproteinase 1 (TIMP-1), amino-terminal propeptide of type III procollagen (PIIINP) and hyaluronic acid (HA). The levels of these markers are then used in an established formula to produce the ELF score which will enable the classification of liver fibrosis in various stages. However, a reference interval for the ELF score meant for the healthy multi-ethnic population is yet to be established. Therefore, the aim of this study was to determine the reference interval for the ELF score in a healthy multiethnic Malaysian population. The ELF score was measured in 181 seemingly healthy blood donors of known gender, age, and ethnicity using an ADVIA Centaur XP automated system. The ELF score was calculated using the algorithm combining TIMP-1, PIIINP and HA values. The reference interval was determined using the nonparametric method following Clinical and Laboratory Standards Institute (CSLI) guidelines (C28-A3). The reference interval (90% confidence interval (CI)) for ELF score for the overall population ranges from 6.08 (5.25-7.02) to 9.85 (9.61-10.87). ELF score of 9.8 - 10.5 recommended by Younossi Z. et al (10.1016/j.cgh.2026.01.016) is used in the guideline to identify patients likely having metabolic dysfunction-associated steatohepatitis (MASH) with F2-F3 fibrosis for pharmacological treatment. ELF score was significantly higher for males versus females (P<.01). ELF score also showed significant age-dependent differences (P<.001). However, there was no significant difference in ELF scores between ethnic groups (P>.05). The reference interval of ELF score helps in assessing and identifying people at high risk of liver fibrosis especially MASLD before they are subjected to a further set of detailed testing. However, factors that may influence the ELF score such as gender, obesity and increasing age, need to be taken into consideration.

Introduction

Liver fibrogenesis, is a dynamic, highly integrated molecular, cellular and tissue process responsible for driving the excess accumulation of extracellular matrix (ECM) components (i.e., liver fibrosis) sustained by an heterogeneous population of hepatic myofibroblasts (Parola & Pinzani, 2019). Fibrosis results from an imbalance between fibrogenesis and fibrinolysis. The predominant aetiologies of liver fibrosis encompass chronic viral hepatitis, alcohol misuse, and metabolic dysfunction-associated steatotic liver disease (MASLD).

MASLD encompasses a spectrum of conditions from simple fatty liver to non-alcoholic steatohepatitis (NASH-fatty changes with inflammation and hepatocellular injury or fibrosis), to advanced fibrosis and cirrhosis (Tacke & Weiskirchen, 2021; Tanwar *et al*., 2020). MASLD is also associated with insulin resistance (Watt *et al*., 2019), type 2 diabetes mellitus (Targher *et al*., 2021), dyslipidaemia (Katsiki *et al*., 2016), obesity (Fan *et al*., 2017), and metabolic syndrome (Radu *et al*., 2023). According to the systematic review and meta-analyses reported by Riazi *et al*. (2022), the overall prevalence of MASLD

worldwide is approximately 32.4%. Unfortunately, the prevalence of MASLD increased over time from 25.5% in or before 2005 to 37.8% in 2016 or later (Riazi *et al*., 2022).

Tateishi *et al*. (2019) indicated that the aetiologies of hepatocellular carcinoma in Asia are shifting from viral to non-viral risk factors, including MASLD. Yip *et al*. (2022) indicated that the prevalence of MASLD in Asia has reached levels comparable to that in the West. The incidence of type 2 diabetes mellitus and obesity in Malaysia is reportedly increasing, suggesting a probable rise in MASLD as well (Jan Mohamed *et al*., 2015), with the current prevalence at approximately 22% (Goh *et al*., 2013). A cross-sectional observational study in Klang Valley, Malaysia revealed that 37.4% of individuals were diagnosed with definite MASLD (Khammas *et al*., 2019). The incidence of MASLD was greater among Indians and Malays than in Chinese (Khammas *et al*., 2019). Comparing the study by Goh *et al*. (2013) and Khammas *et al.* (2019), the six-year interval revealed a 15.4% increase in the prevalence of MASLD among Malaysians.

