Research Article

Paraprotein interferences: Insights from a short study involving multiple platforms and multiple measurands

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Keywords

Multiple myeloma, paraprotein interferences, erroneous results, wet chemistries, Bland Altman plots, regression analysis.

Abstract

Background

Though paraproteinaemic interferences is a well-known phenomenon in clinical chemistry, a large-scale evaluation study involving multiple paraproteinaemic specimens on multiple platforms including multiple measurands with an aim to provide a predictive analysis, is singularly lacking. The present study aims to fill this gap in research.

Material and Methods

This cross-sectional non-interventional observational study involved thirteen paraproteinaemic subjects, determined their gamma globulin characterization and measured their total bilirubin, direct bilirubin, HDL-cholesterol, calcium, inorganic phosphate, iron and unsaturated iron binding capacity (UIBC) levels on a dry chemistry platform (Vitros 350) as the established method and two wet chemistry platforms (AU5800 and Cobas 6000) as the evaluation methods. Data thus generated was analyzed for any significant variation and tested if such variation increased with decreasing albumin/ globulin ratio.

Results

Significant variation between dry chemistry and wet chemistry measurements were obtained for direct bilirubin, HDL and iron on AU5800 with p-values of 0.0009, <0.0001 and 0.0466 respectively. Similarly, discrepant results were obtained on Cobas 6000 for direct bilirubin and iron, with p-values of <0.0001 and 0.0002 respectively. Additionally, UIBC measurements on AU5800 varied significantly with increasing amounts of paraprotein present in the specimen (p-value = 0.0207).

Conclusion

This study emphasizes on predictive analyses to show that paraprotein interferences are fairly common on wet chemistry platforms. Evolving algorithms for monitoring of reaction curves can minimize release of erroneous results due to such interferences.

Introduction

Since the inception of quantitative estimation of measurands in clinical chemistry, analytical interferences have been a bane towards achieving accuracy in tests results. Accordingly, considerable effort has been directed in finding ways to mitigate this shortcoming. In particular, awareness against haemolysis, icterus and lipaemia have been optimized such that most modern auto-analyzers have in-built checks against such interferants. Considerable research is ongoing even against drug interferences [1] and interferences in immunoassays [2]. However, a more insidious and stealthier interferant is often overlooked – that is due to paraproteinaemia. Multiple myeloma, Waldenström's macroglobulinaemia, plasmacytoma, amyloidosis and monoclonal gammopathy of undetermined significance (MGUS) account for majority of the paraproteinaemic cases. Prevalence of these conditions are still unclear, however, the prevalence of MGUS, the most common cause of paraproteinaemia, is approximately 3 – 4 % of individuals > 50 years and 6 – 8 % of individuals > 80 years old. About 70 % of paraproteins are IgG, 15 – 20 % IgM and 10 – 15 % IgA [3].

Among original equipment manufacturers (OEMs), dry chemistry platforms like Vitros (by Ortho Clinical Diagnostics, recently renamed as Quidel Ortho) have made pioneering contributions in ameliorating paraprotein interferences [4], by incorporating a physical barrier (Spreading Layer) in their reaction slides, which filter out the potentially interfering proteins. Such measures have proven to protect against spurious results of inorganic phosphate, bilirubin, electrolytes, lipids etc., as evidenced from peer-reviewed literature [5,6]. Even though there are several instances of research involving the effect of paraproteins on individual measurands, to the best of the author's knowledge, there is a gap in research regarding large-scale comparison involving several measurands and involving two or more platforms to examine the effects of paraprotein interference. The present work-up, is thus an effort to fill up this gap, where thirteen individuals characterized as being tested positive for various forms of monoclonal gammopathies are subjected to testing of seven clinical chemistry parameters viz. Total & Direct Bilirubin, HDL-Cholesterol, Calcium, Inorganic Phosphate, Iron and Unsaturated Iron Binding Capacity (UIBC), in a Vitros 350 dry chemistry platform as the established method and two wet chemistry platforms – AU5800 (Beckman-Coulter) and Cobas 6000 (Roche) – as the evaluation methods.

Materials and Methods

This cross-sectional non-interventional observational study was undertaken at Drs. Tribedi & Roy Diagnostic Laboratory, 93 Park Street, Kolkata, India, which is a private undertaking tertiary-level referral laboratory, from January 2023 to June 2023. Informed consent from the study subjects and ethical clearance from the Drs. Tribedi & Roy Diagnostic Laboratory Ethical Committee was duly undertaken for this study, in accordance with all relevant national guidelines. Study subjects were chosen based on their decreased albumin-globulin ratio as revealed as an incidental finding during routine tests. Furthermore, subjects with known prior liver or kidney disorders, with known dyslipidaemias or anaemia and those under treatment with iron supplements, Vitamin D supplements, bisphosphonate therapy or under treatment for any sort of monoclonal gammopathies were excluded from this subset of decreased A/G ratio population. Venous blood samples were collected observing standard aseptic procedures and transferred to standard gel separator tubes [7, 8]. All samples were tested on the same day of collection.

