CONTENTS

RESEARCH

The Morphological Features of Erythrocytes in Stored Packed Red Cells
(Gambaran Morfologi Eritrosit di Packed Red Cells Simpan)
Dewi Sri Kartini, Rachmawati Muhiddin, Mansyur Arif .................................................. 103–106

Correlation of Advanced Glycation End Products with Urinary Albumin Creatinin Ratio in Patients
(Kenasaban Kadar Advanced Glycation End Products dengan Rasio Air Kemih Albumin Kreatinin di
Pasien Diabetes Melitus Tipe 2)
Debie Anggraini, Rismaiwati Yaswir, Lillah2, Husni ........................................................................ 107–110

Monocyte Lymphocyte Ratio in Dengue Hemorrhagic Fever
(Monocyte Lymphocyte Ratio di Dengue Hemorrhagic Fever)
Dwi Retnoningrum, Purwanto AP .................................................................................................. 111–113

Correlation between NT-proBNP and Left Ventricular Ejection Fraction by Echocardiography in Heart
Failure Patients
(Kenasaban antara Kadar NT-proBNP dan Fraksi Ejeksi Ventrikel Kiri Secara Ekokardiografi di Pasien
Gagal Jantung)
Mutiara DS, Leonita Anniwati, M. Aminuddin .................................................................................. 114–118

Detection of Mycobacterium Tuberculosis with TB Antigen Rapid Test in Pulmonary Tuberculosis
Patients with Four Types of Sputum Sample Preparation
(Deteksi Antigen Mycobacterium Tuberculosis Menggunakan TB Antigen Uji Cepat di Pasien Tuberkulosis
Paru dengan 4 Cara Preparasi Dahak)
Miftahul Ilmiah, IGAA. Putri Sri Rejeki, Betty Agustina Tambunan .................................................. 119–125

Diagnostic Test of Hematology Parameter in Patients Suspect of Malaria
(Uji Diagnostik Tolok Ukur Hematologi di Pasien Terduga Malaria)
Ira Ferawati, Hanifah Maani, Zelly Dia Rofinda, Desywar .................................................................. 126–130

Comparison Results of Analytical Profile Index and Disc Diffusion Antimicrobial Susceptibility Test to
Technical Dedicated Reasonable 300B Method
(Perbandingan Hasil Analytical Profile Index dan Uji Kepekaan Antibiotika Difusi Cakram dengan
Metode Technical Dedicated Reasonable 300B)
IG Eka Sugiartha, Bambang Pujo Semedi, Puspa Wardhani, IGAA Putri Sri Rejeki .................................. 131–137

The Agreement between Light Criteria and Serum Ascites Albumin Gradient for Distinguishing
Transudate and Exudate
(Kesesuaian Patokan Light dengan Serum Ascites Albumin Gradient dalam Membedakan Transudat dan
Eksudat)
Rike Puspasari, Lillah, Efriida ........................................................................................................... 138–140

Correlation between Serum Tissue Polypeptide Specific Antigen Level and Prostate Volume in BPH
(Kenasaban antara Kadar Tissue Polypeptide Specific Antigen Serum dan Volume Prostat di BPH)
Mahrany Graciella Bumbungan, Endang Retnowati, Wahjoe Djatisoesanto ......................................... 141–145
Correlation of Antinuclear Antibody Profile with Hematologic and Renal Disorders in Systemic Lupus Erythematosus
*(Hubungan Antinuclear Antibody Profile dengan Gangguan Hematologi dan Ginjal di Systemic Lupus Erythematosus)*
Chelvi Wijaya, Asvin Nurulita, Uleng Bahrun

Identification of Dengue Virus Serotypes at the Dr. Soetomo Hospital Surabaya in 2016 and its Correlation with NS1 Antigen Detection
*(Identifikasi Serotipe Virus Dengue di RSUD Dr. Soetomo Surabaya Tahun 2016 serta Kenasabannya dengan Deteksi Antigen NS1)*
Jeine Stela Akualing, Aryati Puspa Wardhani, Usman Hadi

Correlation of Coagulation Status and Ankle Brachial Index in Diabetes Mellitus Patients with Peripheral Arterial Disease
*(Hubungan Status Koagulasi terhadap Nilai Ankle Brachial Index Pasien Penyakit Arteri Perifer dengan Diabetes Melitus)*
Lany Anggreani Hutagalung, Adi Koesema Aman, Syanti Syafril