In spite of liver biopsy being an invasive procedure with a potential sampling error and associated morbidity and mortality, it has remained the gold standard method for the pathological diagnosis and assessing the severity of MASLD (Angelini *et al*., 2023). However, several non-invasive strategies have been proposed as reliable screening methods without the need for biopsy as the first line assessment tool. Imaging techniques such as ultrasound, computed tomography scan and magnetic resonance imaging (MRI) scan have also been proposed as adjuvant diagnostics methods of MASLD but their properties for that purpose are very limited (Theel *et al*., 2022).

Besides imaging-based techniques, liquid biopsy using blood and serum samples has emerged as a promising adjuvant to direct tissue biopsy in order to investigate the presence of MASLD (Mann *et al*., 2018; Sookoian & Pirola, 2017). Using the concept of liquid biopsy, several clinical scoring systems based on simple clinical or laboratory indices have been proposed to screen for advanced fibrosis in patients with MASLD and other liver diseases. These include HepaScore®, FibroTest®, FibroMeter®, (Leroy *et al*., 2014),NAFLD fibrosis score (Angulo *et al*., 2007), and the quantification of aspartate aminotransferase (AST) to platelet ratio index (APRI**)** (Yilmaz *et al*., 2011). In a multicentre study carried out by Angulo *et al*. (2007), theNAFLD fibrosis score was clearly able to distinguish advanced fibrosis and hence the suggestion forNAFLD fibrosis score to be utilised as a triaging tool for optimising liver biopsy. However, theNAFLD fibrosis score has not been shown to be effective in differentiating milder forms of fibrosis from the normal or moderate forms (Angulo *et al*., 2007). Over time, there are other models proposed to replaceNAFLD fibrosis scoring. A clinical model proposed by Bazick *et al.* (2015) reported better performance compared to theNAFLD fibrosis score in detecting advanced fibrosis.

On the other hand, the establishment of Enhanced Liver Fibrosis (ELF) score from the ELF test, by Rosenberg *et al*. (2004) (on behalf of the European Liver Fibrosis Group) has shown its ability to further classify liver fibrosis into mild, moderate, and severe categories. The ELF scoring system consists of a panel of three direct serum markers measured by immunoassay method consisting of tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), procollagen III amino terminal peptide (PIIINP), and hyaluronic acid

(HA). There have been two important developments regarding the utility of ELF: (1) The increasing use of ELF as a second line test for patients with elevated FIB4, and (2) The use of ELF to identify patients for pharmacological treatment and for monitoring treatment response (Zobair M Younossi et al, 2026,doi: 10.1016/j.cgh.2026.03.030). However, to implement the ELF scoring system for assessing liver fibrosis, the reference interval for the ELF score for a particular population must be established beforehand. Therefore, the objective of the present study focused on establishing the reference interval in a multi-ethnic population of Malaysia comprising the Malays, Chinese, and Indians. To the best of our knowledge, this is the first study that was conducted to establish a reference interval for the ELF scoring system using blood samples from the multi-ethnic Malaysian population.

Materials and Methods

Study subjects

This cross-sectional study involved 181 healthy blood donors aged between 18–60 years of both genders and varying ethnicity consisting of 77 Malays, 80 Chinese, and 24 Indians. There was a total of 75 males and 106 females in this present study. This study was carried out at the University of Malaya Medical Centre (UMMC), Kuala Lumpur. Participants were validated as healthy based on the pre-donation questionnaires and clinical examination. Clinical records including age, race, gender, body mass index (BMI), alcohol history, and past medical history were collected, recorded, and tabulated. The participants also had their blood tested for plasma glucose level, fasting lipid profile, and renal function test. The assessment of liver enzymes which includes serum alanine aminotransferase (ALT), AST, gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP) was conducted to exclude issues pertaining to liver disorders. Each participant had also undergone screening for hepatitis B, hepatitis C, and HIV. Subjects with abnormal results were then further excluded from the study.

The exclusion criteria implemented in the present study consisted of (I) diagnoses of acute or chronic liver disease, (II) obesity which is defined as having a BMI greater than 27.5kg/m^2, (III) presence of type 2 diabetes mellitus, (IV) hypertension (with systolic and diastolic blood pressure ≥ 140/90 mm Hg), (V) metabolic syndrome, (VI) systemic autoimmune disease and (VII) neoplastic disorder, (VIII) substance abuse or severe alcohol consumption. Informed consent was obtained during the recruitment phase before blood sampling was carried out. The study protocol has been approved by the ethics committee of UMMC [MEC 895.9].