Table 1: Study population

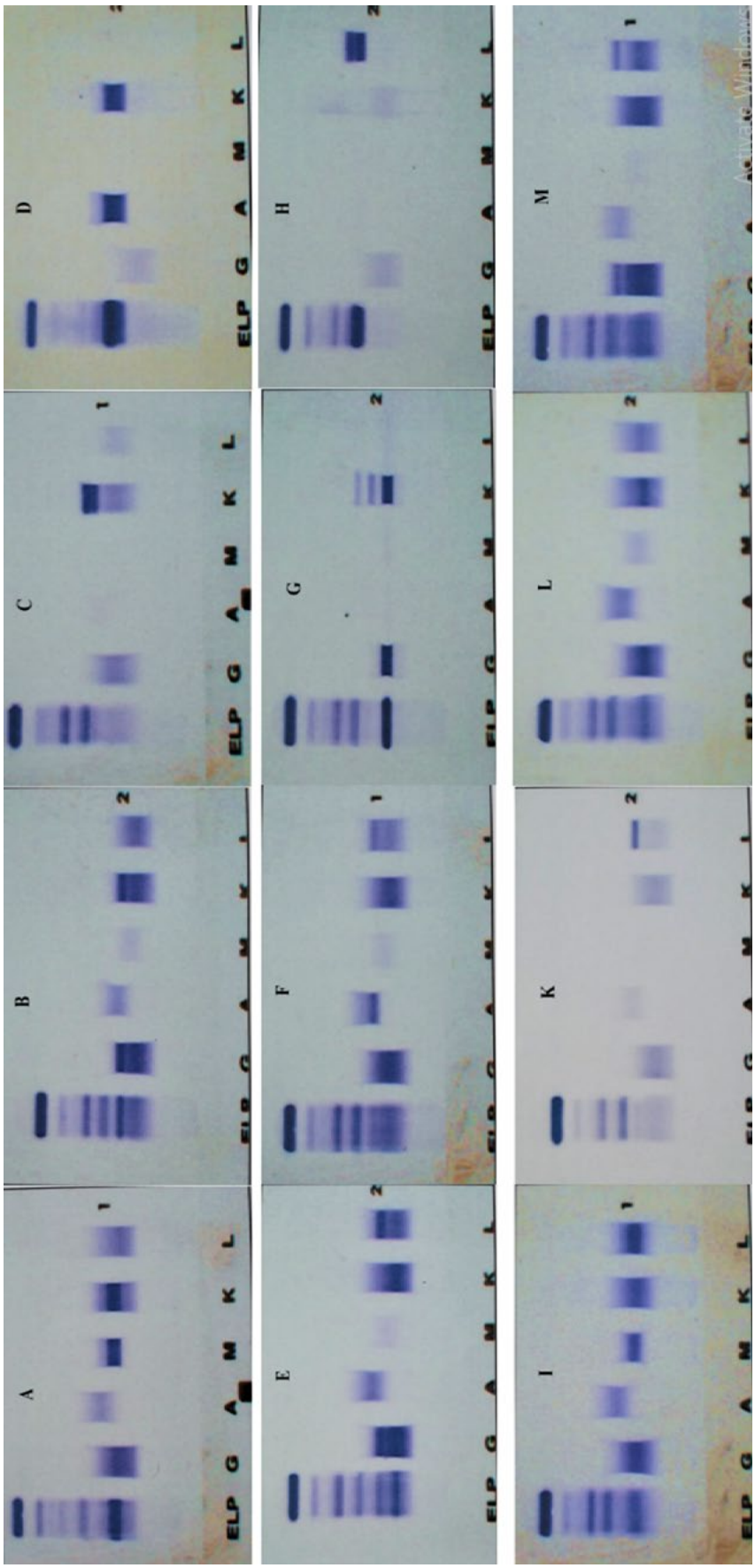
Study Subjects	Demographics	Total Protein, g/dL	Albumin g/dL	Globulin g/dL	A/G Ratio	IFE Characterization
Subject A	Male, 63 yrs, Bengali	13.9	3.1	10.8	0.29	Monoclonal gammopathy of IgM (κ – Light Chain).
Subject B	Male, 52 yrs, North Indian	11.1	2.5	8.6	0.29	Monoclonal gammopathy of IgG (κ – Light Chain).
Subject C	Female, 77 yrs, Bengali	13.9	3.2	10.7	0.30	Monoclonal gammopathy of κ – Light Chain (Two bands seen).
Subject D	Male, 49 yrs. Bengali	9.9	2.8	7.1	0.39	Monoclonal gammopathy of IgA (κ – Light Chain).
Subject E	Male, 65 yrs, Bengali	11.1	3.4	7.7	0.44	Biclonal gammopathy of monoclonal IgG (κ – Light Chain) and monoclonal IgG (λ – Light Chain).
Subject F	Male, 71 yrs, North Indian	14.4	3	11.4	0.26	Monoclonal gammopathy of IgA (λ – Light Chain).

Subject G	Female, 62 yrs, Mongoloid	10.6	3.3	7.3	0.45	Monoclonal gammopathy of IgG (κ – Light Chain) with two additional bands of κ – Light Chain.
Subject H	Male, 78 yrs, Bengali	14.4	1.3	13.1	0.10	Monoclonal gammopathy of λ – Light Chain (Two bands seen).
Subject I	Female, 72 yrs, Bengali	10.4	3.6	6.8	0.53	Monoclonal gammopathy of IgM (λ – Light Chain).
Subject J	Male, 52 yrs, North Indian	9.5	2.8	6.7	0.42	IFE not done.
Subject K	Male, 59 yrs, Bengali	10.3	3	7.3	0.41	Monoclonal gammopathy of λ – Light Chain.
Subject L	Male, 61 yrs, Bengali	11.4	2.8	8.6	0.33	Monoclonal gammopathy of IgA (κ – Light Chain) with a faint monoclonal λ – Light Chain band.
Subject M	Male, 66 yrs, South Indian	12.9	2.3	10.6	0.22	Monoclonal gammopathy of IgG (λ – Light Chain).
Range	49 – 78 yrs.	9.5 – 14.4	1.3 – 3.6	6.7 – 13.1	0.10 – 0.53	
Mean	63.6 yrs.	11.8	2.8	9	0.34	
Median	63 yrs.	11.1	3	8.6	0.32	

IFE: Immuno-Fixation Electrophoresis, IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M.

