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Editorial

From the Secretary's Desk

Respected Senior's & Friends,

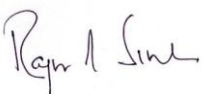
Greetings from ACBI Head office.

After a gap of nearly 16 years, we once again are going to witness an International conference in Clinical Biochemistry & Laboratory Medicine in India. As you all know, Jaipur will be hosting the 15th. Asian Pacific Congress of Clinical Chemistry & Laboratory Medicine from 17th to 20th November 2019. This is a once in a life time opportunity of attending an International conference on Indian soil at Indian rates!! The organizing team has lined up an exemplary scientific program with speakers from all corners of the Globe. The full program is available on www.apfcbcongress2019.org.

Many members have registered and this is a request to those who have not, Register now so as not to miss this grand scientific event.

I wish to inform all members that this year there shall be no Executive Council & General Body meeting of the association. I know that for many of us it will be a shocking news but we all were helpless as we were unable to get a decent time slot to hold our meetings. The APFCB day to day program is packed from morning to evening and also we do not have any room vacant to hold the meetings. This decision was taken after extensive consultation with the Executive Board and all Past Presidents. We also decided that the present EB shall be allowed to continue till next years Kolkata conference.

Looking forward to seeing many of you in Jaipur.



Dr. Rajiv R Sinha
General- Secretary, Editor
ACBI & Editor-in-Chief

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OBITUARY

Dr. K.G. Tanksale
17/09 /1930 - 14/12/2018

Dr. Tanksale obtained his B.Sc. degree from Khalsa College, Mumbai with First Class Honours. He received the Centenary award of Mumbai University. He obtained his M.SC. Degree in Biochemistry from Seth G.S. Medical College, Mumbai in 1957 and was one of the two to get first class. He obtained his Ph.D. from Mumbai University in 1975.

He started his academic career as a lecturer in Chemistry at Ruparel College, Mumbai and moved to T.N.M. College Biochemistry Department in 1960. He became an Asst. Professor in 1963. He was selected by M.P.S.C. as Professor and Head of the Department at Seth G.S. Medical College in 1969, a position he held with distinction until his retirement in 1988. During his long academic career Dr. Tanksale was awarded many honors including the Fellowship of the royal Institute of Chemistry (England) in 1971, selection as W.H.O. Fellow in Clinical Chemistry at Copenhagen and Glaxo award in clinical Chemistry in 1979. He guided 16 students for M.Sc. (by Thesis) and 16 students for Ph.D. in Biochemistry. One of the pioneering members of the Association of Clinical Biochemists of India, he was the past president of the association and was one of the pioneers who gave their valuable support to stabilize the association through its formative years.

May his soul rest in peace

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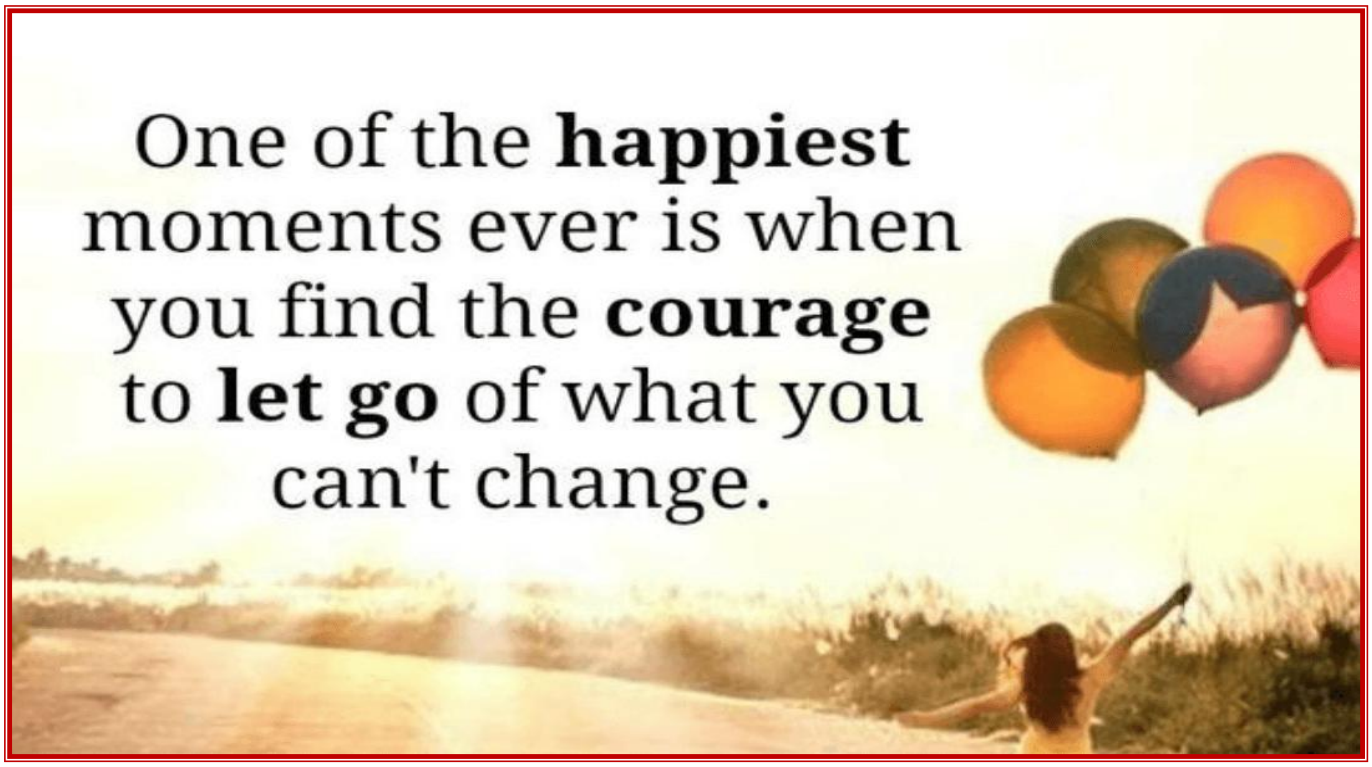

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eJIFCC2018 Vol29 No2 pp105-1110

Vitamin D and its metabolites: from now and beyond.

