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The Biochemistry Chronicles

Hyperbilirubinaemia in Nonicteric Serum

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The Case: On 7th Oct.2016, a blood sample received for liver function tests (LFT) in the afternoon from a 62 yrs male admitted in a medical ward exhibited a total bilirubin (TB) level-7.6 mg/dl in a nonicteric serum despite rechecking by Olympus AU 640 autoanalyzer with Roche/Cobas reagent (Cat.11489194) which had been in use by us from 1999 mainly because of its excellent performances including stability. Anticipating possibility of contamination, a fresh sample was brought and on repetition, a level of 7.4 mg/dl was found. The physical appearance of both the samples were watery. A call was made just in the evening and the following results were shown informing to look into the issue as to whether there was any problem of the autoanalyzer or the TB reagent. (Fig 1)

Approach to the problem: After running nine other samples along with the concerned sample, the same results of TB including other parameters of LFT were found. The report was delivered to the treating doctor at the ward personally explaining that this was false hyperbilirunibaemia. Since the patient was symptom free and his relatives insisted for discharge, just on receipt of this report, the patient was discharged.

Following discharge, after scrutiny of the documents, it was learnt that the patient was admitted to our hospital five days back for bilateral upper back pain with tenderness in the thoracic 11 and 12 vertebrae. X-Ray showed - no bony changes in the thoraco-lumbar region; haematological investigations: Hb-5.9 mg/dl, ESR– 65 mm (1st hr) and WBC count– 4,400/cmm on the next day of admission and one unit of blood was transfused in the same afternoon. The patient became symptom-free on the 4th day. It was seen that the LFT done on the preceding day showed the similar results and the sample was essentially sent for reconfirmation of the following reports.

T.B.	T.P.	Alb.	Glob.	ALP	ALT
7.4	10	2.4	7.6	102	15

In view of high serum total protein with reversal of A:G ratio in three separate samples on two successive days with two samples drawn five hours apart on the 2nd day and raised ESR, a suspicion of multiple myeloma was thought of and accordingly, the X-ray images were reexamined at the Radiology department on the computed tomography to ensure absence of any lytic lesion.The primary sample tube alongwith the blood clot remained in the autoanalyzer overnight.

In the next morning, the same TB programme was made at a different position in the same autoanalyzer with fresh reagents from a separate box and calibrated with QC checkings. Nine samples with various levels of the previous day and the concerned sample were reanalyzed using two positions of TB. Since, no difference was found in any one of the sample, the analytical error by the autoanalyzer and/or the reagents were ruled out. The serum was separated and kept in the freezing chamber of the refrigerator.

Sample	T.B.	T.P.	Alb.	Glob.	ALP	ALT
Unit	mg/dl	g/dl	g/dl	g/dl	U/L	U/L
1st sample	7.6	10.3	2.5	7.8	128	18
2nd sample	7.4	9.7	2.3	7.4	121	12

[T.B. =Total Bilirubin (Roche, RI* : up to 1.2 mg/dl), T.P. = Total Protein(Roche, RI* : 6-8 g/dl), Alb.= Albumin (BC#, RI – 3.5-5.2 g/dl) Glob = Globulin (RI* – 1.5-2.5 mg/dl, A:G = Albumin : Globulin ratio, RI-1.5:1, ALP = Alkaline phosphatase (BC#, RI*- 30-120) ALT = Alanine aminotransferase (BC#, RI* - <50U/L
(*RI =Reference Interval, BC# = Beckman Coulter)
The actual level of total bilirubin appeared to be around 0.3 mg/dl as per visual estimation.

On the 3rd day, repeat TB revealed somewhat lower value and the direct bilirubin by DiaSys reagents which had been in use by our laboratory due to nonavailability of Roche make reagent showed a level of 0.3 mg/dl.

T.B.	D.B.	T.P.	Alb.	Glob.	ALP	ALT
6.7	0.3*	9.4	2.1	9.3	116	14

*mg/dl; D.B. = Direct Bilirubin (DiaSys, RI : <0.2 mg/dl)

Both DiaSys(Cat.108119910704) and Randox (Cat. BR3859) make total bilirubin reagent were arranged on war footing. On the 4th day, DiaSys TB reagent was programmed in the same autoanalyzer at another different position and calibrated with QC checkings. The above sample alongwith nine samples with various levels were simultaneously estimated using both Roche and DiaSys make TB reagents.

