
Assessing the Incidence and Effect of Haemolysis, Lipaemia, and Icterus in Samples for Lipid and Lipoprotein Analysis

Agnes Magwete¹, Florence Marule², Taryn Pillay¹, Donald M. Tanyanyiwa¹

¹School of Pathology, University of the Witwatersrand and NHLS, Johannesburg, South Africa

²National Health Laboratories, Johannesburg, South Africa

Email address:

donaldmoshen@gmail.com (D. M. Tanyanyiwa)

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Abstract: The aims of this study were to determine the frequency, and evaluate the effects of haemolysis, icterus and lipaemia in samples for lipid lipoprotein tests in an academic medical hospital patient population. This was a retrospective study on data from the central Chemical pathology laboratory at the largest academic hospital in Africa. Serum indices (haemolysis, icterus and lipaemia) were available for all the lipid and lipoprotein chemistry specimens analysed over a 4 year (2007 – 2010) period (n=10, 5555). In the study the effects of haemolysis, lipaemia and icterus on the lipid profiles was determined. From a very large sample pool submitted to Chemical pathology laboratory for lipid and lipoprotein tests, serum indices for lipaemia, hemolysis and icterus were analysed. One thousand six hundred and eighteen (15.33%) were identified as having some form of interferent. Results were as follows: lipaemia, 13.92%; hemolysis, 1.17%; and icterus, 0.25%. There were significant differences between the clear and icteric samples in all the measured lipids and lipoproteins except HDL. Icteric samples had lower HDL levels than the clear samples. However, the frequency of icterus and haemolysis serum indices found in this study is lower than reports from other studies. The frequency of lipaemia found in this study constitutes the highest serum index at the hospital. The study concluded that lipaemia, hemolysis, and icterus occur frequently in blood specimens analysed in the laboratory. Therefore, serum indices are important interfering factors, which require an objective assessment.

Keywords: Haemolysis, Icterus, Lipaemia, Lipid and Lipoproteins and Serum Indices

1. Introduction

Interference in biochemical assays due to haemolysis, icterus or lipaemia (HIL) is a common problem in clinical laboratory practice. Up to 22% of samples received in the laboratory may be influenced by endogenous interferences [1]. Three normal serum pools spiked with six increasing concentrations of hemolysate, bilirubin and triglyceride showed that 50% of tested chemical pathology analytes are affected by haemolysis, 29% are affected by lipaemia, and 24% are affected by icterus [2]. Some of these may result in changes in appearance of serum or plasma. However, the availability of online centrifugation as well as the presence of labels that cover primary tubes prevent identification of visible changes [3]. Interference can be spectral for analytes

that are determined in the same wavelength as the absorption spectrum of the interfering substance. Haemoglobin absorbs light strongly at 415nm wavelength while bilirubin absorbs strongly between 340nm and 500nm wavelengths. Assays that use absorbance measurements in one of these ranges will therefore be susceptible to interference. In lipaemia, spectral interference causes light scattering across the visual spectrum (300 to 700 nm) consequently exerting profound effects on turbidimetric and nephelometric assays. Lipaemia also results in differential partitioning of analyte between the polar and aqueous phases of the sample, and the interaction of the lipoprotein particles with assay reagents can all result in interferences [4]. Haemolysis may interfere with analyte measurement through several mechanisms. In haemolysis, the released intracellular constituents and enzymes that participate

in analytical reactions can directly or indirectly result in chemical interference [5]. Icterus refers to the yellow discolouration resulting from increased sample bilirubin concentration. Bilirubin has reducing properties that interfere with reactions that utilise oxidase/peroxidase to produce hydrogen peroxidase [6]. Hydrogen peroxide is an intermediate in many chemical assays including cholesterol and triglycerides, which are therefore susceptible to icteric interference [7].