Thirteen subjects so chosen (Table 1) were subjected to blood tests for Total Protein and Albumin in a Vitros 350 analyzer, which acted as the established platform, for Total & Direct Bilirubin, HDL-Cholesterol, Calcium, Inorganic Phosphate, Iron and Unsaturated Iron Binding Capacity (UIBC) in the Vitros 350, an AU5800 and a Cobas 6000, of which the latter two acted as the evaluation platforms, and for gamma globulin characterization by ImmunoFixation Electrophoresis (IFE) in a Sebia Hydragel conventional electrophoresis platform (Figure 1). One of the subjects declined to undergo the last test. The method specifications of the tests performed have been elaborated in Table 2.

Figure 1: Figure depicts gamma globulin characterization of the subjects.



Subject A: Monoclonal gammopathy of IgM (κ –Light Chain). Subject B: Monoclonal gammopathy of IgG (κ – Light Chain). Subject C: Monoclonal gammopathy of κ – Light Chain (Two bands seen). Subject D: Monoclonal gammopathy of IgA (κ – Light Chain). Subject E: Biclonal gammopathy of monoclonal IgG (κ – Light Chain) and monoclonal IgG (λ – Light Chain). Subject F: Monoclonal gammopathy of IgA (λ – Light Chain). Subject G: Monoclonal gammopathy of IgG (κ – Light Chain) with two additional bands of κ – Light Chain. Subject H: Monoclonal gammopathy of λ – Light Chain (Two bands seen). Subject I: Monoclonal gammopathy of IgM (λ – Light Chain). Subject K: Monoclonal gammopathy of λ – Light Chain. Subject L: Monoclonal gammopathy of IgA (κ – Light Chain) with a faint monoclonal λ – Light Chain band. Subject M: Monoclonal gammopathy of IgG (λ – Light Chain).

Table 2: Assay Characteristics

Measurand	Ortho Clinical Diagnostics		Beckman-Coulter		Roche		Sebia	
	Methodology	**Traceability**	**Methodology**	**Traceability**	**Methodology**	**Traceability**	**Methodology**	**Traceability**
Total Bilirubin	Diazonium salt; reflectance photometry	Jendrassik-Grof; NIST SRM 916	DPD, with Caffeine, Surfactant; conventional photometry	NIST SRM 916a	DPD, with Detergents, stabilizers; conventional photometry	NIST SRM 916; Doumas method	NA	NA
Direct Bilirubin	Polycationic mordant; reflectance photometry	HPLC; NIST SRM 916	DPD,in Acid Medium; conventional photometry	Beckman-Coulter Master Calibrator	Diazo-sulphanilic acid; conventional photometry	NIST SRM 916; Doumas method	NA	NA
HDL-Cholesterol	Phosphotungstic acid-MgCl2 pptn/ cholesterol oxidase; reflectance photometry	CRMLN designated method; NIST SRM 911	Enzymatic Immuno-Inhibition; conventional photometry	US CDC Reference Method	Enzymatic Chemical-Inhibition; conventional photometry	US CDC Reference Ultra centrifugation Method	NA	NA
Calcium	Arsenazo III; reflectance photometry	Flame AAS; NIST SRM 915	Arsenazo III; conventional photometry	NIST SRM 909b L1	NM-BAPTA; conventional photometry	NIST SRM 956c L2	NA	NA
Phosphate	Heteropolymolybdenum blue complex; reflectance photometry	Phosphomolybdate/ phenylenediamine method; NIST SRM 200	UV Molybdate; conventional photometry	Beckman-Coulter Master Calibrator	UV Molybdate; conventional photometry	NERL primary reference material	NA	NA
Iron	Chromazurol B dye; reflectance photometry	Ferene dye; NIST SRM 937	TPTZ (Tripyridyl Triazine); conventional photometry	Beckman-Coulter Master Calibrator	Ferrozine; conventional photometry	NIST SRM 937	NA	NA
UIBC	Iron citrate/ chromazurol B; reflectance photometry	Ferene dye; NIST SRM 937	Nitroso-PSAP; conventional photometry	NIST SRM 937	Ferrozine; conventional photometry	Weighted purified iron SRM	NA	NA
Total Protein	Biuret; reflectance photometry	Biuret; NIST SRM 927	NA	NA	NA	NA	NA	NA
Albumin	Bromocresol green; reflectance photometry	Bromocresol green; NIST SRM 927	NA	NA	NA	NA	NA	NA
Monoclonal Proteins	NA	NA	NA	NA	NA	NA	Agarose gel electrophoresis/ immunofixation; densitometric scanning	NA

The reaction curves of all the results on the two wet chemistry platforms were analyzed; many of the results produced irregular or broken curves (for bilirubins and phosphate) or curves with high extinction coefficients (for iron). Eventually, all the results, both with normal and abnormal reaction curves, were included for data analysis. The data thus generated was analyzed with the help of Microsoft Excel and Statistical Package for Social Sciences (SPSS) software. Goodness-of-fit of comparison data with normal distribution was tested by constructing Quantile-Quantile (QQ) Plot [9], significant differences in comparison data were highlighted by constructing Bland-Altman plots [10] and finally differences in comparison were established by the Passing-Bablok regression analysis model, which assumes no special requirements regarding the distribution of samples and the measurement errors [11].