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ABSTRACT

Total 25-hydroxyvitamin D is currently considered as the most representative metabolite of vitamin D status. There are a multitude of challenges that still deserve to be addressed and despite recent technological advances its determination remains complicated. This current review gives an abbreviated overview of the phases of development that vitamin D metabolite determination has gone through and discusses the difficulties that still require resolving. Furthermore, given the different platforms and methodologies available, the critical issue of standardization and all efforts made as far towards its realization have been discussed. And last but not least, the concepts of 'free' and 'bioavailable' vitamin D along with the 'Vitamin D Metabolism Ratio' have been discussed.

INTRODUCTION

Until recently, 25(OH)-vitamin D (25-OHD) was merely the only vitamin D (VTD) metabolite of interest to explore vitamin D status and metabolism. Unfortunately, the determination of this VTD metabolite, as well as the levels that need to be achieved in healthy or diseased individuals are quite problematic and remain an important matter of debate[1,2]. Recently, other VTD metabolites, like 24,25OH2D, "bioavailable" or "free" vitamin D, cholecalciferol itself and 1,25-OH2D, have emerged as potential new players to better understand the important vitamin D pathway. In this paper, we provide a brief overview on the issues regarding 25-OHD assays and standardization and we will evoke 24,25(OH)2D and Vitamin D ratio (VMR) as potential metabolites of choice to explore vitamin D deficiency.

25- HYDROXY VITAMIN D DETERMINATION

25-OHD is still currently considered as the most representative metabolite of vitamin D status. Unfortunately, its determination remains complicated despite recent technological advances [3]. The reasons why this metabolite is so complicated to be correctly assessed are multiple. First, 25-OHD assays need to recognize 25-OHD2 and 25-OHD3. Second, 25-OHD is a very hydrophobic molecule that circulates bound to vitamin D binding protein (DBP), albumin (ALB) and lipoproteins and a thorough dissociation of the analyte from its ligands is mandatory prior to measurement. This step is particularly complicated for automated immunoassays where, in contrast to radio-immunoassays, binding-protein or chromatographic assays, organic solvents cannot be used for extraction. Hence, automated immunoassays need alternative releasing agents, which do not always achieve total dissociation of 25-OHD. In particular physiological or pathological conditions such as pregnancy, estrogen therapy or renal failure, automated immunoassays often fail to correctly quantify 25OHD [4-7]. Third, 25-OHD2 and 25-OHD3 have different affinity constrants for the carriers & the dissociation step must be highly efficient to obtain an accurate quantification of both forms. Forth, in-vitro

recovery experiments with the molecule give spurious results with immunoassays since it is not clear whether exogenous metabolites bind to all the different carriers in the same proportions as endogenous metabolites. Under-recovery of exogenous 25-OHD has been reported in automated immunoassays[8-9] & even liquid chromatography tandem mass spectrometry (LC-MS/MS) methods[10]. The different methods available for the quantification of 25-OHD use chromatographic separation (HPLC with UV or LC-MS/MS detectors), antibodies or binding-proteins. Binding protein assays have been used in the early eighties and presented clinically acceptable analytical sensitivity and imprecision. They were based on the displacement of H3-labelled 25-OHD and necessitated a chromatographic purification after organic extraction. These home-made methods were very time-consuming and performed in some reference laboratories only. Hence, they have been superseded by radio-immunoassays (RIA) methods. The first commercially available RIA was based on a method described by Hollis et al. in 1993 [11] and the DiaSorin RIA method has been the most widely used method for both routine diagnostic testing as well as for clinical studies until recently. Traditional 25-OHD cutoffs in use today for vitamin D deficiency (either 20 or 30 ng/ml) have been defined on the basis of studies (and meta-analyses of studies) that predominantly used this assay. However, due to the logarithmic increase in 25-OHD requests observed during the last decade, laboratories have opted for more automated immunoassays and less than 1% of laboratories participating in the DEQAS still use this RIA assays nowadays.

The first automated immunoassay for 25-OHD determination was launched in 2001 by Nichols Diagnostics on the Advantage platform. This assay used a competitive legend binding technique with acridinium -ester labeled anti-DBP. Nowadays most of the major in-vitro diagnostic companies have launched their own methods for 25-OHD determination. These methods use a competition design, except the one from Fujirebio on the Lumipulse, which is a non-competitive (sandwich) method based on antimetatype monoclonal antibodies against a hapten–antibody immunocomplex using an ex vivo antibody development system, namely the Autonomously Diversifying Library system, a process which has recently been validated[12]. A large number of studies have evaluated the different automated assays by comparison with RIA, HPLC or, more recently, with LC-MS/ MS methods. Conclusions regarding the accuracy of the assays have also been based on the results of large external proficiency testing programs, such as DEQAS or CAP which now use a reference method to measure the samples sent to the participants, allowing a true calculation of the bias. In a recent study coordinated by the Vitamin D Standardization Program (VDSP) group [13], a set of 50 healthy individuals donor samples were analyzed by 15 different laboratories to provide results for total 25-OHD using both immunoassays and LC-MS/MS methods. The results were compared with those obtained by two reference methods, namely the Ghent University and the U.S. National Institute of Standards and Technology (NIST) methods. Results showed that all but 2 LC-MS/MS achieved VDSP criteria of performance (namely CV $\leq$ 10% and mean bias $\leq$ 5%), whereas only 50% of immunoassays met the criteria. These results can be regarded as optimistic for immunoassays. First, it is obvious from these results that standard deviation around the bias is much more important for immunoassays than LC-MS/MS. As an example laboratory 2a that used an immunoassay and laboratory 10 used a LC-MS/MS method which both presented an excellent mean bias of -1%. But the standard deviation around this bias was 14% for the immunoassay vs. 5% for the LC-MS/MS method. As a consequence, the LC-MS/MS will have 75% of its value within the 5% boundaries whereas the immunoassay will only have 29%. Second, this study has been performed on serum obtained in healthy donors and not in patients. Indeed, patients with