Serum collection

All blood samples were collected according to a standard collection protocol. Approximately, 5 ml of blood were collected in a gel (SST) tube containing clot-activator elements. The samples were centrifuged at 3000 rpm for 5 minutes after being clotted for 30–60 minutes at room temperature. Aliquots of serum samples were stored at -80ºC until analyses were performed.

Biomarker Assays

The sera were processed using ADVIA CENTAUR XP according to the manufacturer's instructions for the quantitative determinations of TIMP-1, PIIINP, and HA. As mentioned earlier, the ELF scoring system provides a single value using an algorithm combining these three serum markers. The ELF score was calculated directly by the instrument employing the following equation:

$$ELF\ score\ =\ 2.278\ +\ 0.851\,ln\,(HA)\ +\ 0.751\,ln\,(PIIINP)\ +\ 0.394\,ln(TIMP-1)$$

Statistics analysis

The statistical analyses were performed using the GraphPad Prism 9 software. The significance level was fixed at $P < 0.05$. Data were displayed as either mean ± standard deviation (SD) or median (interquartile range (IQR)). The error bars in the diagrams represent the IQR (for non-parametric analysis). Reference intervals were calculated as overall for the study population and according to gender, different age groups and different ethnicities. The calculation for the reference interval is based on the recommendations by CLSI C28-A3. According to CLSI C28-A3, the most reliable way to establish a reference interval is to collect data from an adequate number of well-defined reference individuals, with a minimum of 120 samples per partition (e.g., by sex or age group) for non-parametric analysis. However, except for the overall ELF score reference interval calculation, the other variables had less than 120 samples per division of subgroups. As summarized by Ozarda *et al*. (2019), the CLSI EP28-A3c guideline supports the use of robust methods when sample size is limited (< 120), while recommending at least 120 samples for nonparametric reference interval estimation. On that note, for sample sizes below 120, the robust method implemented in MedCalc software was used as an alternative to the percentile method, employing iterative weighting based on the median and median absolute deviation to estimate the lower and upper reference limits. Deemed suitable, hence, the MedCalc version 23.4.0 software was utilised for the determination of reference intervals.

Results

Quantification and comparison of TIMP-1, PIIINP and HA based on gender, age groups, and ethnicity of the participants

The mean ± SD concentrations (ng/ml) of TIMP-1, PIIINP, and HA based on the present population (N = 181) were 200.70 ± 162.30, 7.41 ± 3.02 and 22.11 ± 19.50. The gender comparison in terms of concentrations of TIMP-1 and PIIINP showed no significant differences (P>.05) (Fig. 1A and 1B). It was noted that the quantitated HA levels showed significant gender-dependent differences (P<.001) (Fig. 1C).

Note

The data (not normally distributed) were expressed as median (IQR) and the concentrations of analytes between the two genders were analysed using the Mann-Whitney test.

Among the three analytes, only HA values showed significant age-dependent differences (*P*<.001) (Table 1). The concentration of HA showed an increment with an advancing age pattern. Participants within the age group of 51–60 showed the highest concentration of HA, with a median (IQR) of 26.21 (20.65). The other two analytes which were TIMP-1 and PIIINP showed no significant differences when compared between different age groups (*P*>.05). At the same time, there was no definite pattern in the concentration of TIMP-1 and PIIINP as analysed with increasing age (Table 1).

Table 1
Quantification and comparison of TIMP-1, PIIINP and HA based on age groups.