T.B.	D.B.	T.B.D.	T.P.	Alb.	Glob.
8.1	0.1	0.2*	9.8	2.2	7.6

*mg/dl; TBD = Total Bilirubin (DiaSys, RI : 0.1-1.2 mg/dl)

On the 5th day, analysis with Randox TB reagent was programmed and analyzed in the similar way as above and the TB was reanalyzed with all three namely Roche,DiaSys and Randox make reagents concurrently.

T.B.	D.B.	T.B.D.	T.B.R.
8.4	0.1	0.2	0.4

T.B.R. = Total Bilirubin (Randox, RI : up to 1 mg/dl)

Discussion: Perhaps the abnormal TB level was overlooked seemingly without cross-checking by the visual inspection of the physical appearance of the sample on the preceding day. Direct bilirubin estimation has not been a part of LFT in our laboratory by convention unless wanted. All concerned failed to notice or could not interpret the high total protein with altered A:G ratio leaving aside total bilirubin level. This could probably be a case of early multiple myeloma.

Several samples with variable levels of TB showed comparable results from three different companies but the concerned sample. This perplexing observation of TB with Roche reagent forced to delve into the cause. Reagents used in TB estimation - 2,5-dichlorophenyl diazonium tetrafluroborate (Roche), 2,4 dichloroaniline (DiaSys) and caffeine, sufanilic acid &sodium nitrite (Randox). There was no information about the detergent in Roche and DiaSys reagent. The direct bilirubin estimation of DiaSys used 2,4-dichloroaniline.The probable interference in the estimation of TB only in this case seemed to be the very rare possibility of paraprotein, the reason being Roche solubilizing agent failing to solubilize properly and/or IgG levels exceeding about 2000-3000mg/dl[1,2]. This caused variable precipitation of protein depending on its concentration and isoelectric point at the acidic pH of 1-2 which resulted in increased absorbance instead producing colour proportionate to the concentration of total bilirubin being spuriously measured. The lower levels of total bilirubin in next days could be due to significant degradation of paraproteins and/or IgG, thus causing less interference. In view of the rare discrepancy of results in that particular sample, it would not be prudent to undermine credibility of quality issue of Roche reagent due to non-observation of interference in total bilirubin assay in several multiple myeloma cases over the years using the same reagent kit. The above not only identified a problem but a solution too with variation/similarity of results with reagents from different sources for the same parameter for the first time[3].

Conclusion: False hyperbilirubinaemia was found by Roche reagent very rarely but not so by DiaSys and Randox reagent in a particular sample. Communication with the treating doctor timely and properly prevents creation of problems and confusions. Contamination, requesting for fresh sample, "something wrong somewhere" etc. are common ways of explanation with unconcerned attitudes being the escape route. "Problematic" samples offer unique opportunities for learning. Hence, one must look into the problem inquisitively, nurture the same passionately in an obsessive way and exercise wisdom to unravel the mysteries of unexplained results.

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Hypertriglyceridemia in Nonlipemic Serum

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The case: On 19th Aug. 2008, during daily routine analyses carried out by Olympus AU640 autoanalyzer along with other samples as usual, a serum sample showed 1487 mg/dl triglycerides 'with flagging'(F) using triglycerides reagent kit by the GPO-PAP method (Roche/Cobas reagents, REF 11488872). Physical inspection revealed that the sample was clear and slightly straw coloured as usual without any clot. Still, the serum was recentrifuged , the sample sucked by a micropipette from the primary tube, dispensed into a polystyrene sample cup and checked carefully to rule out presence of any minute particulate matter. Two dilutions – 1 in 2 and 1 in 5 in distilled water were made from the above and all three sample were put in queue one after another along with other routine samples for estimation of triglycerides (TG). The final result of TG was determined to be 1574 mg/dl.