chronic kidney disease, dialysis patients, pregnant women, different ethnic groups, patients in intensive care with fluid shifts present differences in their serum matrix compared to healthy individuals and this can impact the performance of automated 25-OHD immunoassays. Recently, we have shown good clinical concordance between 4 different immunoassays and a VDSP-traceable LC-MS/MS method in healthy subjects. However, significantly poorer agreement with the same LC-MS/MS method has been found in other clinical populations[4, 14]. In the past years, the IFCC has made great efforts to promote standardization of laboratory assays. Indeed, standardization is important to achieve comparable results across different methods and manufacturers. For 25-OHD assays, clinical cut-offs are generally used as target values, and applying common cut-offs on results generated with poorly standardized assays will inevitably lead to inconsistent patient classification and inappropriate therapeutic decisions. Hence, in 2010, the Vitamin D Standardization Program (VDSP) was established to improve the standardisation of 25-OHD assays. The aim of VDSP is that 25-OHD measurements are accurate and comparable over time, location, and laboratory procedure to the values obtained using reference measurement procedures (RMPs) developed at the NIST [15] and Ghent University [16]. As mentioned earlier, a method is considered as standardized if the CV is <10% and the bias <5% [17]. Each candidate receives a set of 10 samples 4 times a year and has to run these samples in duplicate on 2 consecutive days. In January 2018, 27 methods, coming either from IVD companies or clinical laboratories were considered as standardized against the RMPs. However, the proportion of the 40 samples that met the bias criterion (<5%) in 2017 was quite different from one method to the other and ranged from 23 to 85%, with LC-MS/MS methods presenting better results than immunoassays. The list of these standardized methods can be found on the CDC website (http://www.cdc.gov/labstandards/pdf/hs/CDC_Certified_Vitamin_D_Procedures.pdf). Although substantial progress has been made, a range of important issues like standardization of 25-OHD2 & 24,25-(OH)2D as well as improvement of (immuno) assays performance on samples from diseased patients or subjects from different ethnic groups still needs to be achieved. It may thus be tempting to think that immunoassays are outdated and that LC-MS/MS should replace these methods. There are clear limitations to this simplistic view. Indeed, performing a LC-MS/MS is complex and needs experienced and very well trained people. Notably, extensive validation of the LC-MS/MS and sample preparation are of extreme importance. To run a LCMS/MS is much more complicated than "crash the proteins, inject and obtain the results in 2 minutes". A detailed review on how complex running a LC-MS is out of the scope of this present paper, but can be found in a previous report [18]. Finally, laboratories that run LC-MS/ MS do not run "the" reference method, even if their method is certified by the VDSP. As an illustration of this assertion, one can see that some VDSP-certified LC-MS/MS methods present a percentage of samples out of the bias criterion that is lower than immunoassays and much lower than other LC-MS/MS. Also DEQAS results show that LC-MS/MS methods present CVs that are as high as immunoassays.

24, 25-(OH) 2D DETERMINATION AND THE VITAMIN D METABOLITE RATIO

One advantage of LC-MS/MS methods over immunoassays is the possibility to simultaneously quantify 25-OHD and 24,25-(OH)2D allowing to calculate the 25-OHD/24,25-(OH)2D ratio, also known as the Vitamin D Metabolism Ratio (VMR). Indeed, some light has recently been shed on the potential interest of this vitamin D metabolite to better reflect vitamin D deficiency [19]. In summary, CYP24A1, the enzyme allowing the degradation of 25-OHD and 1,25-(OH)2D into 24,25-(OH)2D and 1,24,25-(OH)3D sees its expression increased when there is an increased binding and activation of the VDR in response to 1,25-

-(OH)2D into 24,25-(OH)2D and 1,24,25-(OH)3D sees its expression increased when there is an increased binding and activation of the VDR in response to 1,25-(OH)2D [20]. Hence 24,25-(OH)2D concentration may thus reflect VDR activity which is not really the case with 25-OHD. It has recently been demonstrated that lower 24,25-(OH)2D concentration and lower VMR were associated with increased hip fracture risk in community-living older men and women, whereas 25-OHD was not associated with hip fracture risk. Another point of interest with 24,25-(OH)2D and VMR is that, although concentrations of 25-OHD and 24,25-(OH)2D strongly correlate with each other and are both lower in black Americans than in whites, blacks and whites have equivalent median VMR values [21]. In CKD patients, Bosworth et al have shown that 24,25-(OH)2D was better associated with PTH than 25-OHD or 1,25-(OH)2D [22]. These findings are of great interest but still need to be confirmed by other studies. On the other hand, it is clearly demonstrated that biallelic mutations in CYP24A1 led to idiopathic infantile hypercalcemia [23], a phenotype characterized by profound hypercalcemia, suppressed intact parathyroid hormone, hypercalciuria and nephrocalcinosis. Many heterozygous mutations of CYPA24A1 have recently been described [24]. If they are associated with a less dramatic phenotype than homozygous mutations, patients suffering from these mutations often present with hypercalcemia, suppressed PTH and renal stones [25]. Hence, in patients presenting with a non-parathyroid hypercalcemia (without evident clinical cause), CYP24A1 mutations should be investigated and simultaneous 24,25-(OH)2D & VMR ratio should be measured. A ratio higher than 50, or even 80 should lead to a genetic research of a CYP24A1 mutation. Again, this measurement should be standardized. Fortunately, one candidate reference measurement procedure (RMP) has been published [26] & NIST standard reference material (SRM) 2972a includes 4 standards with certified values (unfortunately, these 4 values are very close to each other) [27]. DEQAS data report that about 10 laboratories provide 24, 25-(OH) 2D results. These data show quite a large variability, which can partially be attributed to the low concentration of the analyte, but also to the lack of ongoing standardization program. This latter will be (probably) even more important than the 25-OHD itself since small variations in 24, 25-(OH) 2D have a dramatic impact on the VMR.