Automated platforms have incorporated accurate measurement of spectral interferences due to endogenous substances such as hemoglobin, bilirubin and lipids. The analysers detect the interferences and produce semi quantitative unitless index values for hemoglobin, bilirubin, and intralipids [8]. Serum indices are calculations of absorbance measurements that produce a semi-quantitative level of icterus, haemolysis and lipaemia [9, 10]. This can be achieved by the use of bichromatic wavelength pairs, like 505/480nm (range-1 for icterus), 600/570nm (range-2 for haemolysis) and 700/660nm (range-3 for lipaemia). Absorbance of a compound or chromogen is measured at two wavelengths; one wavelength is used to estimate the magnitude of the test value and the other wavelength assesses the interfering substances. The general formula used in the calculation of serum indices is shown in Appendix 1 [11].

In intravascular haemolysis, red blood cells in circulation release haemoglobin into the plasma. Haemolysis has been associated with hyperlipidemia, due to increased triglyceride synthesis and decreased catabolism [12]. The lipoprotein changes of liver disease have been known for many years and early studies were concentrated on cholestatic liver disease because of the associated high plasma lipid levels [13, 14]. Development of icterus in cholestasis has been demonstrated to have high cholesterol [15].

There are two basic protocols used to evaluate interference. One involves spiking the potential interfering substance into samples and the results compared with unspiked samples. The alternative method involves obtaining samples containing the interfering substance and results from the assay being evaluated compared with results from a highly specific assay not susceptible to interference [4]. The alternative method has the advantage of showing the interference as they occur in patient samples. The alternative method is less commonly used because of the high number of samples required to identify numerous patient samples with the interfering substance and the requirement for a highly specific measurement procedure. The study assessed the frequency of the endogenous interfering constituents in samples sent for lipid and lipoprotein analysis as well as their effects on the lipid and lipoprotein results.

2. Method

2.1. Study Population

This was a retrospective evaluation of lipid profiles in 10,555 patients with Total cholesterol >5.2mmol/L analysed in Chemical Pathology at Chris Hani Baragwanath Academic Hospital in South Africa, from 1 January 2007 to December

2010. The Chemical Pathology department serves the largest tertiary academic hospital in the southern hemisphere and more than 30 surrounding clinics. It handles the largest volumes compared to any individual laboratory in both the private and public sector in South Africa. The laboratory is accredited by the South African National Accreditation System (SANAS) and participates in the Royal College of Pathologists of Australasia (RCPA) EQA program.

2.2. Analytical Procedures

Blood samples collected into Becton, Dickinson (BD) serum separator tubes for the determination of TC, TG, LDL, and HDL. Measurements of lipid profiles were on a Roche Modular diagnostic platform according to the specification of the manufacturers: Roche Diagnostics (Risch-Rotkreuz, Switzerland). Total cholesterol (TC) was measured using a three step colorimetric, enzymatic method with the formation of red colour when hydrogen peroxide reacts with 4-aminophenazone and phenol. Triglyceride (TG) was measured based on the lipase-catalysed hydrolysis of TG to glycerol and fatty acids. The glycerol portion of the TG molecules after hydrolysis is used to determine the TG concentration. Low Density Lipoprotein (LDL) was calculated using the Friedewald Equation ($LDL = (TC - HDL - (TG/2.22))$ mmol/L [16]. High Density Lipoprotein (HDL) was measured using the homogeneous enzymatic colorimetric method. The performance targets in terms of the CV for all the analytes were within the acceptable CV for Roche Modular.

2.3. Assessing Effects of Interfering Substances

The alternative method was used to evaluate the effects of interfering substances on lipid profile by comparing lipid profiles with different serum indices reports to lipid profiles from samples without (clear) interfering substances.

2.4. Data Analysis

Microsoft Excel was used to capture the lipid levels data. Statistical analyses were performed using Graphpad Prism (Version 5, Graphpad Software Inc. San Diego, CA) and STATA (Version 11, StatSoft, USA) statistical programs. Column statistics provided the statistical analysis, which included a descriptive statistics summary. Normality was tested using Shapiro-Wilk Test and Analysis of Variance (ANOVA) to compare the three datasets. Statistical significance for the analysis was defined as $p < 0.05$.