The Difference of Plasma D-dimer Levels in Acute Myocardial Infarction with and without ST Elevation
*(Perbedaan Kadar D-dimer Plasma di Infark Miokard Akut dengan ST Elevasi dan Tanpa ST Elevasi)*
Desi Kharina Tri Murni, Adi Koesoema Aman, Andre Pasha Ketaren

Fructosamine and Glycated Albumin in Patients with Type 1 Diabetes Mellitus During Ramadhan Fasting
*(Fruktosamin dan Albumin Glikat di Pasien Diabetes Melitus Tipe 1 yang Menjalankan Puasa Ramadhan)*
Vinzy Yulina, Sidarti Soehita, Muhammad Faizi, Budiono

Diagnostic Test on the Fourth Generation Human Immunodeficiency Virus in HIV Suspects
*(Uji Diagnostik Human Immunodeficiency Virus Generasi Keempat di Terduga HIV)*
Sofitri, Ellyza Nasrul, Almurdy, Efrida

Correlation of Neutrophils/Lymphocytes Ratio and C-Reactive Protein in Sepsis Patients
*(Kenasaban antara Rasio Neutrofil/Limfosit dan C-Reactive Protein di asien Sepsis)*
Henny Elfira Yanti, Fery H Soedewo, Puspa Wardhani

Differences of Lymphocyte Proliferation Index After Culture Filtrate Protein 10 Stimulation in Patients with Active and Latent Tuberculosis and Healthy Individuals
*(Perbedaan Indeks Proliferasi Limfosit Pascastimulasi Culture Filtrate Protein 10 di Pasien Tuberkulosis Aktif, Laten dan Orang Sehat)*
Binar R. Utami, Betty Agustina T, Suprapto Ma’at

**LITERATURE REVIEW**
Glycated Hemoglobin A1c as a Biomarker Predictor for Diabetes Mellitus, Cardiovascular Disease and Inflammation
*(Glikasi Hemoglobin A1c sebagai Petanda Biologis Peramal Diabetes Melitus Penyakit Kardiovaskular dan Infamasi)*
Indranila KS

**CASE REPORT**
Erythroleukemia
*(Eritroleukemia)*
Ailinda Theodora Tedja, Riadi Wirawan

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Rismawati Yasirw, July Kumalawati, Mansyur Arif, Rahayuningsih Dharma, Nurhayana Sennang Andi Nanggung, AAG. Sudewa, Ninik Sukartini, Tahono, M. Yolanda Probohesodo
GLYCATED HEMOGLOBIN A1C AS A BIOMARKER PREDICTOR FOR DIABETES MELLITUS, CARDIOVASCULAR DISEASE AND INFLAMMATION

(Glikasi Hemoglobin A1c sebagai Petanda Biologis Peramal Diabetes Melitus Penyakit Kardiovaskular dan Inflamasi)

Indranila KS

INTRODUCTION

Glycated Hemoglobin (HbA1c) was discovered in the late 1960s, as a biomarker of glycemic control it has gradually increased over the last four decades.¹ Glycated hemoglobin is used for monitoring of glucose control in diabetic patients and was proposed used in routine clinical laboratories around 1977.² Glycated haemoglobin (HbA1c) identifies the average of plasma glucose concentration and commonly used in the relation to diabetes.³ HbA1c is formed when haemoglobin joined with glucose in the blood, this explains why HbA1c is differs from blood glucose levels for diagnosing diabetes.⁴ Traditionally, HbA1c has been thought to represent average blood sugar levels for over a period of time. It is limited to an average lifespan of a red blood cell over the entire 120- days.⁵ RBCs do not undergo lysis at the same time, so HbA1C is taken as a
limited measure of 3 months. The higher the HbA1c level in patients, the greater the risk of developing diabetes-related complications. Measuring HbA1c can detect and predict the average level of glucose and the severity of CVD and inflammation.

DISCUSSION

HbA1c and DM

Glycated hemoglobin is a long term control of glycaemia. HbA1c and glucose are complementary information and both are used to assess and individual’s glycemic status. Red blood cells in the human body survive for 120 days before renewal and HbA1c will reflect the average blood glucose levels over that duration. A prospective multinational study documented a linear relationship between HbA1c and mean blood glucose. HbA1c target for people with diabetes to aim for 48 mmol/mol (6.5%) (see Table 1).

