DOI: 10.7860/JCDR/2020/44408.13968

Original Article

Biochemistry Section

Estimation of Glycated Haemoglobin by Nephelometry, Ion Exchange Resin and High Performance Liquid Chromatography: A Cross-sectional Study

MANGALORE BALAKRISHNA PRATHIMA[1], SHRIDHAR RESHMA[2], SUSHITH[3], PRATHIKSHA SHETTY[4], D'SA JANICE[5], BHUVANESH SUKHLAL KALAL[6], GOPAL R MADAN[7]

ABSTRACT

Introduction: Diabetes mellitus cases are continually rising all over the world. Glycated haemoglobin (HbA1c) used as a diagnostic test to measure long-term average glycaemic control in diabetic patients.

Aim: To assess the precision and reproducibility of the Ion Exchange Column Chromatography Resin (IECR) method and nephelometry in comparison to High-Performance Liquid Chromatography (HPLC) with respect to the estimation of HbA1c.

Materials and Methods: A cross-sectional comparative study was conducted on 50 blood samples, collected from the diabetic subjects at the Department of Biochemistry and their HbA1c values were estimated by HPLC based BioRad D-10, nephelometry and IECR techniques. HPLC was used as a gold standard method, to evaluate the sensitivity and specificity of nephelometry and IECR techniques. Data were expressed as mean±standard deviation and intraclass Correlation Coefficient and Pearson correlation were calculated.

Results: The mean age of the study subjects was 53.06±7.67 years. The mean plasma HbA1c levels were 8.16±2.9, 7.62±2.5 and 7.84±2.5 and mean estimated Average Glucose (eAG) was 187.00±84.4, 172.0±76.8, and 289.46±199.9 by HPLC, Nephelometry and IECR, respectively. Compared with HPLC, nephelometry had excellent correlation (r-value 0.925); p<0.001) and IECR (r-value 0.869; p<0.001).

Conclusion: Nephelometry and IECR both had better performance and showed a greater concordance with gold standard HPLC. Therefore, nephelometry and IECR can be used as an alternative assay for HbA1c estimation.

Keywords: Diabetes mellitus, Enzymatic assay, India, Test performance

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterised by high blood glucose (hyperglycaemia) resulting from insufficient insulin production or defects in insulin action, or both. It affects almost all the body organs and prolongs hyperglycaemia can lead to life-threatening complications and defects [1].

HbA1c is a haemoglobin-glucose complex that is formed non-enzymatically inside the cell [2]. Determination of HbA1c is a good index of blood glucose in a given past period. HbA1c levels are the means of blood sugar of last 8-12 week., therefore it is used as gold standard for monitoring patients' blood sugar level [3]. Results of HbA1c measuring are used in the management of diabetic patients. Hence, the agreement of results between different HbA1c measuring methods and kits is critical in medical decision making [4].

A single glucose determination gives a value which is true only at the time when the blood sample is drawn and measurement of HbA1c correlated with an individual's mean blood glucose over the preceding eight to 12 weeks [5]. HbA1c is unaffected by diet, Insulin or exercise on the day of testing and thus reflects the average glucose level over the last several weeks thereby, giving a status of the long term metabolic control of glucose in individuals [6]. HbA1c is now widely recognised as an important test for the diagnosis of diabetes mellitus and is a reliable indicator of the efficacy of therapy. Increased levels of HbA1c are directly linked with a greater risk of complications from diabetes. Therefore, the guidelines also recommend the monitoring of HbA1c [7].

Clinical laboratories use different techniques such as liquid chromatography, electrophoresis, boronate affinity, and ion-exchange chromatography, immunoassay and spectrophotometry are used for accurate measurement of HbA1c [8,9]. These methods are based on molecular charge or structure or chemical formulation. HPLC is considered "gold standard" and routinely used methods that have high validity, accuracy, and stability [10]. However, the different types of haemoglobin variants on HbA1c can influence the results [11]. Additionally, the cost of HPLC device, tedious and time-consuming sample processing which requires skilled personal, professionals are looking for an alternative method to detect and monitor HbA1c which is cheaper and cost-effective for all laboratories [12]. Several studies have compared different methods with HPLC however, results are still not well satisfied due to the inconsistency of HbA1c [4,13-16]. A rather alternate validated method is required to detect HbA1c.