CONCLUSION

The assessment of vitamin D status is a changing landscape [19]. Although 25-OHD is still recommended as the marker of choice by virtually all scientific bodies growing evidence indicates significant limitations that hamper the utility of this analyte in clinical practice. Issues related to the use of 25-OHD include analytical aspects and the interpretation of results. While in normal individuals the agreement of results generated with automated assays is improving, comparability of results in distinct populations, such as children, pregnant women, hemodialysis patients or intensive care patients, remains problematic. The relationships between 25-OHD and various clinical indices are also rather weak and not consistent across races. Recent studies have provided new insights in physiological and analytical aspects of vitamin D that may change the way how we will assess vitamin D status in the future. The VMR (25-OHD/24,25-OH2D ratio), but also 'free' and 'bioavailable' vitamin D are all interesting markers that have expanded our knowledge about vitamin D metabolism and some of these analytes may now be considered for routine use (at least in specialized centers).

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A Woman with Pancreatitis and Hypertriglyceridemia

Ayesha Farooq, Angela Treml, Jessica M. Colón-Franco

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CASE DESCRIPTION

A woman presented to the emergency department with abdominal pain, nausea, and vomiting. On examination, she had abdominal tenderness & a medical history of diabetes mellitus type 1, hypertension, end-stage renal disease, liver disease (hepatic steatosis), hypothyroidism, chronic obstructive pulmonary disease, & congestive heart failure. The patient denied alcohol use or abuse. Lipase was 680 U/L (reference interval, 13–60 U/L) and triglycerides were >4425 mg/dL [reference interval, <150 mg/dL (50 mmol/L)]. These results, along with clinical and radiologic findings, suggested a diagnosis of hypertriglyceridemia-mediated pancreatitis. The patient was treated with insulin & heparin, but triglycerides remained >4425 mg/dL. Consequently, plasmapheresis was used to reduce the serum triglyceride concentrations on day 3 posthospital presentation, but triglycerides persisted at >4425 mg/dL (Table 1). The transfusion medicine team noticed the appearance of clear plasma during the next plasmapheresis course and called the laboratory to investigate.

TABLE1. : Triglycerides and glycerol measurements.

	Reference interval	Results				
		Day 1	Day 2	Day 3	Day 5	Day 9
Triglycerides, mg/dL (mmol/L)	<150 (3.69)	>4425 (50)	>4425 (50)	>4425 (50)	3971 (44.87)	1315 (14.86)
Triglycerides, CDC method, mg/dL (mmol/L)	<150 (3.69)		6620 (74.8)			
Triglycerides, glycerol-corrected, mg/dL (mmol/L)	<150 (3.69)		151 (1.71)			
Glycerol, calculated, mg/dL (mmol/L)	3.5–32.7 (0.04–0.37)		6469 (73.1)			
		Before apheresis		After apheresis		

Triglyceride testing in a reference laboratory followed (Table 1). The CDC method also revealed markedly increased triglycerides at 6620 mg/dL (74.8 mmol/L). However, triglycerides were 151 mg/dL (1.7 mmol/L) in a glycerol-corrected triglyceride assay (Roche Triglyceride/Glycerol Blanked Reagent, Roche Diagnostics). Glycerol concentrations were calculated (triglycerides, CDC - triglycerides, glycerol corrected) to be 6469 mg/dL (73.1 mmol/L) [reference interval, 3.5–32.7 mg/dL (0.04–0.37 mmol/L)]. In other words, 6469 mg/dL (73.1 mmol/L) of the 6620 mg/dL (74.8 mmol/L) in this sample represented free glycerol and not triglyceride-derived glycerol.

QUESTIONS TO CONSIDER

1. What is measured in blanked and non blanked triglyceride tests?
2. What conditions could lead to these findings?
3. How can the laboratory identify falsely increased triglycerides?

ANSWER WITH DISCUSSION ON PAGE: 22

NEWS FROM BRANCHES/ZONES

SOUTH REGIONAL CONFERENCE OF ACBI.

"THE LABORATORY AND DIABETES MELLITUS – NEW CHALLENGES, NEW CONCEPTS, NEW MEASURES" 25th of April, 2019 Organized by: Department of Biochemistry, Sir Ganga Ram Hospital, New Delhi & Association of Clinical Biochemists of India - Delhi Chapter

The Department of Biochemistry, Sir Ganga Ram Hospital (SGRH), under the aegis of Delhi Chapter of the Association of Clinical Biochemistry of India (ACBI), conducted the 8th Annual CME titled "THE LABORATORY AND DIABETES MELLITUS – NEW CHALLENGES, NEW CONCEPTS, NEW MEASURES" on 25th of April, 2019. It was very well-attended by scientists, laboratorians and clinicians from all over Delhi/NCR. It is our belief that patient care is enhanced when clinicians and laboratorians function synergistically. Hence, our scientific sessions included academicians as well as practicing physicians of our hospital as well as clinicians from other reputed hospitals of Delhi/NCR and abroad.

Dr. Seema Bhargava, Chairperson & Senior Consultant, Deptt. Of Biochemistry, SGRH and Vice President, ACBI, addressed the gathering with a warm welcome note.