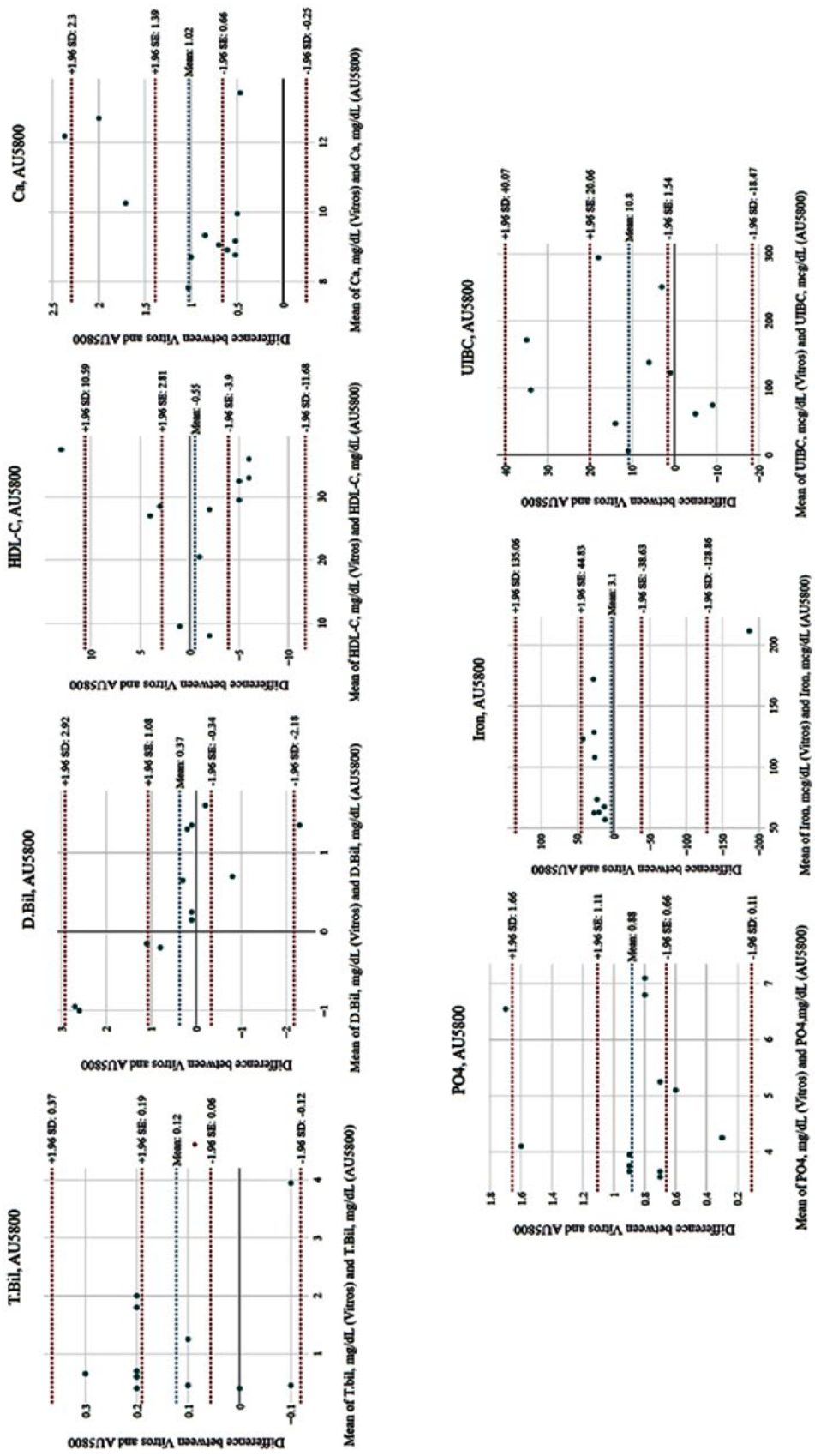
Table 3: Comparison of Test Methods

Evaluation of Variation w.r.t. Established Method								
		T.Bil	**D.Bil**	**HDL**	**Calcium**	**I. Phosphate**	**Iron**	**UIBC**
AU5800 w.r.t. Vitros 350								
Range of Variation (In Absolute Values)		0 – 40 %	7.1 – 1150 %	7.4 – 29.6 %	4.9 – 17.7 %	6.8 – 32.6 %	15.1 – 158.5 %	0.8 – 91.7 %
Regression Analysis	Intercept	-0.16	-0.86	2.26	0.634	-0.61	-90.2	-6.7
	Slope	0.04	1.22	-2.11	-0.165	-0.06	0.8	0
	p-Value	0.2745	**0.0009**	**<0.0001**	0.1364	0.5494	**0.0466**	0.585
Cobas 6000 w.r.t. Vitros 350								
Range of Variation (In Absolute Values)		0 – 228.6 %	0 – 75 %	0 – 57.1 %	4.6 – 14.5 %	0 – 20.3 %	7.7 – 23.7 %	0 – 83.3 %
Regression Analysis	Intercept	0.06	-0.22	-2.5	-0.883	-0.21	4.8	-15.8
	Slope	0.12	0.43	0.1	-0.001	-0.08	-0.3	0
	p-Value	0.3868	**<0.0001**	0.5977	0.9902	0.4022	**0.0002**	0.9242
Evaluation of Variation w.r.t Variation in A/G Ratio								