The nephelometry methodology is a reliable, time-saving and non-tedious and is for estimation of HbA1c following National Glycohaemoglobin Standardisation Program guidelines [17,18]. It measures the scattered light from the surface of latex particles [19]. The analyser provides direct results in percentage HbA1c and it does not need haemoglobin measurement. Hence, the automated system has been adopted and it results in excellent reproducibility among laboratory settings. It has the advantages of accuracy, sensitivity and low cost compared to HPLC [20]. IECR is a method of molecular separation based on their charge. It is less time consuming and considered a more reliable method of HbA1c detection [21].

Therefore, the present study was taken up to evaluate the analytical performance of the most common cost-effective technique i.e., nephelometry, and ion-exchange chromatography, in comparison with the gold standard HPLC method for estimation of glycated HbA1c.

MATERIALS AND METHODS

A cross-sectional comparative study between three methods HPLC, Nephelometry and IECR was conducted on diabetic patients who were referred to the central diagnostic laboratory facility of hospital, AJ Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India, for HbA1c estimation from April 2016 to October 2016. The study was approved by the Institutional Ethics Committee (AJEC/REV/12/2016). Sample size was calculated using formula: n= {n0/(1+((n0-1)/N))}. Where, n-adjusted sample size; n0-the initial sample size; N-the population size. Using this sample size minimum sample required to obtain 80% power was 34 and present study sample size was 50.

Diabetic patients, aged above 20 years, without any history of long-term complications were included in the study. Pregnant women and patients with anemia, renal diseases, hepatic diseases, cardiac diseases, and hypertension were excluded from the study. The informed consent was obtained from each subject and a blood sample (2 mL) was collected in EDTA vacutainer and stored at -4°C.

Biochemical Parameters

HbA1c was estimated by HPLC D-10 (Bio-Rad Laboratories, Hercules, CA, USA), nephelometry (MISPA i2, AGAPPE Diagnostics GmbH, Switzerland) and the IECR method. HPLC D-10 system works on chromatographic separation of haemoglobin fractions. It is an automated system where the samples are loaded directly, diluted, and injected into the analytical cartridge. The separation of haemoglobin-based on its ionic interactions with the cartridge and the analytical flow path is measured at 415 nm [22].

Nephelometry utilises an antigen-antibody interaction method to determine HbA1c in whole blood. Non-specific adsorption of HbA1c to latex particle forms a complex with mouse anti-human HbA1c monoclonal antibodies. Anti-mouse IgG antibody interacts with the monoclonal antibodies and agglutination occurs. The quantity of agglutination is measure by nephelometry, standard curve are made and used to measure the percentage of HbA1c [23,24].

In the IECR method, the IECR separator is used to separate charged amino acid side chains based on its electrostatic interactions and HbA1c and non-glycosylated fraction is eluted out [25]. Maintaining the HPLC as a gold standard method, IECR and nephelometry method were evaluated in the terms of sensitivity and specificity. The subjects were grouped based on their HbA1c levels as follows: Group 1: Below 6%; Group 2: 6-9%; and Group 3: Above 9% [4]. estimated Average Glucose (eAG) was calculated using previously reported formula eAG=(28.7×HbA1c-46.7]) mg/dL [26].

STATISTICAL ANALYSIS

Statistical analysis were done using Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL) version 11.0. All quantitative data were expressed as mean±Standard Deviation (SD). Intraclass Correlation Coefficient and Pearson correlation were calculated. Receiver Operating Characteristic (ROC) curve, sensitivity, and specificity were calculated for all three methods. The p-value <0.05 was considered to be statistically significant.

RESULTS

The mean age of the study subjects were 53.06±7.67 years. Majority of the study subjects were between the age group of 51-60 years [Table/Fig-1]. The biochemical parameters of the study group are shown in [Table/Fig-2]. Most of these parameters fall under normal category with no significant differences.