After checking the requisition, it was evident that the above sample was from 58 year old male with a diagnosis of cerebrovascular accident. Immediately recalling the principle of GPO-PAP method by which finally the amount of released glycerol after enzymatic reaction is measured and possible management by oral glycerol to reduce intracranial tension, an enquiry was made over intercom telephone to the ward and the same was confirmed by the nursing staff. After completion of testing of all morning samples, the patient was seen in the ward and the treatment sheet scrutinized. It was learnt that oral glycerol was administered through nasogastric tube at a dose of 30 ml eight hourly and the blood sample was taken about 90 minutes after the 4th dose.The report was delivered with a note against TG – (? interference by glycerol; repeat investigation at least 24 hrs after the last dose of oral glycerol).

Laboratory experiments: About 1 ml of the glycerol administered to the patient was brought to the laboratory for testing. The sample was very viscous for which the micropipette tip needed to be cut to widen the opening to ensure correct sucking. Estimation of TG was done after diluting the glycerol as an unknown sample and it was found to have an unbelievably high level. In order to ascertain the level of TG which was falsely measured so in the glycerol solution itself and to investigate the degree of its interference in the TG assay by deliberate artificial contamination in serum and plasma, about 2 ml of glycerol was taken from a new bottle subsequently from the same ward to carry out the following study next day. After returning to the department, 20 serum samples from indoor and 20 heparinized samples from OPD patients were pooled in two plastic containers before discarding. These were mixed well, kept at room temperature for 30 minutes and then at 4^0C in the refrigerator for about 18 hours after which the pooled samples were kept at room temperature for about two hours with intermittent shaking to allow autoagglutination mainly and thereafter centrifuged. The pooled serum and plasma samples were denoted as sample Y and sample Z respectively.

The highly viscid solution of glycerol was primarily diluted to 1 in 100 in distilled water (Sample X) from which three more samples were made - sample 1 (1 in 1000 dilution) , sample 2 (1 in 1500 dilution) and sample 3 (1 in 2000 dilution). Sample 4, 5 and 6 were prepared by adding 100 ul of sample X to to 900 ul, 1400 ul and 1900 ul respectively of sample Y. Sample 7, 8 and 9 were prepared by adding 100 ul of sample X to 900 ul, 1400 ul and 1900 ul respectively of Sample Z.

Due to very high levels exceeding the linear range of measurement of TG (1000 mg/dl) in sample 1, 4, 7 and 8 showing as 1323F, 1365F, 1423F and 1020F mg/dl respectively, other experimental samples were prepared as follows. Sample 1A, 4A, 7A and 8A were prepared by diluting 1 in 2 in distilled water of sample 1, 4, 7 and 8 respectively.

All these samples i.e. sample 1A, 2, 3, 4, 4A, 5, 6, 7, 7A, 8, 8A and 9 including sample Y and

sample Z together were subjected to analyses for TG as a separate set of samples consecutively eleven times within about 40 minutes to check precision by temporarily keeping the routine analyses of patients' samples in abeyance. The results are shown in Table 1.

The level of TG was calculated by taking average of sample 1A, 2 and sample 3 due to its remarkably high level in sample X for technical reasons with the variation of 0.84% from the mean in the setting of a maximum 0.48 % CV error. A concentration of about 13, 50, 500 mg/dl 'TG' was found in glycerol solution by the above system of analyses. The initial level of TG in the pooled serum and plasma were 190 and 113 mg/dl respectively. Table 2 showed that the recovery of glycerol was nearly 100% in sample no 2, 3 and 5 but somewhat higher up to a maximum 22% in others taking into account of TG levels of both in plasma and serum in the concerned sample.

Conclusion: Although only a few cases of false hypertriglyceridaemia resulting from positive interference by temporary high glycerolaemia have been reported due to oral glycerol administration, the above was reported for the first time with two salient information **i**. a concentration of about 13,50,500 mg/dl falsely measured TG was found in glycerol solution indicating proportionate increase in TG level to the degree of glycerolaemia and **ii**. the recovery of added glycerol in distilled water was almost 100% but there was some positive bias more with plasma than serum.[1] Analysis with fresh sample showing different result does not establish "wrong report"/ "laboratory error" of the previous one particularly if reported meticulously due to absence or variable presence of interfering substance(s) and consequently, its matrix effect in the sample at the time of blood collection. Each apparently abnormal/doubtful result of any sample offers an unique opportunity for learning if delved into passionately.