Dr. Mamta Kankra Consultant, Dept. Of Biochemistry, SGRH & Delhi Representative, ACBI, moderated the inaugural session, which began with lamp lighting by our chief guest, Guest of Honor and the Chairperson of our department. In the CME an annual newsletter was released wherein the new tests introduced and departmental highlights were detailed. Dr Seema Bhargava, on behalf of Dept. of Biochemistry, welcomed all the dignitaries on the dais and the delegates. The President ACBI**, Dr. L M Srivastava (Consultant Advisor, Deptt of Lab Medicine, Kolmet Hospital)** extended a warm welcome to all the attendees & motivated all the young scientists with his words of encouragement.

The CME started with the YOUNG SCIENTISTS' PRESENTATIONS which was moderated by **Dr. Parul Singla**, Consultant, Dept of Biochemistry, SGRH and Dr. Reetika Saini. There were eight high quality presentations given by young researchers from various medical colleges and institutes of Delhi/ NCR. The session was judged by **Dr. K K Srivastava (President, Delhi Chapter of ACBI and former Director DIPAS, New Delhi), Dr Anju Jain (Director Prof and Head, Dept of Biochemistry, LHMC) and Prof Arif Ali, (formerly head, Dept of Biosciences Jamia Milia Islamia, New Delhi).**

Our Chief Guest for the CME was **Dr S P Byotra (Chairperson, Deptt. Of Medicine, Director Labs & Vice Chairman, Board of Management, SGRH) and Dr Kusum Verma (Dean GRIPMER and Chairperson Cytopathology, SGRH)** was our Guest of Honor. They addressed the gathering by highlighting the role of labs in patient care. They all appreciated the high quality work being done in the Department of Biochemistry, SGRH and gave a detailed overview of Sir Gangaram Hospital. Dr. Kusum Verma also briefed everyone about the certificate course on '**TOTAL LABORATORY QUALITY MANAGEMENT & INTERNAL AUDITOR- ISO 15189:2012'** being started by the Department of Biochemistry under the aegis of GRIPMER.

The first scientific session was moderated by **Dr. Manushri Sharma**, Associate Consultant, Dept of Biochemistry, SGRH, where she invited **Dr. Anjali Manocha, Senior Consultant, Deptt. Of Biochemistry, SGRH and Secretary, ACBI, Delhi Chapter,** to deliver a talk on **'Challenges in POCT Glucose Monitoring'**.

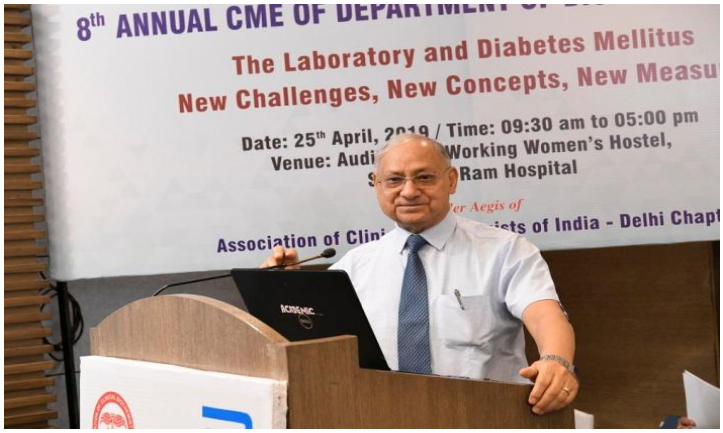
This was followed by a talk on '**Methodologies for HbA_{1C} Measurement- A Comparative Analysis' by our Chairperson, Dr. Seema Bhargava.**

In the second session, which was held post lunch, **Dr Seema Bhargava** introduced our keynote speaker **Prof. William Garry John, Head, Clinical Biochemistry and Immunology, Norfolk & Norwich University Hospital, Norwich, UK, who spoke on 'The Laboratory and Diabetes Mellitus: New Challenges, New Concepts, New Measures'.**

This lecture was followed by two more highly informative talks, where **Dr. Mamta Kankra** invited **Dr. Atul Gogia (Sr. Consultant, Deptt. of Medicine, SGRH),** who **spoke on guidelines for Diagnosis of Diabetes Mellitus- ADA versus WHO Guidelines'** & **Dr. Subhash Wangnoo (Sr. Consultant Endocrinologist, Apollo Centre For Obesity, Diabetes**

& Endocrinology, Indraprastha Apollo Hospital, New Delhi), who **spoke on 'Role of Genetics in Diagnosis and Management of Diabetes Mellitus.**

Dr. Parul Singla, then invited **Dr. Douglas Chung (Marketing Manager Clinical Chemistry, Abbott Diagnostics Division, Asia Pacific)** to deliver the final lecture of the session, which was on **"Performance & Clinical Utility of HbA_{1C} Enzymatic Assay"**.



The session concluded with the prize distribution for the young Scientists' presentation. As **first prize**, the **Prof L M Srivastava Gold Medal** (which was instituted to encourage academic activities of young scientists), a certificate of appreciation and Rs. 2000/- were awarded to **Dr. Nitesh Mishra**, PhD Student, Biochemistry, AIIMS, New Delhi, for his presentation titled "Identification of viral envelope glycoproteins with potential to serve as Clade C based immunogens from HIV-1 infected pediatric elite neutralizers". The **second prize** of a certificate of appreciation and Rs. 1500/- was awarded to **Dr. Devanjan Dey**, PhD Student, Biochemistry, AIIMS, New Delhi for his presentation titled "Using human fetal neural stem cells and oligodendrocytes as a disease model to delineate the pathogenesis of cerebral palsy". The **third prize**, consisting of a cash prize of Rs. 1000/- and a certificate of appreciation, was awarded to **Dr. Sagar Verma**, PhD student, Deptt. Of Research, SGRH for his presentation titled "A cell-based model to study ALS pathogenesis". Rest of the participants were given a **consolation prize** of Rs. 500 each and a certificate of appreciation. The prize money is sponsored by Prof. L M Srivastava every year, to encourage the young research scholars. The CME concluded with a vote of thanks given by **Dr Mamta Kankra**, followed by tea and refreshments.