<table>
<thead>
<tr>
<th>HbA1c</th>
<th>%</th>
<th>mmol/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Below 6.0%</td>
<td>Below 42 mmol/mol</td>
</tr>
<tr>
<td>Prediabetes</td>
<td>6.0% to 6.4%</td>
<td>42 to 47 mmol/mol</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6.5% or over</td>
<td>48 mmol/mol or over</td>
</tr>
</tbody>
</table>

Glycated hemoglobin (HbA1c) is a long-term glycaemic control, lowering of HbA1c, by improving HbA1c by 1% (or 11 mmol/mol) for people with type 1 diabetes or type 2 diabetes, cuts the risk of microvascular complications Retinopathy; Neuropathy; Diabetic nephropathy by 25% and lowering the risk of macrovascular complication. Research has also shown that people with type 2 diabetes who reduce their HbA1c level by 1% are: 19% less likely to suffer cataracts, 16% less likely to suffer heart failure, 43% less likely to suffer amputation or death due to peripheral vascular disease. HbA1c is commonly used as a gold standard index of glycemic control in the clinical setting, it is recommended to bring HbA1c lower than 7.0% is order to prevent the development and progression of chronic diabetic complications. HbA1c examination has been received to assess the results of the treatment and be able to assess disease control DM so as to prevent microvascular and macrovascular diabetes complications. Higher amounts of glycated hemoglobin, indicate poorer control of blood glucose levels and have been associated with cardiovascular disease, nephropathy, neuropathy, retinopathy and inflammation.

HbA1c and CVD

Diabetes is associated with increased in risk of cardiovascular disease. Several studies have demonstrated a positive relationship between elevated HbA1c and outcome in the Acute Coronary Syndrome (ACS), Acute Myocardial Infarction (AMI), heart failure, pancreatitis and even patients after coronary artery bypass surgery and Drug-Eluting Stent (DES) implantation with and without primary DM.

Although consistent evidence have supported that optimal control of HbA1c at a target value, can confer to a lower incidence of microvascular complications, and the associations of high levels of HbA1c with macrovascular complications such as stable coronary disease remains controversial. Previous studies have demonstrated a positive correlation of high HbA1c levels with severity of Coronary Artery Disease (CAD). Others associated glycated hemoglobin as an intermediate glycaemic marker, a potent atherogenic protein and the risk of cardiovascular disease. Intensive glycemic control has been shown to reduce the development of CVD as well as diabetic microangiopathy during long-term follow up in patients with both type1 and type 2 diabetes.

Recent clinical trials aimed at reducing HbA1c levels in patients with DM type 2 have failed to show an additional benefit on CVD outcome. Reducing HbA1c below the normal 6% would reduce the rate of cardiovascular events in Type 2 Diabetes. HbA1c is used as a reliable tool for not only diagnosing DM but also identifying individuals at high risk of cardiovascular events with and without DM. Cavallet al. indicate that CVD was associated with increased age, longer diabetes duration, higher HbA1c and fibrinogen in men.

Hong et al. clearly suggest that high level of plasma HbA1c (>6.5%) is an independent predictor for presence and severity of CAD as well as the early outcome of patients with stable angina. Another trial by ACCORD determined that reducing HbA1c below 6.0% would reduce the rate of cardiovascular events. Persistent elevations in blood sugar and HbA1c increase the risk of long-term vascular complications of diabetes such as coronary disease, heart attack, stroke, heart failure, kidney failure, blindness, erectile dysfunction, neuropathy, gangrene and poor wound healing.

Impaired lipid metabolism results from uncontrolled hyperglycemia has been implicated in cardiovascular complications, HbA1c is an useful biomarker for
identifying lipid profile, glycaemic control and risk of cardiovascular complication.\textsuperscript{31}

**HbA1c AND INFLAMMATION**

Inflammation has been suggested to play a central role in developing atherosclerosis. Diabetes is known as a risk factor for atherosclerosis, also associated with increased level of sensitive markers of subclinical systemic inflammation.\textsuperscript{32}

Wu et al.\textsuperscript{33}, shows elevated C-Reactive Protein (CRP) were associated with higher level of circulating HbA1c. Higher level of HbA1c was related to elevated inflammatory markers such as C-reactive protein (CRP), fibrinogen and white blood cell count, which were routinely available and well established predictors of future mortality. Therefore, it might provide meaningfully predictive value than either alone used.\textsuperscript{33}

Hyperlipidemia was considered to be present in patients with fasting total cholesterol (TC) ≥200 mg/dL or triglyceride (TG) ≥150 mg/dL. The underlying hypothesis of the current results might consist that the high levels of HbA1c were not only associated with long-term disorder of glycolipid metabolism but also connected with low-grade systematic inflammation and atherosclerotic plaques progress.\textsuperscript{34}