3. Results

A total of 10,555 lipid profiles were identified, and accordingly classified under one of the three serum indices. Samples with a clear appearance constituted 84.67%, lipaemic serum index 13.92%, haemolysed serum index 1.16% and icteric serum index. 0.25%. The semi quantitative value for the serum index was not taken into account. The column statistics show the median (95%CI) values (Table 1)

for the measured lipoprotein parameter in each serum index category.

Table 1. Median (95%CI) values of lipid profiles levels.

		Clear	Haemolysed	Icteric	Lipaemic
TC	Median ((95% CI)	5.80 (6.10 – 6.15)	6.00 (6.12– 6.49)	7.10 (7.16– 10.95)	6.10 (6.51– 6.71)
TG	Median ((95% CI)	1.60 (1.89 – 1.96)	1.80 (1.97– 2.49)	2.50 (2.06– 3.91)	2.85 (3.74– 418)
LDL	Median ((95% CI)	3.20 (3.25 – 3.31)	3.55 (3.23 – 3.66)	4.20 (3.66 – 5.77)	3.60 (3.80 – 3.97)
HDL	Median ((95% CI)	1.20 (1.27 – 1.29)	1.30 (1.21 – 1.38)	1.15 (1.00 – 1.33)	1.20 (1.24 – 1.28)

TC - Total Cholesterol; TG – Triglyceride; LDL – Low-density lipoprotein; HDL – High-density lipoprotein

Using the Student's t-test, measured parameters in the clear (control) samples were lower than the haemolysed, icteric and lipaemic samples except HDL which was lower in the icteric sample with a median (95%CI) of 1.15mmol/L (1.00 – 1.33mmol/L) compared to the clear sample median (95%CI) of 1.20mmol/L (1.27 – 1.29 mmol/L). Figures 1 to 4 demonstrate the difference between the measured lipoprotein levels in the clear sample compared to the haemolysed, icteric and lipaemic samples respectively.

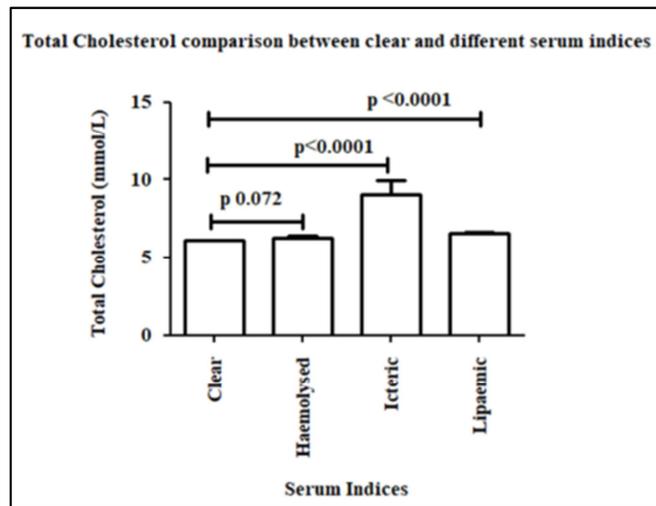


Figure 1. A significant positive ‘interference’ was observed across all the serum indices in the measurement of Total cholesterol. Difference between the clear and haemolysis index was not significant ($p\ 0.072$) and a small difference between the means of 0.180mmol/L . Difference between the clear and icteric sample index was significant ($p < 0.0001$) and difference between the means of 2.908mmol/L . Difference between the clear and lipaemic sample index was significant ($p < 0.0001$) and difference between the means of 0.484mmol/L .