AU5800 w.r.t. A/G ratio								
Regression Analysis	Intercept	14.4	78.99	8.302	11.52	16.08	3.357	75.26
	Slope	15.3	634.8	22.03	-6.375	3.628	101.3	-172.3
	p-Value	0.6551	0.5289	0.3334	0.6603	0.8719	0.458	**0.0207**
Cobas 6000 w.r.t. A/G Ratio								
Regression Analysis	Intercept	48.8	2.085	22.83	9.025	18.87	25.72	67.92
	Slope	-11.31	92.34	-12.66	0.9345	-23.04	-26.22	-135.3
	p-Value	0.9467	0.1621	0.7785	0.9428	0.2456	0.1103	0.0653

Results

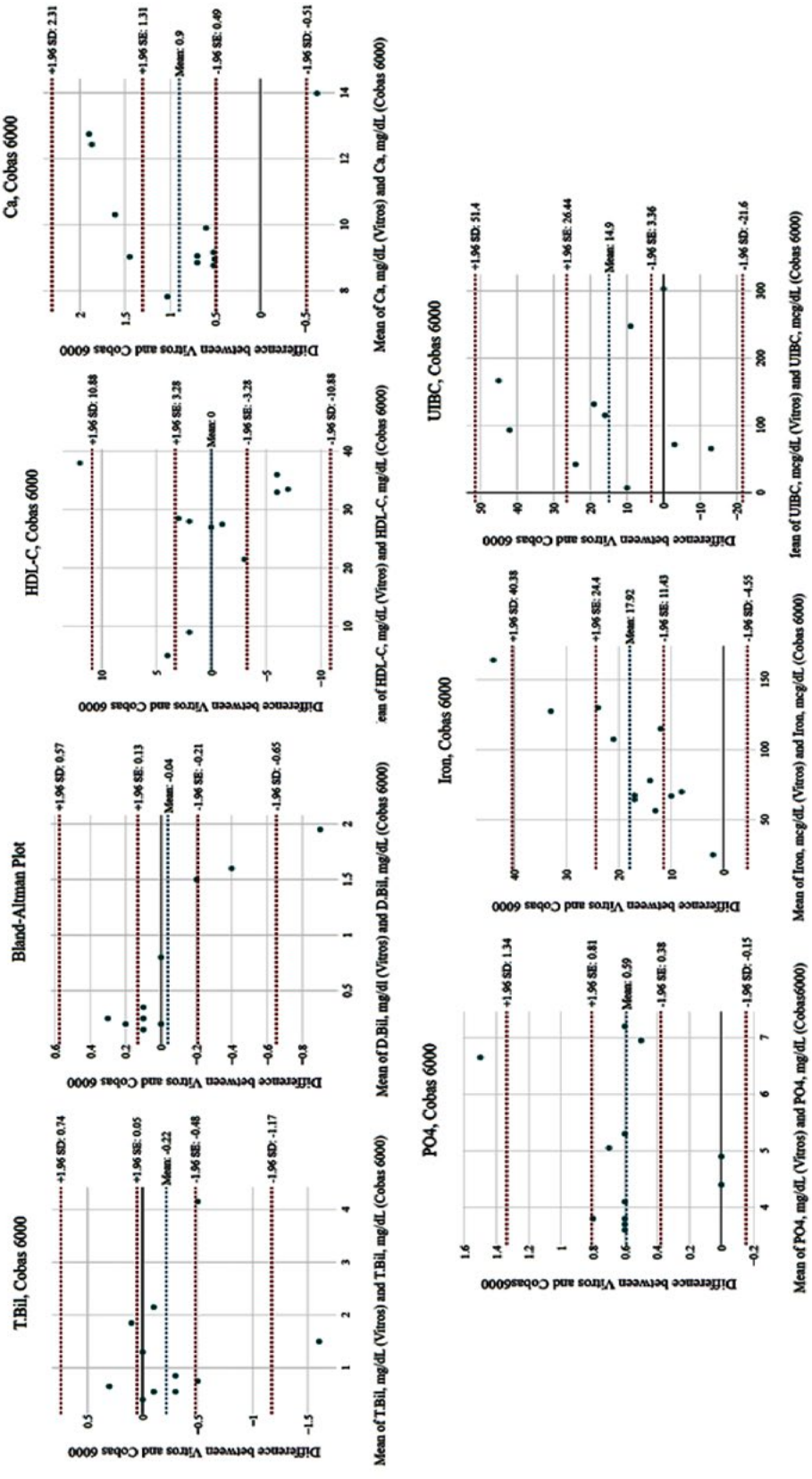
Data analysis was done with two research questions as the basis: 1) Whether the evaluation methods (wet chemistry platforms AU5800 and Cobas 6000) display a significant variation with respect to the established method (dry chemistry platform Vitros 350), and 2) Whether the evaluation methods display an increasing variation w. r. t. decreasing A/G Ratio. The findings of the analysis are summarized in Table 3, and Figures 2 and 3.