Analytes	**Age group**	**Range**	**Median (IQR)**	***P*-value**
TIMP-1	≤ 20	32.40–215.50	178.80 (101.20)	*P*>.05
	21–30	41.60–2230.00	193.10 (38.70)	
	31–40	51.60–597.70	199.60 (57.10)	
	41–50	115.40–292.10	197.20 (71.20)	
	51–60	123.90–274.60	196.50 (71.60)	
PIIINP	≤ 20	2.20–16.06	6.77 (4.24)	*P*>.05
	21–30	2.36–15.80	7.04 (3.26)	
	31–40	2.16–17.82	6.28 (3.62)	
	41–50	3.99–18.58	6.88(3.40)	
	51–60	3.37–15.64	7.14(3.60)	
HA		2.79–14.52	7.99 (3.65)	*P*<.001
	≤ 20	3.67–58.40	10.97 (8.69)	
	21–30	3.41–101.90	17.43 (20.41)	
	31–40	7.51–104.60	22.88 (24.48)	
	41–50	8.96–121.70	26.21 (20.65)	
	51–60			

Note: All the data were not normally distributed. Therefore, the Kruskal Wallis test was conducted for all three analytes (based on age groups).

Based on Table 2, it was apparent that there were no significant changes in the concentrations of TIMP-1, PIIINP and HA in terms of ethnicity. Based on the data obtained, we noted that the Malay participants showed the highest readings for TIMP-1 and PIIINP, the Indian participants showed the highest reading for HA.

Table 2
Quantification and comparison of TIMP-1, PIIINP and HA based on ethnicity.

Analytes	Ethnicity	Range	Median (IQR)	*P*-value
TIMP-1	Chinese	32.40–292.80	192.60 (41.70)	*P*>.05
	Indian	51.60–271.60	201.00 (87.00)	
	Malay	41.60–2230.00	196.70 (56.90)	
PIIINP	Chinese	2.20–18.58	6.85 (3.71)	*P*>.05
	Indian	3.36–16.35	6.26 (3.51)	
	Malay	2.16–17.34	7.06 (3.43)	
HA		3.26–96.42	17.08 (17.30)	*P*>.05
	Chinese	3.41–51.34	18.85 (21.52)	
	Indian	2.79–121.70	13.26 (14.79)	
	Malay			

Note: All the data were not normally distributed. Therefore, Kruskal-Wallis test was conducted for all three analytes (based on ethnicity).

Quantification and comparison of ELF score based on gender, age groups, and ethnicity of the participants

The reference interval (90% CI) for ELF score for the overall population ranges from 6.08 (5.25–7.02) to 9.85 (9.61–10.87). The ELF score was significantly higher for males versus females (*P*<.01) as shown in Table 3. Higher ELF score has been observed among the male participants as compared to females (*P*<.01). The distribution of the ELF score by age group is also shown in Table 3. There was a statistically significant difference between age groups as determined by Kruskal Wallis test (*P*<.001). The median (IQR) values of ELF score in Malays, Chinese and Indians were 7.96(0.79), 8.25(0.79) and 8.22(1.02) respectively. There was no significant difference in ELF score observed between the different ethnic groups (Table 3).

Table 3
Calculation of ELF score reference interval.

Variable		N	Reference Interval	Mean ± SD	Median (IQR)	90% CI		*P*-value
						Lower limit	Upper limit	
Total		181	6.08–9.85	8.16 ± 0.81	8.11(0.90)	5.25–7.02	9.61–10.87	
Gender								
	Male	75	7.01–9.57	8.38 ± 0.75	8.26 (0.92)	6.77–7.27	9.28–9.83	*P*<.01
	Female	106	6.70–9.42	8.00 ± 0.82	7.96 (0.78)	6.50–6.92	9.16–9.66	
Age								
	≤ 20	15	5.98–9.31	7.34 ± 0.89	7.63 (0.57)	5.24–6.82	8.47–9.84	[a]*P*<.001
	21–30	65	6.87–8.95	7.84 ± 0.62	7.90 (0.56)	6.64–7.11	8.71–9.18	
	31–40	36	6.64–9.62	8.24 ± 0.88	8.19 (0.67)	6.24–7.13	9.15–10.11	
	41–50	34	7.57-9.58[a] 7.49-9.59[b]	8.58 ± 0.61	8.57 (0.80)	7.27–7.87 7.26–7.74	9.27–9.88 9.29–9.86	
	51–60	31	7.62-9.75[a] 7.45-9.69[b]	8.68 ± 0.65	8.64 (0.86)	7.29–7.96 7.24–7.81	9.41–10.08 9.38–10.07	
Ethnicity								
	Malay	77	6.50–9.46	8.06 ± 0.88	7.96 (0.79)	6.23–6.81	9.15–9.76	*P*>.05