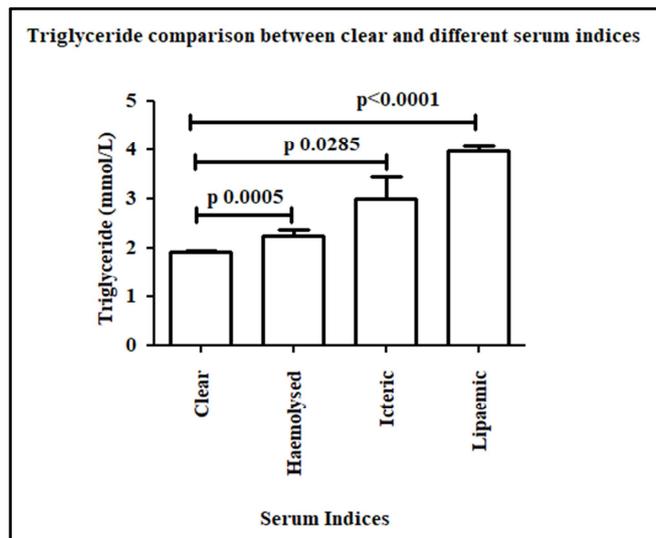


Figure 2. A significant positive ‘interference’ was observed across all the serum indices in the measurement of Triglyceride. Difference between the clear and haemolysis index was significant ($p\ 0.0005$) and difference between the means of 1.059mmol/L . Difference between the clear and icteric sample index was significant ($p\ 0.0285$) and difference between the means of 0.306mmol/L . Difference between the clear and lipaemic sample index was significant ($p < 0.0001$) and difference between the means of 2.037mmol/L .

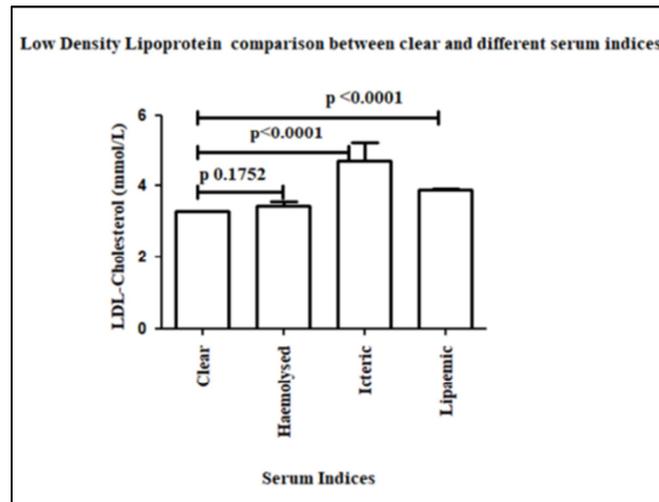


Figure 3. Positive 'interference' was observed across all the serum indices in the measurement of low-density lipoprotein. Difference between the clear and haemolysis index was not significant (p 0.1752) and difference between the means of 0.162mmol/L. Difference between the clear and icteric sample index was significant (p <0.0001) and difference between the means of 1.433mmol/L. Difference between the clear and lipaemic sample index was significant (p < 0.0001) and difference between the means of 0.5974mmol/L.

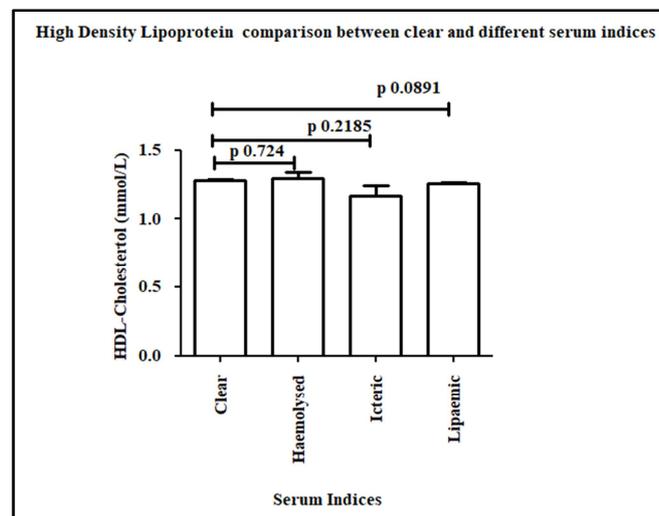


Figure 4. Positive 'interference' was observed between clear serum index and the haemolysis and lipaemic indices but negative interference against icteric index in the measurement of high-density lipoprotein. Difference between the clear and haemolysis index was not significant (p 0.7244) and difference between the means of 0.0152mmol/L. Difference between the clear and icteric sample index was not significant (p 0.2185) and difference between the means of 0.1146mmol/L. Difference between the clear and lipaemic sample index was not significant (p 0.0891) and difference between the means of 0.0224mmol/L.