Figure 2: Figure depicts Bland-Altman plots of paired data between Vitros 350 (Established Method) and AU5800 (Evaluation Method)



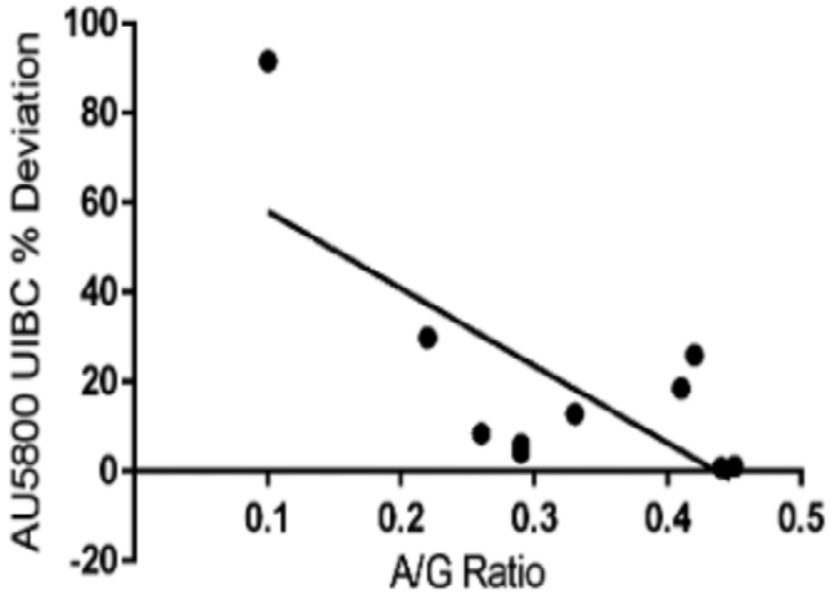
Top row (Left to right) includes Total Bilirubin, Direct Bilirubin, HDL-Cholesterol and Calcium. Bottom row (Left to right) includes Inorganic Phosphate, Iron and Unsaturated Iron Binding Capacity. Means of paired data are plotted on the x-axis and differences of the same data are plotted on the y-axis. Mean of differences is along the blue dotted line and the uppermost and lowermost red dotted lines denote the 1.96 SD limits.

Figure 3: Figure depicts Bland-Altman plots of paired data between Vitros 350 (Established Method) and Cobas 6000 (Evaluation Method).



Top row (Left to right) includes Total Bilirubin, Direct Bilirubin, HDL-Cholesterol and Calcium. Bottom row (Left to right) includes Inorganic Phosphate, Iron and Unsaturated Iron Binding Capacity. Means of paired data are plotted on the x-axis and differences of the same data are plotted on the y-axis. Mean of differences is along the blue dotted line and the uppermost and lowermost red dotted lines denote the 1.96 SD limits.

Figure 4: Figure depicts Passing-Bablok Regression Analysis of variation of Unsaturated Iron Binding Capacity measurements on AU5800 w.r.t



Vitros 350 as a function of changes in A/G ratio. The equation obtained was [UIBC] = -172.3*(A/G ratio) + 75.26, with a p-value of 0.0207. Thus, the increase in variation of UIBC measurements with decreasing A/G ratio is significant.

On the first question, a cursory look at the data reveals that the bilirubins and iron profile present the problem areas: AU5800 exhibits a variation of 7.1 – 1150% for Direct Bilirubin, 15.1 – 158.5% for Iron and 0.8 – 91.7% for UIBC; Cobas 6000 reveals a variation of 0 – 228.6% for Total Bilirubin and 0 – 83.3% for UIBC. In particular, the two wet chemistry platforms occasionally threw up bizarre results for the bilirubins, sometimes even in the negative. For example, for a Total Bilirubin of 0.7 mg/dL measured on Vitros350, the corresponding value on Cobas 6000 was 2.3 mg/dL; for a Direct Bilirubin of 0.2 mg/dL measured on Vitros350, the corresponding value on AU5800 was 2.5 mg/dL (more than Total Bilirubin of 0.5 mg/dL measured on the same AU5800!); for a Direct Bilirubin of 0.3 mg/dL measured on Vitros350, the corresponding value on AU5800 was -2.3 mg/dL; and so on. Since none of the data sets exhibited normality on the Q-Q Plots, they were subjected to transformation, mostly logarithmic, but sometimes Box-Cox or inversion, until normality was achieved. Bland-Altman Plots were constructed along two panels – on the first, variation of the test results of the respective measurands on AU5800 vis-à-vis Vitros 350 were plotted (Figure 2), while on the next, variation of test results of the same measurands on Cobas 6000 vis-à-vis Vitros 350 were plotted (Figure 3). Regression analysis at this level was done by plotting the results of the respective measurands in two sets, viz. Vitros 350 vs. AU5800, and Vitros 350 vs. Cobas 6000. Regression analysis with the assumption that both the data sets are same returned significant p-values (< 0.05) for Direct Bilirubin, HDL-Cholesterol and Iron in AU5800 and for Direct bilirubin and Iron in Cobas 6000, indicating that the measurands mentioned have significant variation vis-à-vis the Vitros 350 values.