The mean of HbA1c by the HPLC method was higher than that of nephelometry and IECR. The mean HbA1c of group 1 was higher (p<0.05) when it was estimated by the IECR method, whereas it was found that the HbA1c measured in groups 2 and 3 was higher when estimated by HPLC. The mean eAG was significantly higher in all groups when it was estimated by the IECR method [Table/Fig-3].

Age (years)	Male	Female	Frequency
40 and below	3	2	5
41-50	4	8	10
51-60	21	12	35
Total	28	22	50

[Table/Fig-1]: Age distribution in the cases.

Parameter	Mean±Standard deviation	Normal reference range
Pulse rate (bpm)	76.8±8.9	60-100
Systolic BP (mmHg)	124.5±19.8	Upto 120
Diastolic BP (mmHg)	76.4±8.5	Upto 80
Hb (%)	11.4±2.2	Males:13.8 -17.2; Females:12.1-15.1
FBS (mg/dL)	150.2±76.4	70-110
RBS (mg/dL)	152.5±62.8	70-140
PPBS (mg/dL)	200±81.9	Upto 140
Blood Urea (mg/dL)	27.4±14.5	12-40
Serum Creatinine (mg/dL)	1.2±1.0	Males: 0.7-1.4; Females: 0.6-1.2
Serum uric acid (mg/dL)	4.5±1.7	Males: 3.4-7; Females: 2.5-6
Sodium (mEq/L)	131.0±7.3	135-145
Potassium (mEq/L)	4.1±0.5	3.5 -5
Chloride (mEq/L)	97.7±6.5	95-105
Total bilirubin (mg/dL)	0.9±0.9	Upto 1.2
Direct bilirubin (mg/dL)	0.3±0.5	<0.3
Total protein (g/dL)	6.5±0.6	6-8
Serum albumin (g/dL)	3.6±0.4	3.5-5.5
Serum globulin (g/dL)	2.9±0.6	2.0-3.5
AST (IU/L)	33.6±21.3	5-40
ALT (IU/L)	24.9±14.7	5-40
ALP (IU/L)	239.5±165.5	20-140

[Table/Fig-2]: Clinical and biochemical parameters of the overall study group. Hb: Haemoglobin; FBS: Fasting blood sugar; RBS: Random blood sugar; PPBS: Post Prandial blood sugar; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase

Considering a cut-off of 6.5% the sensitivity, the specificity of HPLC, Nephelometry, and IECR was calculated using the Receiver Operator Curve (ROC). Area Under Curve (AUC) for HPLC was 1 (95% confidence interval (CI) 0.93 to 1); for nephelometry 1 (95% CI 0.93 to 1); and for IECR was 0.863 (95% CI 0.73 to 0.94) [Table/Fig-4,5].

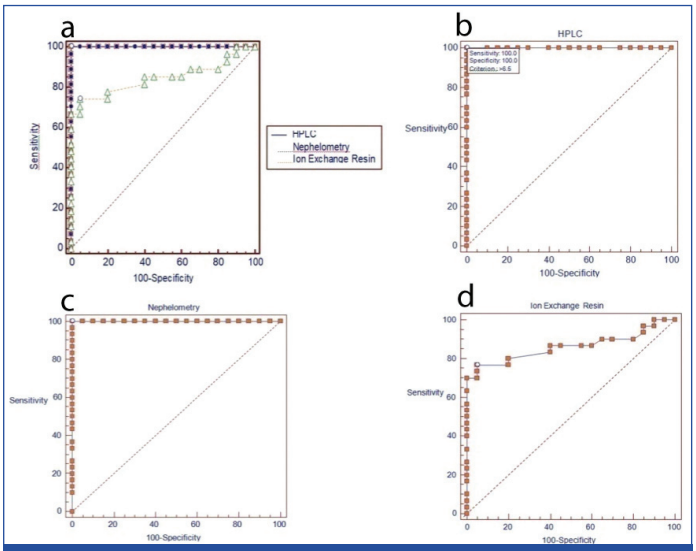
The intra-class correlation was performed to determine the reliability of HPLC and IECR [Table/Fig-6a,7]. High similarity was observed between HPLC and nephelometry (r-value 0.925) [Table/Fig-6b]. Nephelometry percentage shows significant fair agreement (r-value 0.756) with IECR% (p-value is <0.001) which was statistically highly significant [Table/Fig-7]. A high similarity was observed between nephelometry and the IECR method. Pearson correlation of Fasting Blood Sugar (FBS) showed a significant positive correlation with HbA1c estimated with HPLC (r=0.562; p-value <0.001), nephelometry (r=0.556; p-value <0.001), and IECR method (r=0.538; p-value <0.001). The sensitivity and specificity were 100% and 95%, 100% and 81% and 97% and 80% for HPLC, Nephelometry, and IECR respectively [Table/Fig-8].