4. Discussion

During the period of 2007 to 2010 there were 10,555 lipid profiles with TC >5.2mmol/L which were classified under one of four serum indices, clear, haemolysis, icteric and lipaemic. Samples with a clear appearance constituted 84.67%, lipaemic serum index 13.92%, haemolysed serum index 1.16% and icteric serum index. 0.25%. The 1.16% frequency of haemolysis found in this study is lower than reports from other studies [1, 17]. The reduction in haemolysis can be attributed to improved venesection method. There was significant hypertriglyceridaemia in haemolysed samples compared to other indices. However, there was no significant difference in TC, LDL and HDL between the clear and haemolysed samples. Low TC as well

as low levels of, HDL and LDL were reported in patients with sickle-cell disease [18].

The 13.92% frequency of lipaemia found in this study constitutes the highest serum index at the hospital. However, the frequency in this study is lower than centers where intravenous lipid infusions are commonly used [19]. The other cause for lipaemia is non-fasting as well as inadequate fasting [20]. There were significant differences between the clear and lipaemic samples in all the measured lipids and lipoproteins except HDL. Lipaemia was the only serum index with some tendency towards significance (p 0.0891).

The 0.25% frequency of icterus serum index found in this study is lower than reports from other studies [1]. Icterus is possibly the only serum index directly linked to the underlying pathology associated with its appearance [21]. There were significant differences between the clear and icteric samples in

all the measured lipids and lipoproteins except HDL. Icteric samples had lower HDL levels than the clear samples. This finding is contrary to high cholesterol levels due to lipoprotein X found in autoimmune liver disease causing interference in lipoprotein and lipid measurements [22]. Another study derived an equation used to estimate an icterus interference - free value for some analytes including Triglycerides and cholesterol measured in icterus samples [23].

5. Conclusion

In a very large sample of lipid profiles, the frequency of serum indices and their effect on lipid and lipoprotein measurements were assessed. Based on current records, the study provides the first evidence that icterus negatively affects HDL measurement. The study further confirmed findings that haemolysis positively affects TG levels. Further studies with semi quantitative grading of the serum indices would provide additional information.

Recommendations

Every effort must be made to observe sample pre-analytical requirements to avoid presence of endogenous interfering constituents.

Strength and Limitations

- (1) The study looked at more than 10 555 lipid profile results.
- (2) Retrospective studies are generally less expensive than prospective studies.
- (3) The main limitation is that retrospective aspect may introduce selection bias and information bias.
- (4) The less common method used to investigate interferences in patient samples was employed

Conflict of Interest

The authors declare that they have no competing interests.

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None.

Declarations

Ethical approval: CHBAH Medical Advisory Committee, Department of Health and University of the Witwatersrand Ethics Committee.

Author Contributions

Agnes Magwete: Writing and revision of the manuscript.
Donald M Tanyanyiwa: Generated hypothesis, wrote manuscript and corresponding author.
Florence Marule: Collected and compiled research data.

Taryn Pillay: Analysed data, and edited manuscript

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Appendix

General Formula for calculating serum indices.

$$\text{Haemolysis index} = \frac{(\Delta\text{Abs}2) - B \times ((\Delta\text{Abs}3))}{A}$$

$$\text{Lipaemic index} = \frac{(\Delta\text{Abs}3)}{C}$$

Icteric index

$$= \frac{(\Delta\text{Abs}1) - E \times \{(\Delta\text{Abs}2) - B \times (\Delta\text{Abs}3)\} - F \times (\Delta\text{Abs}3)}{D}$$

A = scaling factor for haemoglobin

B = corrects haemoglobin measurement for lipaemia

C = scaling factor for lipaemia

D = scaling factor for bilirubin

E = corrects bilirubin measurement (absorbance) for haemoglobin

F = corrects bilirubin measurement for lipaemia

DABs1,2,3 = absorbance values of the 480–505 nm, 570–600 nm and 660–700 nm bichromatic readings, respectively, in relation to the blank absorbance.

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