On the second question, percent variation w. r. t. the established method was plotted against A/G ratio to determine the correlation, if any. In this level of data analysis, regression plotting was done on A/G ratio vis-à-vis percent deviation of the respective measurands on AU5800, or alternately, on Cobas 6000. None of the measurands produced any significant p-value, except UIBC measured on AU5800 (Figure 4, p-value = 0.0207). This signifies that the variation of UIBC measurement on the AU5800 platform increases with the decrease in A/G ratio.

All data generated or analysed during this study are included in this published article and its supplementary information file.

Discussion

Research regarding interference due to paraproteinaemia was already in the upswing by the 1980s, with an important communiqué emerging in 1980 about interference in thyroxine measurements [12] and two seminal papers appearing in 1986 regarding urea [13] and creatinine [14]. In fact, the last paper kickstarted such a flurry of research that almost all the major OEMs modified their Jaffé creatinine methods, and many major laboratories around the world have shifted to enzymatic creatinine assay. So much so, that it was decided against including creatinine and urea in the present study, because of the glut of information already available. Regarding information about paraprotein interferences in measurement of measurands

included in this study, a general consensus of the researchers [15-31] can be stated as 1) interferences due to paraproteins lack reproducibility across samples with similar paraproteins and across methods, and sometimes even in the same sample, and 2) interferences due to paraproteins are not proportional to the amount of paraprotein present in the sample. This implies that, even if comparison data between an established method and an evaluation method is satisfactory in one instance, such concordance may not be reproducible on repeat testing of the same sample set. Despite recognizing this fallacy, most studies till date have relied only on comparison data of actual runs. While not discarding real data altogether, the present study has put some emphasis on a regression model such that reliable predictions can be arrived at regarding general trends.

Paraprotein interference on measurement of bilirubin is a well-known phenomenon. In the present study, in view of the significant variation observed in D Bil estimation on both the wet chemistry platforms (AU5800 p-value = 0.0009, Cobas 6000 p-value ≤ 000.1, Table 3), four observations can be elucidated: 1) interference cannot be predicted based on the type of paraproteinaemia; e. g. T. Bil measured 0.7 mg/dL was falsely measured as 2.3 mg/dL on Cobas 6000 in a subject with monoclonal gammopathy of IgA (λ – Light Chain) (Subject F, Table 1); D. Bil measured 0.4 mg/dL was falsely measured as -2.3 mg/dL on AU5800 in a subject with monoclonal gammopathy of IgM (κ – Light Chain) (Subject A, Table 1); D. Bil measured 0.2 mg/dL was falsely measured as 2.5 mg/dL on AU5800 in a subject with monoclonal gammopathy of IgA (λ – Light Chain) (Subject F, Table 1); D. Bil measured 0.3 mg/dL was falsely measured as -2.3 mg/dL on AU5800 in a subject with monoclonal gammopathy of IgM (λ – Light Chain) (Subject I, Table 1). Reaction curves of all these improbable results were found to be broken or irregular. 2) interference in a sample on one platform is non-transferable to the other platform, e.g., in the above instances corresponding measurements in the other wet chemistry platform correlated well with the dry chemistry results; 3) interference is not correlated to the concentration of the measurand, in this case, bilirubin and 4) interference is not proportional to the amount of paraproteins present, as the absolute percentage variation of the wet chemistry vis-á-vis dry chemistry results does not correlate inversely with the A/G ratio (Table 3). As explained by King et al [30] and Madenci et al [31], such interferences in bilirubin measurement by diazotization are usually due to precipitation of proteins in the extremely acidic pH of the reaction mixture.

As regards to HDL-Cholesterol, the present study has found significant variation between AU5800 measurements and the established Vitros 350 values, with a p-value of <0.0001, though the range of variation in absolute percentage (7.4 - 29.6 %) was unremarkable. Variation of results of Cobas 6000 was found to be not significant. This finding seemed to be a bit perplexing because a brief review of existing literature pointed to the problem specifically against the Roche HDL-C reagent used in Cobas 6000 [16,18–20]. However, on a detailed enquiry with the OEM, it was noted that the HDL-C kit in use during 2003 – 04, when the articles were published, were of 2nd generation. The current lot of reagents is from the 4th generation, which has undergone several modifications, some of which were to address the issue of paraprotein interference. On the question as to why the performance of the AU5800 HDL-C platform faltered is difficult to explain, especially when the reaction curves were found to be normal, but the method being based on immuno-inhibition of non-HDL fraction of lipoproteins may serve as an indicator for further research.