Note: The Mann-Whitney test was carried out for the gender-based analysis whereas for age and ethnicity, the Kruskal Wallis analysis was carried out. [a]The Kruskal Wallis test for age-based analysis was followed by a post-hoc test, the Dunn`s multiple comparisons test. Pairwise comparisons showed significant differences between a) ≤ 20 and 31–40 age groups (*P*<.01), b) ≤ 20 and 41–50 age groups (*P*<.001), c) ≤ 20 and 51–60 age groups (*P*<.001), d) 21–30 and 41–50 age groups (*P*<.001) and e) 21–30 and 51–60 age groups (*P*<.001). For partitions with small sample sizes, reference intervals were primarily estimated using the robust method[b], with parametric estimates additionally provided for comparison where normality assumptions were satisfied[a].

Variable		N	Reference Interval	Mean ± SD	Median (IQR)	90% CI		*P*-value
						Lower limit	Upper limit	
	Chinese	80	7.04–9.48	8.28 ± 0.73	8.24 (0.91)	6.79–7.31	9.19–9.78	
	Indian	24	6.82–9.67	8.11 ± 0.81	8.22 (1.02)	6.28–7.38	9.19–10.23	

Note: The Mann-Whitney test was carried out for the gender-based analysis whereas for age and ethnicity, the Kruskal Wallis analysis was carried out. [a]The Kruskal Wallis test for age-based analysis was followed by a post-hoc test, the Dunn`s multiple comparisons test. Pairwise comparisons showed significant differences between a) ≤ 20 and 31–40 age groups (*P*<.01), b) ≤ 20 and 41–50 age groups (*P*<.001), c) ≤ 20 and 51–60 age groups (*P*<.001), d) 21–30 and 41–50 age groups (*P*<.001) and e) 21–30 and 51–60 age groups (*P*<.001). For partitions with small sample sizes, reference intervals were primarily estimated using the robust method[b], with parametric estimates additionally provided for comparison where normality assumptions were satisfied[a].

Discussion

The discovery of ELF score has provided an alternative tool for the screening and aiding in the diagnosis of liver fibrosis (Rosenberg *et al*., 2004). ELF score has also been shown to be able to classify patients into those with significant MASLD and without (Guha *et al*., 2008). An appropriate reference interval is undoubtedly required to validate the ELF score in a disease population and hence the aim of this study. The influencing factors such as gender, age and ethnicity were also taken into consideration when establishing the reference interval.

The ELF scoring system consists of a panel of three direct serum markers measured by immunoassay method consisting of TIMP-1, PIIINP, and HA. TIMP-1 inhibits peptidases, preventing ECM degradation and potentially promoting fibrogenesis (Lachowski *et al*., 2019). PIIINP serves as a marker for early fibrogenesis and inflammation and helps differentiate between simple steatosis and more advanced liver conditions like non-alcoholic steatohepatitis (Mosca *et al*., 2019; Polyzos *et al*., 2019). Hepatic stellate cell activation leads to an abnormal ECM fibre deposition (Garbuzenko, 2022), and serum HA levels help identify severe fibrosis in MASLD patients (Suzuki *et al*., 2005). These three markers together assess ECM changes.

In our study, we found that the ELF reference interval for the total population ranged from 6.08 to 9.85 and it was significantly higher in males (median (IQR): 8.26(0.92)) than in females (median (IQR): 7.96(0.78)). Studies by Lichtinghagen *et al*. (2013), Yoo *et al*. (2013), and Dellavance *et al*. (2016) further confirmed that the reference intervals are influenced by gender-factor where the ELF score reference interval is generally higher in males. The ELF score reference intervals and corresponding 90% CIs stratified by gender in the present study demonstrate good agreement with those reported by Lichtinghagen *et al*. (2013) (Table 4), with overlapping intervals suggesting consistency across populations, albeit with minor shifts in the lower and upper limits.

At the individual assay level, Lichtinghagen *et al*. (2013) reported that both PIIINP and HA values showed significant gender-dependent differences. In our study, we found that only HA was significantly higher in males compared to females (Fig. 1). In agreement, a Nigerian study postulated that serum HA level was significantly higher among healthy males compared to females (Ihegihu *et al*., 2018).