Reference:
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Table 1: The levels of triglycerides measured by GPO-PAP method

Sample No	2	3	5	6	9	Y	Z	1A	4A	7A	8A
Mean ± SD (mg/dl)	889 ± 4.3	678 ± 2.9	961 ± 3.8	754 ± 3.9	815 ± 4.1	190 ± 0.9	113 ± 0.6	681 ± 2.9	709 ± 2.6	743 ± 2.9	527 ± 2.3
CV%	0.48	0.42	0.39	0.51	0.5	0.47	0.5	0.42	0.36	0.39	0.43

(n=12 for sample 2, 3, 5, 6, 9, Y & Z ; n=11 for sample 1A, 4A, 7A and 8A)

Table 2: Recovery as triglycerides by GPO-PAP method from artificial contamination of sample by glycerol

Sample No	2	3	5	6	9	1A	4A	7A	8A
Actual Level (mg/dl)	902	675	900	675	675	675	676	676	451
Observed Level (mg/dl)	885	674	966	762	821	681	709	754	527
% Recovery	98.11	99.85	107.3	112.9	121.6	100.88	104.88	111.53	116.85

AMBICON WB 2022-'Broadening our Horizons'

by Dr. Manali Sinharay, Associate Professor, CNMC,Kolkata.

Introduction:

Venue: This year the annual conference of West Bengal chapter of Association of Medical Biochemists of India (AMBI) was hosted on 8th and 9th September at Novotel, Kolkata.

Theme and Preparations: The theme of the conference was "Broadening our Horizons". Prior to the event, some committees were formed with special responsibilities assigned to some members for the successful conduction of the conference. The advisory committee comprising Prof (Dr.) Soma Gupta, Prof (Dr.) Indranil Chakraborty and Prof. (Dr.) Debes Ray had been constant guiding force in every small chores. Their compassionate words as well as stern directions were indispensable for the success of the conference. Dr Indranil Basu, the Organizing Secretary handled his office with great diligence. Dr. Priyanka Datta was the Programme Co-ordinator of AMBICON 2022 who accomplished the painstaking job excellently. Scientific committee members were Dr. Debasmita Bandopadhyay, Dr. Rituparna Maji and Dr. Manali Sinharay, who performed their assigned tasks meticulously with superb coordination between them. Dr. Chinmoy Ghosh and Dr. Soumika Biswas handled excellently the most difficult office of Treasurers. Dr. Sanghamitra Chakraborty was the Souvenir editor who kept her commitment in getting us a beautiful souvenir that AMBICON WB has seen. Dr. Satwika Sinha and Dr. Sanchayan Sinha managed the Registration of delegates marvellously. The Hospitality committee members Dr. Sharmistha Chatterjee, Dr. Pinaki Saha, Dr. Piali Roy Chowdhury, Dr. V Loknathan, Dr. Viveka Roy Srivastava executed their undertakings diligently. Dr Quazi Md. Tajuddin and Dr. Arkajit Dasgupta managed the travel co-ordination with great endeavour.

Delegates: Delegates from our state, national as well as international delegate including many stalwarts belonging to the fraternity of medical biochemistry had attended the program. There was an extravaganza of events for two days. Research Presentations by dignitaries, faculties and post graduate trainees along with an excellent panel discussion on a pertinent topic marked the occasion. The scientific sessions imparted insightful cognizance of various facets of Biochemistry. Indeed, it broadened our horizons in pursuit to reach the stars in the limitless space of medical science.

Events:

Day 1:

Day 1 commenced with faculty presentations by Dr. Suman Chatterjee, Dr. Satwika Sinha, Dr. Sanchayan Sinha and Dr. Ananya Ghosh on their exemplary research works on Electrolyte imbalance in Fluoritic individuals in Fluoride endemic zone, Vitamin D And Insulin Resistance in Obesity, Alteration of enzymes causing cardiac damage in Covid 19 infection and Best software for Prenatal screening using maternal markers respectively. The session was chaired by Prof. Dr. Ajanta Roy, an eminent teacher in the field of Biochemistry with an eversmiling face.