Pseudohypercalcaemia is an oft-repeated reporting in the field of research on paraprotein interference [21,22]. In comparison with the o-cresolphthalein complexone (OCPC) method, which uses an alkaline medium, or the NM-BAPTA method, which also uses a neutral to alkaline medium, the Arsenazo III method, which uses an acidic medium, seems to be the more common culprit. But the present study, which examines the Arsenazo III method on AU5800 (Range of variation 4.9 – 17.7 %, Table 3) and the NM-BAPTA method on Cobas 6000 (Range of variation 4.6 – 14.5 %, Table 3), fails to reveal any significant discrepancy vis- á-vis the dry chemistry results. Also, no significant variation was found with varying degrees of paraproteinaemia. The reason for such concordance is difficult to determine but may be due to periodic revision and upgradation of the reagent constituents, especially on the Arsenazo III platform. After all, the two articles quoting pseudohypercalcaemia on Arsenazo III platforms were a couple of decades old! Variation in results of inorganic phosphate due to presence of paraproteins is an active area of research for almost three decades [23–26]. Various researchers have reported both falsely increased and falsely decreased results, mostly on measurements carried out on single serum specimens. In the opinion of the current author, such variation should not be prefixed as hyper- or hypo-, but rather be characterized as a variable variability, because of the irreproducible nature of the mechanism of interference. Despite extensive reportage of such variations, the present study has failed to produce significant variations between inorganic phosphate results on wet chemistry and dry chemistry platforms (Table 3). Reason for such concordance is difficult to pin-point; it may be surmised that the active intervention of the OEMs over the years to react to the continued reportage of variations and thereby modify their respective methods has likely yielded desirable results. It may be pertinent to mention here that though most of the wet chemistry results of inorganic phosphate were congruent with those of the dry chemistry results, many of the reaction curves in the former platforms were broken or irregular. Like the bilirubins, interferences of paraproteins on iron estimation have been an active area of research for long [27–29]. In the present study, the findings regarding iron and unsaturated iron binding capacity (UIBC) mirrored those of bilirubins, especially on the AU5800 platform. Many of the reaction curves were abnormal, with very high extinction coefficients. The range of variation of iron measurements on AU5800 was unacceptably large (15.1-158.5 %), with a significant p-value (0.0466) of the regression analysis.

Though the range of variation of iron measurements on Cobas 6000 was relatively low (7.7 - 23.7 %), regression analysis revealed a significant variation with a p-value of 0.0002. Like the bilirubins, the reactions for iron measurement takes place in extreme acidic matrices (pH 1.7 in AU5800, pH < 2.0 in Cobas 6000), and as explained by Bakker [27] and Dorizzi et al [28], such extreme manipulations of pH and ionic strengths may precipitate the paraproteins in the sample, causing turbidimetric interferences in measurement of iron concentrations. In contrast to iron, UIBC is usually measured in an alkaline environment (pH 8.1 in AU5800, pH 8.4 in Cobas 6000); consequently, comparability of UIBC results between wet chemistry and dry chemistry platforms were within acceptable limits. However, when percentage variation of UIBC results were plotted against A/G ratio, regression analysis returned significant results for AU5800 (p-value = 0.0207) but not for Cobas 6000, indicating that variation of UIBC results on AU5800 increases with decrease in A/G ratio. This is the singularly positive finding among all the measurands in this category of analyses (percent variation vs. A/G ratio).

A word or two about Bland-Altman plots may be pertinent in this discussion. Utility of BA plots in medical research is no doubt undeniable but it appears to the present author that mere visual inspection of BA plots may sometimes be misleading, a fact which was acknowledged by the authors themselves in a later article [32]. Applying the thumb rule that comparison data is acceptable when ~90% of the points lie between ± 2 SD of a BA plot, without analyzing the accompanying regression data, would have led to an erroneous conclusion in this study that all the measurands correlate well between the established and evaluation methods (Figures 2 and 3). This is particularly true when the sample size is small, as in the present study.

Finally, there are no qualms in acknowledging the drawbacks of this study. The first and obvious shortcoming is the sample size. Thirteen is woefully low a sample size for method comparison studies and the author acknowledges it as such. However, it must be kept in mind that the specific requirement of abnormality in the samples (presence of paraproteins) would always render gathering enough samples a tall ask. As such, the author treats this study as a sentinel survey and intends to build upon it for broader research in near future. Secondly, criticisms might arise as to why other relevant measurands were excluded from the study. The reason is twofold: firstly, there were sample volume constraints and secondly, a broader research protocol is intended to be set in near future dedicated fully to the study of paraprotein interferences. Thirdly, it might be argued that the evaluation methods should have been compared with the corresponding reference (or definitive) methods, instead of comparing with dry chemistry methods. This is a valid point but access to reference methods, mostly confined to reference laboratories, is almost out of reach for routine clinical laboratories like the one where the present study was conducted. In short, the study is not flawless, but it reasonably demonstrates important findings regarding paraprotein interferences, facts which are vindicated by a very recent study [33], which states, "...the paraproteins interfered maximally with direct bilirubin, total bilirubin, iron and Total Iron Binding Capacity (TIBC) assays."

Conclusion

The present study reveals that interferences due to presence of paraproteins are fairly common especially for parameters like Direct Bilirubin, HDL-Cholesterol, Iron and Unsaturated Iron Binding Capacity on the wet chemistry platforms evaluated. Though preventing the occurrence of such interferences seems impossible, they can be detected before release of erroneous results on inspecting the reaction curves when suspicions arise regarding results discrepant with the patient demographics or clinical findings. Development of feasible algorithms which can detect irregularities of reaction curves and prevent release of the corresponding results, without significantly impacting the throughput of the system, remains a challenge.

Declarations

Ethics Approval and Consent to Participate

Ethics approval for this study was obtained from Drs. Tribedi & Roy Ethical Committee and informed and written consent was obtained from the subjects for this study, in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

Consent for Publication

Written informed consent was obtained from the subjects for publication of their electrophoretograms and health data.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing Interests

The author declares that he has no competing interests.

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Author Contributions

Rajarshi Sarkar has conceptualized, collected data, analyzed data, prepared a literature review, written the manuscript and prepared the tables and figures for this study.

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