The prevalence of MASLD increases with age, particularly in post-menopausal women (Wang *et al*., 2021). The prevalence of MASLD peaked at 50–69 years of age (Wong *et al*., 2023). In a recent survey, almost 20% of American children (age 2–19 years) were obese (Stierman *et al*., 2021) and at least a quarter of these children will also have MASLD (Yu *et al*., 2019). Therefore, in distinguishing the reference intervals for the ELF scoring system, age as a confounding factor was taken into consideration. Comparing the ELF scores of the different age groups, we can observe a significant gradual increase as the age advances, and it peaked at 51–60 years old category. Similarly, the quantification of HA showed significant difference among the age groups where the concentration of HA was seen increasing with advancing age. Ihegihu *et al*. (2018) stated that in each age group, there were significant positive correlations between serum HA and age.

Table 4
The reference intervals/range, mean and median of ELF scores in previous studies.

Study population	Location of study	Major findings	Remark	References
Healthy subjects (N = 40), 20 men and 20 women, Caucasian race)	Czech Republic	Median: 8.27 Reference range: 7.14–9.55	The results were expressed in median and reference range in 95% CI (calculated using the robust method according to the CLSI C28-A3 protocol). The study utilised the ADVIA Centaur immunoassay analyser.	Jabor *et al*., 2018
Blood donors (N = 949), 501 men and 448 women	Colsan - Benevolent Association of Blood Collection in São Paulo, Brazil	The mean ELF scores were significantly different between female (mean ± SD: 8.53 ± 0.75) and male groups (mean ± SD: 8.76 ± 0.76) and also according to age strata ($p < 0.001$). The range of the ELF score was 6.52–10.85.	The results were expressed in mean ± SD and range (minimum and maximum). The study utilised the ADVIA Centaur immunoassay analyser.	Dellavance *et al*., 2016
Healthy controls (N = 67)	Italy	Mean ± SD: 6.36 ± 0.77	The result was expressed in mean ± SD. The study utilised the ADVIA Centaur immunoassay analyser.	Catanzaro *et al*., 2013
Healthy controls (blood donors) (N = 400), 187 men and 200 women	Hannover Medical School, Germany	The total ELF score reference range was calculated non-parametrically as 6.72 (90% CI 6.58–6.84) to 9.79 (90% CI 9.45–10.01). Non-parametrically calculated gender specific reference values (90% CIs in parentheses) were: 7.04 (6.98–7.12) to 9.85 (9.45–10.17) for men and 6.58 (6.43–	The partition of subgroups was evaluated by the standard normal deviate test according to CLSI-standard C28-A3. The study utilised the ADVIA Centaur CP immunoassay analyser.	Lichtinghagen *et al*., 2013

Note: The studies listed above utilised the Siemens algorithms: using the ADVIA Centaur, ADVIA Centaur CP and ADVIA Centaur XP systems as an advanced automated immunoassay analyser: 2.278 + 0.851 ln(HA) + 0.751 ln(PIIINP) + 0.394 ln(TIMP1).

Study population	Location of study	Major findings	Remark	References
		6.72) to 9.31 (9.11–9.45) for women.		
Healthy subjects (N = 183), 72 men and 111 women	South Korea	The normal ELF range was 5.95–8.73. The normal range of men was 6.72–8.93 and that of women was 5.69–8.67.	The ELF value was calculated using the range between the 5th and 95th percentiles. The study utilised the ADVIA Centaur XP immunoassay analyser.	Yoo *et al*., 2013
Note: The studies listed above utilised the Siemens algorithms: using the ADVIA Centaur, ADVIA Centaur CP and ADVIA Centaur XP systems as an advanced automated immunoassay analyser: 2.278 + 0.851 ln(HA) + 0.751 ln(PIIINP) + 0.394 ln(TIMP1).				

Based on our analysis of the present study and the previous studies noted in Table 4, the reference interval of ELF score obtained from the present study is within the range of scores reported by previous studies where Yoo *et al*. (2013) reported the lowest lower limit of ELF score (5.95) for healthy participants whereas Dellavance *et al*. (2016) reported the highest upper limit for ELF score (10.85) for healthy ELF reference interval score. Such comparison was made using the reference interval or range reported by the concerning papers.