The scientific session was followed by the inaugural ceremony which is the essence of every conference. The elegant inauguration event and souvenir publication was done by Prof. (Dr) Debashis Bhattacharya, Director of Medical Education, West Bengal, Prof. Dr. Prasanth Vishwanath, President of AMBI, Prof Dr. H. N. Das, President, AMBI West Bengal, Prof. Dr. Tapan Mukhyopadhay, Vice President, AMBI West Bengal, Prof (Dr.) Soma Gupta, Secretary, AMBI West Bengal and Dr Indranil Basu, Organizing secretary of AMBICON 2022.

Prizes were awarded to the highest scorer in MD Biochemistry 2022 to Dr. Rima Rudra from R.G Kar Medical College and to Sayan Banerjee, MBBS student of Calcutta National Medical College,Kolkata for scoring highest marks in Biochemistry in MBBS 2022. Prof. Dr. Mohan Chandra Mondal, an_esteemed teacher and evergreen personality in the field of Biochemistry received Lifetime achievement

award. Prof. Dr. Prasanth Vishwanath, President of AMBI and Director & Coordinator, IQAC and JSS Academy of higher education and research, Professor, Department of Biochemistry, JSS Medical College, Mysuru delivered an excellent oration on Gut Microbiome and its implications in Health and disease. It was Prof Dr. Shyamoli Biswas Oration and the Chairperson was Prof. Dr. Subimal Chaudhuri, an eminent Biochemist. This was followed by a didactic lecture by Dr. Saptarshi Bishnu, MD, DM Hepatology; Consultant Hepatologist & Gastroenterologist on Approach to newly diagnosed patient of fatty liver. It was Dr. Kanika Mandi Memorial Oration and the Chairperson was Prof. Dr. C.R. Maity, a prestigious personality in Biochemistry.

Just before lunch Dr. Arghya Chattopadhyay, MD, DM (Rheumatogy) Assistant professor, Department of Rheumatology, North Bengal Medical College delivered an illuminating presentation on Lab in Rheumatology- Confounders and clues. It was Dr. Aruna Bhattacharya Memorial Oration and the Chairperson was Prof. Dr.Mohan Chandra Mondal.

A gala lunch was followed by second session of Faculty Presentation which was chaired by -Prof. Dr. Debasish Basu, HOD, Biochemistry, Medical College, Kolkata. Dr V Lokanathan, Dr. Manali Sinharay, Dr. Debojyoti Bhattacharjee, Dr Sudeshna Ray delivered their extensive research work on Foetal distress, Medical education, Adrenal incompetency and the relatedness of Depression with Vitamin D and Cortisol.

The sessions opened for Corporate talks where national and international representatives conveyed their presentations. Post graduate trainees and Faculties who presented their Posters were Dr. Tousif Rabi, Dr. Mousumi Das, Dr. Rik Swarnakar, and Dr. Satarupa Basu and faculty Dr. Priya Mondal. The Judge was Prof. (Dr.) Shanta Saha, an adorable personality in Biochemistry.

The attraction of the day was the Panel Discussion by eminent panelists - Prof (Dr.) Indranil Chakraborty, Prof (Dr) Swati Bhattacharyya, Dr. Indira Bhaskar Biswas, Prof (Dr.) Mousumi Mukhopadhyay, Prof. (Dr.) Pallab Basu and esteemed moderator: Prof (Dr.) Soma Gupta on Teaching of Medical Biochemistry as per new curriculum by NMC. AMBI West Bengal General Body meeting was held at the end and new members were chosen for important posts in AMBI West Bengal Chapter. The academic sessions ended successfully only to open for the jamboree- the Banquet dinner where everyone feasted and relished to the fullest.