TELANGANA STATE BRANCH:

The Department of Biochemistry, Nizam's Institute of Medical Sciences, Hyderabad conducted the Association of Clinical Biochemists of India (ACBI) Telangana State Chapter **one day CME and Hands-on Workshop on " Hemoglobinopathies" on 10th August, 2019.** Dr. I. Krishna Mohan, Associate Professor, Department of Biochemistry was Organizing Secretary, and Dr. M. Vijaya Bhaskar, Professor of Biochemistry was the Organizing Chairman. About 150 Delegates from Telangana State attended for CME and 50 Delegates attended the Hands-on Workshop. Professor S. Rammurti, Dean of Nizam's Institute of Medical Sciences and other dignitaries inaugurated the CME and Hands-on Workshop.

The following topics were discussed in the morning session of CME. "Introduction of Hemoglobinopathies" by Dr. N.N. Sreedevi, Asst. Professor, Dept. of Biochemistry, Nizam's Institute of Medical Sciences, Hyderabad. Second was "Prevalence & Current Concepts of Laboratory Approach towards Hemoglobinopathies & A1c" by Dr. Kushnooma Italia, Product Specialist for A1c & Thalassemia, Bio-rad Laboratories. Dr. G. Somayajulu, Consultant & Technical Advisor, DDRC, Hyderabad talked on "Hemoglobinopathy: My Encounters" followed by the topic, "Molecular Analysis in hemoglobinopathies" by Dr. Ashwin Dalal, Head, Diagnostic Division, CDFD, Hyderabad. The lectures were followed by a panel discussion conducted with 3 speakers and 3 other subject experts from Internal medicine, Medical genetics, and Biochemistry departments. The members then felicitated Senior Biochemistry Faculty, Dr. A. S. Kanagasabapathy, Dr. L. Vasantha, Dr. G. Somayajulu, Dr. K. Ambica Devi and Dr. B. Prabhakar Rao. Following the lunch break, Dr. I. Krishna Mohan, Associate Professor, Dept. of Biochemistry presented the topic "Identification of Abnormal Hemoglobin variants by HPLC Technique". The Hands-on Workshop on Hemoglobinopathies was conducted between 2.20 – 5.00 pm for 50 Workshop participants. Different abnormal hemoglobinopathy variant samples were analyzed and showed the patterns to the participants. Workshop participants actively participated in the workshop and learned HPLC technique and identified the various abnormal hemoglobin variants in the patient blood samples.

WEST BENGAL BRANCH

Symposium on "Molecular Diagnostics & Therapeutics" on June 21-22, 2019 At College of Medicine & JNM Hospital, WBUHS- Report

Molecular diagnostics has seen much growth in the clinical setting, providing rapid and sensitive approaches for the detection and monitoring of a wide range of human ailments. Linking the approaches to chemical genomics and molecular therapeutics should provide an expanding repertoire of targeted therapeutics for clinical evaluation. In this context, Department of Biochemistry, College of Medicine & JNM Hospital (COMJNMH), WBUHS, with Association of Clinical Biochemists of India (ACBI) organized a Symposium on "Molecular Diagnostics & Therapeutics" on June 21-22, 2019. The symposium was highly integrated basic science and translational components in four areas: structural biology; drug discovery, development and delivery; pharmacology and pharmacogenomics; and oncogenic signaling.

Inauguration of the program started with Saraswati Vandana followed by welcome address by Prof Keshav Mukhopadhya, Principal COMJNMH. Prof. Subir Kumar Das, HOD, Biochemistry, COMJNMH felicitated the dignitaries on the dias. Prof. L. M. Srivastava, President, ACBI had stressed on development of National Reference Range for diagnostic parameters as well on the development of affordable diagnostic kits for investigations. Prof. Pradeep Kr Mitra, Hon'ble Director of Medical Education, Govt of West Bengal, urged for development of early predictive markers to identify disease progression through molecular diagnosis. Prof Saumitra Das, Hon'ble Director, National Institute of Biomedical Genomics (NIBMG), Kalyani had elaborated on integration of basic science with medicine towards development of better world. Prof. Sankar Ghosh, Hon'ble Vice Chancellor, University of Kalyani further emphasized how collaboration between basic scientists with clinicians benefits common people to combat disease, particularly with reference to North-East India. He urged to develop an education hub at Kalyani using all leading Institutes, & excel in academics and health care. Prof. Rajen Pandey, Hon'ble Vice Chancellor of the West Bengal University of Health Sciences (WBUHS), suggested to develop excellence in academics and research for healthcare within the frame work of ethical guidelines. In Scientific session, Prof Sourav Pal, Hon'ble Director, Indian Institute of Science Education and Research (IISER), Kolkata, focused how different disciplines of education is involved in the development of better world. Prof. Saumitra Das, Director, NIBMG elaborated the understanding of biology of Hepatitis C virus for the past two decades. Prof. Sankar Ghosh, Hon'ble Vice Chancellor of University of Kalyani discussed about the plasma cf-NA as a potential diagnostic marker as well as a promising, less-invasive tool for early detection & monitoring of several human diseases like cancer, stroke, trauma, myocardial infarction, autoimmune disorders, & pregnancy-associated complications. Dr. Mriganka Mouli Saha from College of Medicine & JNM Hospital, WBUHS, Kalyani showed the utility of cffDNA using the methylation-dependent DSCR3 and RASSF1A markers along with total cell free DNA (cf-DNA) in maternal serum by HYP2 marker in predicting preeclampsia, intrauterine growth restriction.

Prof. Abhijit Saha, Centre Director, UGC-DAE Consortium for Scientific Research, Kolkata presented some selective facile techniques using soft chemical and radiation chemical approaches to synthesize good quality colloidal nanocrystals. Prof. Anindya Sundar Ghosh from IIT, kharagpur has inferred that the copper nanoparticles (CuNPs) & biosurfactin stabilized silver nanoparticles (AgNPs) also serve as good candidates for inhibiting bacterial population, either individually or in combination with other antibiotics.