Based on Table 4, it is apparent that not a particular reference range of ELF is applicable to the entire population in the world. Most of these studies were conducted using European populations. Therefore, this further validates that the present study is the first to address the application of ELF score reference range using blood samples from the multi-ethic Malaysian population, which could also represent the Southeast Asian populations. Malaysia has a multi-ethnic population comprising of Malays, Chinese and Indians. It is important to know if ethnic groups have any influence on the ELF value. According to Wai *et al*. (2020), theNAFLD fibrosis score has a lower sensitivity in patients of South Asian descent, since most of them had a lower BMI and were younger than Caucasian patients with a similar disease stage, and thus had a lower score. In our study, we found no significant difference in the ELF score or individual markers' values in all 3 ethnic groups. In agreement with our study, another multicentre study of southeast Asians (Malaysian and Chinese) and Caucasians showed that ethnicity did not affect the performance of the non-invasive tests performed (Fu *et al*., 2020). We propose that the present reference interval of ELF score which is 6.08–9.85 can be implemented to represent the different ethnic groups in our Malaysian population.

ELF score can serve as a guide for clinicians when screening populations at high risk of developing liver disorders especially MASLD before subjecting them to further detailed set of tests. ELF score also plays a significant role in monitoring disease progression and clinical outcomes. Our study was limited by its relatively small sample size. A larger number of subjects in each ethnic group will most probably provide a more definitive value in determining the ethnicity specific reference interval.

Reports describing reference ranges of the ELF score, based on HA, PIIINP and TIMP-1 measured on the Siemens Centaur System are scarce. The categorisation of the ELF score for fibrosis staging and liver cirrhosis, according to Lichtinghagen *et al*. (2013), Jabor *et al*. (2018) and Vali *et al*. (2020) is summarised in Table 5.

Table 5
The categorisation of ELF score and its interpretation.

ELF Test Score	Interpretation
< 7.7	Likely none or mild fibrosis
7.7–9.8	Moderate fibrosis
> 9.8	Likely severe fibrosis
> 11.3	Liver cirrhosis

As noted in Table 4, one must take into consideration that the values of the ELF score in apparently healthy persons are mostly in the area of weak fibrosis. The overlap of values in healthy volunteers and patients with weak fibrosis is not surprising, as the ELF score (based on continuous variables of serum indices) is compared with categorical variables (based on liver biopsy) (Jabor *et al*., 2018).

The present study has several limitations that should be considered when interpreting the findings. First, the overall sample size (N = 181) is relatively small compared to previous studies, such as Lichtinghagen *et al*. (2013) (N = 400) and Dellavance *et al*. (2016) (N = 949), which may limit the robustness and generalisability of the derived reference intervals. Second, further stratification of the cohort into subgroups based on gender, age, and ethnicity resulted in smaller sample sizes within each category, potentially reducing the statistical power and precision of subgroup-specific reference intervals. Certain subgroups (of variables) had limited sample sizes (e.g., n = 15 and n = 24), which may affect the stability and generalizability of the reference intervals. Although the robust method was applied as recommended for small samples, its performance may be constrained in very small datasets. Finally, this study was conducted at a single centre, which may not fully capture population variability or provide for adequate number of patients for each ethnic group. Therefore, multi-centre studies involving more diverse populations with a larger number of subjects per ethnic group would be preferable to enhance the external validity of the findings.

Conclusion

In conclusion, ethnicity does not have a significant effect on the reference interval for the ELF score, but we would suggest a gender and age specific reference interval to be used. These factors need to be considered when interpreting the ELF score. The clinical utility of ELF score as a non-invasive marker of hepatic fibrosis should also be assessed by doing a correlation study between the ELF score and liver

biopsy which is beyond the scope of our study. This can strengthen the findings from the various studies on the clinical usefulness of the ELF score.

Declarations

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Funding Declaration

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Human Ethics and Consent to Participate

Written informed consents were obtained from the participants during the recruitment phase before blood sampling was carried out. The study protocol has been approved by the ethics committee of UMMC [MEC 895.9].

Conflicts of Interest

The authors declare no conflict of interest.