DISCUSSION

HbA1c has been used as a gold standard for assessing mean glycaemia and also a measure of risk for the development of diabetes mellitus related complications [10]. The study compared the analytical performance of the estimation of HbA1c by three different

Parameter	Method	Mean±SD <6% (Group 1)	Mean±SD 6-9% (Group 2)	Mean±SD >9% (Group 3)	Mean±SD Overall
HbA1c	HPLC%	5.16±0.5	7.38±0.7	11.63±1.3	8.16±2.9
	Nephelometry%	4.97±0.5	7.22±0.76	10.47±1.65	7.62±2.5
	Ion exchange resin%	5.99±0.8	7.18±1.57	10.13±2.71	7.84±2.5
eAG	HPLC%	101.0±14.4	164.73±22.3	286.78±39.7	187.00±84.4
	Nephelometry%	95.64±14.3	160.0±22.1	270.60±32.43	172.0±76.8
	Ion exchange resin%	108.0±24.7*	215.73±39.1**	522.22±135.3**	289.46±199.9**

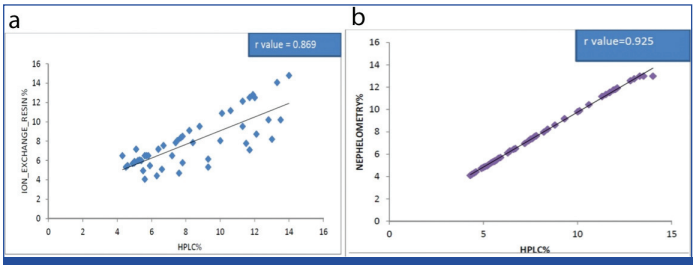
[Table/Fig-3]: Mean HbA1c and eAG of three methods amongst HPLC, nephelometry, and IECR.
*p<0.05; **p<0.01; HPLC: High performance liquid chromatography; HbA1c: Glycated haemoglobin; eAG: Estimated average glucose; SD: Standard deviation; Group 1, 2, 3 are based on HbA1c concentration



[Table/Fig-4]: Receiver Operator Curve (ROC) of HbA1c estimation by HPLC and IECR method.

Method	The area under the curve	Standard error	Asymptotic significance*	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
HPLC	1	0	<0.001	0.929	1.000
Nephelometry	1	0	<0.001	0.929	1.000
Ion exchange resin	0.863	0.0545	<0.001	0.733	0.942

[Table/Fig-5]: ROC of HbA1c% estimation by HPLC, nephelometry and IECR.
*p-value<0.001 is considered statistically significant



[Table/Fig-6]: The intra-class correlation coefficient between (a) HPLC and ion exchange resin, (b) HPLC and nephelometry method.
HPLC: High-performance liquid chromatography

	Intra-class correlation*	F value	95% confidence interval		p-value
			Lower bound	Upper bound	
HPLC-Ion exchange resin	0.869	7.706	0.771	0.926	<0.001
Nephelometry-Ion exchange resin	0.756	4.062	0.570	0.862	<0.001
HPLC-Nephelometry	0.925	14.819	0.856	0.959	<0.001

[Table/Fig-7]: Intra-class correlation coefficient between nephelometry and ion exchange resin.