Day 2:

The second and the last day of AMBICON 2022 commenced with oral paper presentations from the young medical biochemists of our organisation whose research spree added a new dimension to our subject and constitute the future of our organisation AMBI WB . The Judges were Prof Dr. H. N. Das and Prof. Dr. Tapan Mukhyopadhay. Post graduate trainees - Dr. Sankar Narayan Maity, Dr. Mir Mozzaffar, Dr. Pritilata Saha, Dr. Asis Bhattacharya, Dr. Binay Ray, Dr. Sadhak Roy, Dr. Soham Biswas, Dr. Sukanya Mukherjee, Dr. Debasish De, Dr. Debi Mallick, Dr. Animesh Sardar Singh, Dr. Sangita Chanda, Dr. Sekh Rajib, Dr. Debraj Bhattacharya, Dr. Nirupoma Bag presented their research works.

Prof. Dr.Susanta Banerjee, ex DME of West Bengal delivered an elaborate talk on pertinent facts of medical field. Third session of Faculty Presentations marked the day with excellent and illuminating presentations, the Chairperson being Prof. Dr. Pinaki Sarkar, an energetic personality in Biochemistry. Dr Indranil Basu, Dr. Sharmistha Chatterjee, Dr. Arindam Ghosh, Dr Surankita Sukul and Dr Anirban Ganguly presented their research works on Retrospective assessment of ANA by IFA and Immunoblot in patients of polyarthritis, a Brief report on Comparison of the LDL-Cholesterol concentrations calculated by Friedwald formula vs that obtained by direct estimation, visceral adiposity index and lipid accumulation product index: the promising role in assessing cardio metabolic risk in non-obese patients of PCOS, a comparative pilot study using conventional nested PCR and an in-house real-time PCR

High-risk Human Papillomavirus 16/18 in oral mucosa and cervix of sexually active women and evolution of Cell Culture Models for Medical Research: From 2D to3D and Organ on Chips respectively.

After the delegates had relished a mouth-watering lunch, a corporate talk was presented. Prof. (Dr.) Debes Ray, Professor, Department of Biochemistry, NRS Medical College with his talk on Implementing Six Sigma Metrics for Performance Evaluation of Clinical Laboratory Services excited the neurons of many who will be trying their level best to implement them in days to come. It was Prof. Dr. Pradip Saha Memorial Oration and the Chairperson was Dr. Pradyot Sen, a respected teacher in Biochemistry.

Conclusion:

The conference ended with Prize distribution of paper and poster presentation. In oral presentation The first prize was awarded to Dr. Pritilata Saha of Murshidabad Medical College, 2nd prize went to Dr.Sankar Narayan Maity of IPGMER, Kolkata and the 3rd prize was won by Dr. Sukanya Mukherjee of NRS Medical College, Kolkata. The prize for poster presentation was awarded to Dr. Mousumi Das. All good things come to an end for better beginnings. At the end there was the Valedictory & Vote of thanks by Organizing Secretary, Dr.Indranil Basu who thanked all AMBIANS to let him be a part of the combined hard work as a team of AMBI West Bengal.

AMBI West Bengal Chapter this year had a gala conference which owes its success to every member. We wish to grow and keep our spirits high to represent ourselves well in next upcoming National and State Conferences. Let's pledge to continue our 'Kinship with Biochemistry' in the limitless vista of medical science.


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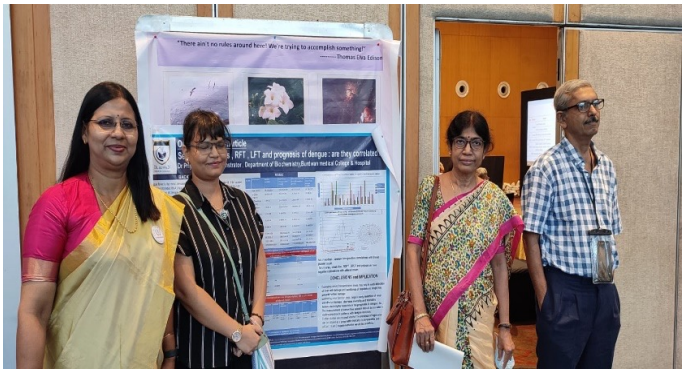


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