Prof Suman Kapur from BITS-Pilani, Hyderabad showed a significant increase in urinary gallic acid concentration in obese subjects in comparison to normal and overweight subjects. With the advent of new and improved technologies, Prof. Maitree Bhattacharyya, Director, Jagadis Bose National Science Talent Search (JBNSTS) highlighted this aspect in understanding correlation among oxidative stress, autophagy markers and insulin resistance may provide an understanding of the molecular mechanism for the development of new therapeutic strategy for Type 2 diabetes mellitus. Dr. Priyadarshi Basu from NIBMG identified the NAFLD-initiating molecular changes including increased fatty acid import, development of cellular stress, and activation of PI3K-AKT pathway in the early stages of disease spectrum.

Dr. Amit Kunwar, from Bhabha Atomic Research Centre (BARC), Mumbai showed that 3,3'-diselenodipropionic acid (DSePA), a selenocystine derivative known to prevent radiation pneumonitis through intraperitoneal route can retain its activity through oral route. The anti-pneumonitic effect of DSePA was attributed to the lowering of PMN-induced oxidants, maintenance of glutathione peroxidase activity and subsequent suppression of NF-kB/IL-17/G-CSF/neutrophil axis in the lung of irradiated mice. Dr. Sandeep Singh from NIBMG, Kalyani hypothesized that the intratumoral functional heterogeneity exhibited by oral cancer cells is driven by distinct sub-populations of oral-stem-like cancer cells (Oral-SLCCs). Dr. Sutapa Mukherjee from Chittaranjan National Cancer Institute (CNCI), Kolkata concluded that reversal of acquired chemoresistance by phenethyl isothiocyanate (PEITC) targeted Aurora Kinases potentiated breast cancer cells towards paclitaxel induced apoptosis.

Dr. Abhijit De from Tata Memorial Centre (TMC), Navi Mumbai suggested that future drug design or screen strategy should aim both arms of STAT3 pathway to completely abrogate the oncogenic function of STAT3. Dr. Kartiki V. Desai, NIBMG, Kalyani discussed the utility of genomic data to identify potential targets, studying their biology& combining these data to find potential gene based signatures that could be prognostic, (or even better) and predictive of treatment response. Dr. Ashwin Dalal from the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad discussed on Next Generation Sequencing in diagnosis of genetic disorders. Dr. Amitava Sengupta from CSIR-IICB reported that MBD3 loss in human primary Acute myeloid leukemia (AML) associated with leukemic NuRD, an ATP-dependent chromatin remodeling complex that regulates epigenetic architecture and cellular identity. Dr. Pritha Ray from ACTREC, Mumbai revealed the crucial role of IGF1R signaling in promotion of chemoresistance and metastasis in epithelial ovarian cancer cells. Dr. M.K. Ghosh from CSIR-IICB, encapsulated indole derivative 3,3´-diindolylmethane (DIM) in PLGA nanoparticles and tagged with novel peptide designed against SSTR2 receptor on its surface for targeted delivery to the tumor site. Dr. Rituparna Sinha Roy from IISER, Kolkata have engineered protease-stabilized facial lipopeptides for intracellular delivery of siRNA in functional form for breast cancer treatment.

Tuberculosis is a disease of antiquity. But, it remains a global threat with 1.6 million deaths per year and 10 million infected people estimated in 2017. While Dr. Indranil Halder from COMJNMH, Kalyani has discussed on Molecular Diagnosis of Tuberculosis, Dr. Bhaswati Pandit from NIBMG, Kalyani, suggested that plasma cytokines and chemokines could be used as immunological markers for diagnosing active TB disease and for monitoring effective anti-tuberculosis therapy.

Dr. Partho Sarothi Ray from IISER, Kolkata had shownthat the time-dependent biphasic expression of miR-125b, an oncomiR, contributes to the pulsatile expression of p53 in response to DNA damage. Dr. Neelam Shirsat from ACTREC, Mumbai observed that the WNT subgroup tumors having excellent survival rates have the most distinguishing microRNA profile with a number of miRNAs like miR-193a, miR-148a,

miR-224 over expressed almost exclusively in these tumors. Expression of these miRNAs bring about reduction in malignant potential of medulloblastoma cells by reducing anchorage-independent growth and/or invasion potential & thus have therapeutic potential. Dr. Neelam Shirsat also studied circulating miRNAs in sera from prostate cancer patients for early detection & during follow-up of the patients in the course of treatment in addition to PSA that is the only marker available presently. Dr. Malancha Ta from IISER, Kolkata investigated the impact of physiological fever-like temperature on Wharton's jelly-derived MSCs (WJ-MSCs) & concluded that NF-kB pathway might be playing an active role in determining the thermosensitivity of WJ-MSCs under febrile temperature condition. Dr. Snehasikta Swarnakar from CSIR-IICB, found that the increased activity of MMP-3 & -9 with endometriosis progression in human. Ectopic tissues from stages III & IV human endometriosis patients showed increase in MMP-13 & MMP-7 activity as compared to non endometriotic individual. Furthermore, epidermal growth factor receptor (EGFR) is required for MMP7 upregulation. Dr. A.V.S.S. Narayana Rao from BARC attempted towards developing a method for determining the EGFR gene status. Dr. Radhakrishnan R Nair from Rajiv Gandhi Centre for Biotechnology (RGCB), Trivandrum, observed that using quantitative reverse transcriptase polymerase chain reaction (qrtPCR) & reporting against known standard BCR-ABL transcripts on normalized values have the best possibility of monitoring treatment response in Chronic Myeloid Leukemia (CML), undergoing therapy by tyrosine kinase inhibitors (TKIs)with a simple blood draw. Dr. Sucheta P Dandekar, showed potent anticancer activity of honey against cervical cancer cells. Dr. K Indira Priyadarsini from BARC, explained the unique biological activities of curcumin. Dr. Sudip Kumar Datta from AIIMS, New Delhi showed Two important genes activated by vitD are Nrf2 and the anti-ageing gene Klotho, both of which have multiple roles in maintaining the integrity of cellular signalling systems.