Test	HPLC	Nephelometry	Ion exchange resin
Sensitivity	100%	100%	97%
Specificity	95%	81%	80%
Positive predictive value	97%	89%	80%
Negative predictive value	100%	100%	97%
Accuracy	98%	93%	89%

[Table/Fig-8]: Sensitivity, specificity, positive and negative predictive values, and accuracy of three tests.

methods that are currently in practice. It shows the agreement between the HPLC, nephelometry, and IECR method in measuring HbA1c and was found to be good but there was variability amongst HbA1c values measured in the IECR method.

IECR correlated well with HPLC, with a 97% sensitivity and 80% specificity which probably reflects the decreased precision of the IECR method, although even in this case the relationship was highly significant which was similar to Hamwi A et al., study [27]. The best correlation with HPLC was found with nephelometry with a sensitivity of 100% and a specificity of 81%. The present study results were consistent with the Rukmini MS et al., [25], where the HPLC and IECR were compared for HbA1c quantification and showed sensitivity and specificity of 94% and 62.4% as in present study it was 97% and 80%, respectively [Table/Fig-8].

Amongst the subjects, the mean and standard deviation of the HbA1c value was found to be higher for group 1 when estimated by the IECR method. Whereas, the mean and standard deviation of HbA1c measured in groups 2 and 3 were higher when measured by HPLC. These findings were in accordance with a study done by Gautam N et al., [28]. This indicates that maximum patients were under the risk of developing complications related to diabetes mellitus and also reflect poor management of diabetic patients in this region because of ignorance, poverty, poor health education, unawareness about diabetes control programs, etc., [29,30].

The present study showed a better function and excellent agreement of nephelometry to HPLC compared with the IECR method. Nephelometry showed significant fair agreement with IECR (p<0.001). The findings were in accordance with the studies of Al-Lawati JA and Al-Lawati AM and Ankush RD et al., [31,32]. Good relationship and concordance between the different methods as indicated in other studies support the reliability of properly used different methods. Nephelometry shows excellent correlation with the reference HPLC method (r=0.925; p<0.001). IECR shows good correlation with reference HPLC (r=0.869; p<0.001). Eckerbom S et al., showed an even better correlation, when they analysed 131 patient samples analysed on the Bio-Rad Diamat system and with the Mono S column for HbA1c show that higher levels of HbA1c are achieved with the Diamat system [21]. These could be due to different chromatographic efficiency among HPLC and Diamat system.

Although HPLC is considered as "gold standard" for the measurement of HbA1c, it has several disadvantages such as cost, assay times and requirement of special laboratory skill [11]. Therefore, the alternate reliable method is needed which has a close strong correlation with HPLC. This quick method is helpful to obtain

the report during patients' visits without any delay and that gives better opportunities for clinical decision-making and changes in the treatment regimen [26].

Limitation(s)

Small sample size, variables confounding factors that affect HbA1c in patients with diabetes such as diabetic stage, residual insulin and age factor. In addition, sensitivity and specificity of each equipment may affects the results.

CONCLUSION(S)

Although HPLC is considered to be the gold standard method for the estimation of HbA1c, the present study shows an excellent correlation of nephelometry and IECR method with HPLC. Compare with, HPLC, both methods are fast, cheaper, reliable, and non-tedious methods compared to HPLC. It may be useful to make early clinical decision-making and changes in the treatment regimen thereby can reduce the huge economic burden of diabetes patients with lower socioeconomic status.

Acknowledgement

The authors are thankful to all the patients and volunteers who participated in this study.