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Figures

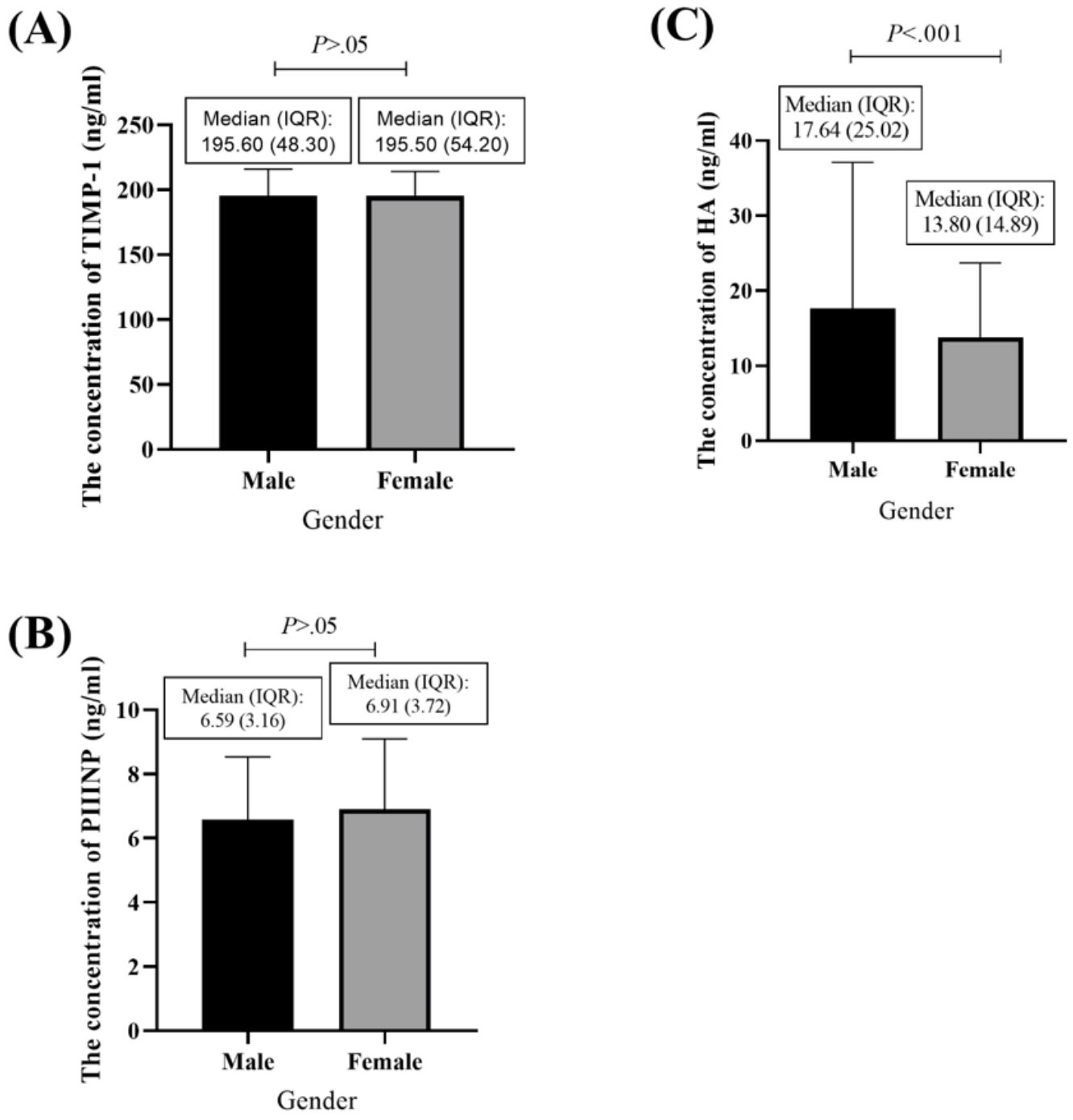


Figure 1

The comparison of A) TIMP-1, B) PIIINP and C) HA based on gender.

Note: The data (not normally distributed) were expressed as median (IQR) and the concentrations of analytes between the two genders were analysed using the Mann-Whitney test.