Dr. Avijit Hazra from IPGMER, Kolkata discussed the pitfalls, beyond those associated with statistical calculations, in the evaluation of diagnostic tests. About 150 students, researchers, scientists and faculty members from various parts of India attended & actively participated in this program. The symposium was supported by the CSIR, DAE-BRNS, DBT, Immunology Foundation & MCI. Dr. Tanmay Saha & Dr. Mrityunjoy Halder compared the entire program. The symposium ended with vote of thanks.

ACBI BENEVOLENT FUND

AN APPEAL

The Executive Council and GB were concerned to know the fact that one of our very senior members is suffering due to lack of money for his treatment and upkeep. For such situation many organizations have created 'Benevolent' fund to assist their members in dire need. We should also have compassion when any of our members are in need of help. Therefore the G.B. has decided to create a Fund to help our needy members and has sanctioned Rs. 50,000 from ACBI account for this fund. The IJCB Board has also decided to contribute Rs. 25,000. Many members have agreed to send money for the fund. Dr. B.C. Harinath has contributed Rs. 17000 which includes the money he got as recipient of ACBI-A.J. Thakur award for Distinguished Clinical Biochemist. Some have sent Rs. 1000 / 2000 /3000 as their contribution.

I solicit your support and appeal you to send money for this noble work as much as you like. The money be sent to the Treasurer, Association of clinical Biochemists of India, Biochem-Lab, East Boring Canal Road, Patna - 800001 by bank draft in the name of "ACBI Benevolent Fund" payable at Patna. The names of Donors are published in News Bulletin.**Dr.**

Dr. Rajendra Prasad

President

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ANSWER & DISCUSSION

From Page:

CLINICAL CHEMISTRY CLINICAL CASE STUDY

A Woman with Pancreatitis and Hypertriglyceridemia

Discussion

Most triglyceride tests used in clinical laboratories do not measure triglyceride itself but the glycerol hydrolyzed from the triglyceride by the action of lipase. This is followed by another sequence of enzymatic steps resulting in color formation & spectrophotometric measurement. In this method, any free glycerol in the sample contributes to the measured triglyceride amount. Increased free glycerol in a sample will falsely increase triglycerides unless a blanking method is used. In the glycerol blanking method, the difference of the non corrected and glycerol-corrected triglyceride results represents the concentration of free glycerol. Although once a matter of debate, offering a glycerol-blanked triglyceride assay is not clinically necessary under most circumstances and would add unnecessary cost. Significant increases of free glycerol are uncommon in inpatient & outpatient populations (1–3). Among laboratories enrolled in proficiency testing for triglycerides from the College of American Pathologists, only 7% report results using glycerol-blanked methods.

The laboratory results were suspicious for pseudohypertriglyceridemia. The workup of suspected pseudohypertriglyceridemia begins with measuring triglycerides after glycerol blanking, followed by

Identifying the source of hyperglycerolemia, whether endogenous or exogenous (Table 2). Glycerol kinase deficiency (GKD)2 is the most commonly reported cause of increased glycerol concentrations in patients (4). GKD is a rare X-linked recessive disorder affecting fewer than 200000 individuals in the US. It can occur isolated with or without synonyms or combined, involving adrenal dysfunction or Duchene muscular dystrophy (5). The clinical presentation of GKD ranges from asymptomatic to critical metabolic crisis. GKD is identified in patients with chronic hypertriglyceridemia [usually <1000 mg/dL (11.3 mmol/L)] not responding to intensive exercise regimens, dietary modifications, & multiple triglyceride-lowering medications, with absence of metabolic syndrome signs, later found to have pseudohypertriglyceridemia (4). The diagnosis of GKD is mostly a diagnosis of exclusion, and testing for glycerol kinase activity is not necessary for diagnosis (5). Patients with GKD show varying degrees of glycerol kinase activity in leukocytes, and various mutations have been identified.

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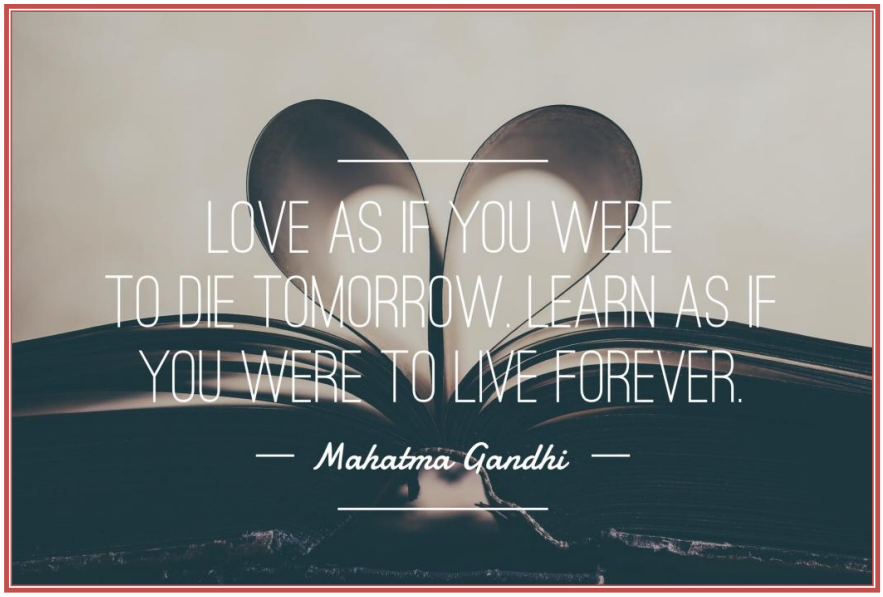

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