REFERENCES

[1] Diagnosis and classification of diabetes mellitus. Diabetes care. 2013;36 Suppl 1:S67-74. Epub 2013/01/04.
[2] Little RR, Roberts WL. A review of variant haemoglobins interfering with haemoglobin A1c measurement. J Diabetes Sci Technol. 2009;3(3):446-51. Epub 2010/02/11.
[3] Kojic Damjanov S, Deric M, Eremic Kojic N. Glycated haemoglobin A1c as a modern biochemical marker of glucose regulation. Medicinski Pregled. 2014;67(9-10):339-44. Epub 2014/12/31.
[4] Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. Biomarker Insights. 2016;11:95-104. Epub 2016/07/12.
[5] Chehregosha H, Khamseh ME, Malek M, Hosseinpanah F, Ismail-Beigi F. A view beyond HbA1c: Role of continuous glucose monitoring. Diabetes therapy: Research, treatment and education of diabetes and related disorders. 2019;10(3):853-63. Epub 2019/05/01.
[6] Bacchi E, Negri C, Trombetta M, Zanolin ME, Lanza M, Bonora E, et al. Differences in the acute effects of aerobic and resistance exercise in subjects with type 2 diabetes: Results from the RAED2 randomized trial. PloS one. 2012;7(12):e49937. Epub 2012/12/12.
[7] Bajaj S. RSSDI clinical practice recommendations for the management of type 2 diabetes mellitus 2017. Int J Diabetes Dev C. 2018;38(Suppl 1):01-115. Epub 2018/03/13.
[8] Weykamp C. HbA1c: A review of analytical and clinical aspects. Annals of Laboratory Medicine. 2013;33(6):393-400. Epub 2013/11/10.
[9] Jia W. Standardising HbA1c-based diabetes diagnosis: Opportunities and challenges. Expert Review of Molecular Diagnostics. 2016;16(3):343-55. Epub 2015/12/19.
[10] Davis JE, McDonald JM, Jarett L. A high-performance liquid chromatography method for haemoglobin A1c. Diabetes. 1978;27(2):102-07. Epub 1978/02/01.
[11] Dam AR, Ahuja AJ, Singh M, Singh R. Limitation of HPLC methodology for HBA1c estimation. Indian J Pathol Microbiol. 2013;56(4):483-84. Epub 2014/01/21.
[12] Shetty R, Rai M, Chandrashekar R, Kalal BS. Diabetogenic effect of gluten in Wistar albino rats: A preliminary preclinical screening. Medicine and Pharmacy Reports. 2020;93(1):47-52. Epub 2020/03/07.
[13] Özçelik F, Yiğiner O, Serdar MA, Kurt I, Öztosun M, Arslan E, et al. Comparison of three methods for measurement of HbA1c. Türk Biyokimya Dergisi (Turkish Journal of Biochemistry-Turk J Biochem). 2010;35(4):344-49.
[14] Karami A, Baradaran A. Comparative evaluation of three different methods for HbA1c measurement with high-performance liquid chromatography in diabetic patients. Advanced Biomedical Research. 2014;3:94. Epub 2014/05/07.
[15] Razi F, Rahnamaye Farzami M, Ebrahimi SA, Nahid M, Bigdeli MG, Sheidaei A, et al. Comparative analytical performance of various HbA1c assays in Iran. Archives of Iranian Medicine. 2016;19(6):414-19. Epub 2016/06/14.
[16] Tesija Kuna A, Dukic K, Nikolac Gabaj N, Miler M, Vukasovic I, Langer S, et al. Comparison of enzymatic assay for HBA1C measurement (Abbott Architect) with capillary electrophoresis (Sebia Minicap Flex Piercing Analyser). Laboratory Medicine. 2018;49(3):231-38. Epub 2018/03/13.
[17] Barrot A, Dupuy AM, Badiou S, Bargnoux AS, Cristol JP. Evaluation of three turbidimetric assays for automated determination of haemoglobin A1c. Clinical Laboratory. 2012;58(11-12):1171-77. Epub 2013/01/08.
[18] Hoelke B, Gieringer S, Arlt M, Saal C. Comparison of nephelometric, UV-spectroscopic, and HPLC methods for high-throughput determination of aqueous drug solubility in microtiter plates. Analytical Chemistry. 2009;81(8):3165-72. Epub 2009/03/26.
[19] Ackerman E, Rosevear JW. Immunonephelometric assays. Medical Progress Through Technology. 1979;6(2):81-90. Epub 1979/01/30.
[20] Armbruster DA, Overcash DR, Reyes J. Clinical chemistry laboratory automation in the 21st Century-Amat Victoria curam (Victory loves careful preparation). The Clinical Biochemist Reviews. 2014;35(3):143-53. Epub 2014/10/23.
[21] Eckerbom S, Bergqvist Y, Jeppsson JO. Improved method for analysis of glycated haemoglobin by ion exchange chromatography. Annals of Clinical Biochemistry. 1994;31 (Pt 4):355-60. Epub 1994/07/01.
[22] Chandrashekar V. Hb A1c Separation by high performance liquid chromatography in haemoglobinopathies. Scientifica. 2016;2016:2698362. Epub 2016/03/19.
[23] Sun Q, Zheng W, Lin C, Shen D. A Low-cost micro-volume nephelometric system for quantitative immunoagglutination assays. Sensors (Basel). 2019;19(20). Epub 2019/10/12.
[24] Koivunen ME, Krogsrud RL. Principles of immunochemical techniques used in clinical laboratories. Laboratory medicine. 2006;37(8):490-97.
[25] Rukmini MS, Ashritha, Nishmitha P, Yalla D, Christy A, Manjrekar P. Analytical calibre of high performance liquid chromatography and ion exchange chromatography resin methods in estimation of glycated haemoglobin: A comparitive study. Biomed Res. 2017;28(4):1765-69.
[26] Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1C assay into estimated average glucose values. Diabetes Care. 2008;31(8):1473-78. Epub 2008/06/10.
[27] Hamwi A, Schweiger CR, Veitl M, Schmid R. Quantitative measurement of HbA1c by an immunoturbidimetric assay compared to a standard HPLC method. Am J Clin Pathol. 1995;104(1):89-95. Epub 1995/07/01.
[28] Gautam N, Dubey RK, Jayan A, Nepaune Y, Padmavathi P, Chaudhary S, et al. Comparative study of glycated haemoglobin by ion exchange chromatography and affinity binding nycocard reader in type 2 diabetes mellitus. Nepal Med Coll J: NMCJ. 2014;16(2-4):103-08. Epub 2014/12/01.
[29] Shah S, Kalal BS. Oxidative stress in cervical cancer and its response to chaemoradiation. Turk J Obstet Gynecol. 2019;16(2):124-28. Epub 2019/07/31.
[30] Nishana E, Bhat SS, Sahana KS, Hegde SK, Bhat V, Kalal BS. Estimation of salivary sCD14 in children with early childhood caries in association with pneumonia. Reports of Biochemistry & Molecular Biology. 2019;8(2):132-38. Epub 2019/12/14.
[31] Al-Lawati JA, Al-Lawati AM. The utility of fasting plasma glucose in predicting glycosylated haemoglobin in type 2 diabetes. Annals of Saudi Medicine. 2007;27(5):347-51. Epub 2007/10/09.
[32] Ankush RD, Suryakar AN, Ankush NR. Hypomagnesaemia in type-2 diabetes mellitus patients: A study on the status of oxidative and nitrosative stress. Indian J Clin Biochem: IJCB. 2009;24(2):184-89. Epub 2009/04/01.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Biochemistry, A. J. Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India.
2. Associate Professor, Department of Biochemistry, A. J. Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India.
3. Professor and Head, Department of Biochemistry, A. J. Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India.
4. Postgraduate, Department of Biochemistry, A. J. Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India.
5. Assistant Professor, Department of Biochemistry, A. J. Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India.
6. Assistant Professor, Department of Pharmacology, A. J. Research Centre, A. J. Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India.
7. Assistant Professor, Department of Biochemistry, A. J. Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Bhuvanesh Sukhlal Kalal,
Assistant Professor, Department of Pharmacology, A. J. Research Centre, A. J. Institute of Medical Sciences and Research Centre, Mangaluru-575004, Karnataka, India.
E-mail: bhuvanesh611@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Mar 23, 2020
- Manual Googling: Jun 02, 2020
- iThenticate Software: Aug 04, 2020 (20%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Mar 21, 2020**
Date of Peer Review: **Apr 28, 2020**
Date of Acceptance: **Jun 05, 2020**
Date of Publishing: **Sep 01, 2020**