Association between HbA1c and fibrinogen level were also found in patients with non-insulin dependent diabetes and between HbA1c and white blood cell count.\textsuperscript{35} Diabetic patients have increased level of markers of inflammation and have a relationship between this inflammation markers and HbA1c. HbA1c within the normal range indicate an early association between degree of glycemia, inflammation and atherosclerosis.\textsuperscript{36} Some studies have demonstrated that when the glycated hemoglobin (HbA1c) is <8.0%, the proliferative function of CD4 T lymphocytes and their response to antigen is not impaired.\textsuperscript{37}

**HbA1c IN MOLECULAR ASPECT**

Glycation of immunoglobulin occurs in patients with diabetes in proportion with the increase in HbA1c and this may harm the biological function of the antibodies. However, the clinical relevance of these observations are not clear, since the response of antibodies after vaccination and to common infections are adequate in patients with DM.\textsuperscript{38}

Some studies have detected a deficiency of the C4 component in DM, reduction of C4 is probably associated with polymorphonuclear dysfunction and reduced cytokine responses.\textsuperscript{39} Mononuclear cells and monocytes of diabetic people secrete less interleukin-1 (IL-1) and IL-6 in response to stimulation by lipopopysaccharides.\textsuperscript{40} Others studies reported that the increase of glycation could inhibit the production of IL-10 by myeloid cells, as well as that of interferon gamma (IFN-\(\gamma\)) and Tumor Necrosis Factor (TNF-\(\alpha\)) by T cells. Glycation would also reduce the expression of class I Major Histocompatibility Complex (MHC) on the surface of myeloid cells, impairing cell immunity.\textsuperscript{41}

Hyperglycemia also decreased mobilization of polymorphonuclear leukocytes, chemotaxis, and phagocytic activity, block the antimicrobial function by inhibiting glucose-6-phosphate dehydrogenase (G6PD), increasing apoptosis of polymorphonuclear leukocytes and reducing polymorphonuclear leukocyte transmigration through the endothelium.\textsuperscript{42}

**INTERPRETATION OF RESULTS**

Glycated hemoglobin (HbA1c) is formed by a nonenzymatic reaction occurring between glucose and the N-end of the beta chain. This forms a Schiff base which is converted to 1-deoxyfructose. This rearrangement is an example of an Amadori rearrangement.\textsuperscript{43} When blood glucose levels are high, glucose molecules attach to the hemoglobin in red blood cells, the longer hyperglycemia occur in blood, the more glucose binds to hemoglobin in the red blood cells and the higher the glycated hemoglobin.\textsuperscript{44}

Glycated hemoglobin within the red cell, reflects the average level of glucose to which the cell has been exposed during its life-cycle. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term plasma glucose regulation.\textsuperscript{45}

Laboratory results may differ depending on the analytical technique, the age of the subject, and biological variation among individuals. Two individuals with the same average blood sugar can have HbA1c values that differ by as much as 3 percentage points.\textsuperscript{46}

Results can be unreliable in many circumstances as for example after blood loss, after surgery, blood transfusions, anemia, high erythrocyte turnover, chronic renal or liver disease, after administration of high-dose vitamin C, and erythropoetin treatment.\textsuperscript{47}

A review of the UKPDS, Action to Control Cardiovascular Risk in Diabetes (ACCORD), ADVANCE and Veterans Affairs Diabetes Trials (VADT) estimated that the risks of the main complications of diabetes (diabetic retinopathy, diabetic nephropathy, diabetic neuropathy and macrovascular disease) decreased by approximately 3% for every 1 mmol/mol decrease in HbA1c.\textsuperscript{48}
REFERENCE RANGE

In healthy young people is about 30–33 mmol/mol (4.9–5.2 DCCT%). Higher levels of HbA1c are found in people with persistently elevated blood sugar, as in DM.[1] The 2010 American Diabetes Association Standards of Medical Care in Diabetes added the HbA1c ≥48 mmol/mol (≥6.5 DCCT%) as another criteria for the diagnosis of diabetes. The International Diabetes Federation and the American College of Endocrinology recommend HbA1c values below 48 mmol/mol (6.5 DCCT%), while the American Diabetes Association recommends HbA1c below 53 mmol/mol (7.0 DCCT%) for most patients. Lower-than-expected levels of HbA1c can be seen in people with shortened red blood cell lifespan, such as with glucose-6-phosphate dehydrogenase deficiency, sickle-cell disease, or any other condition causing premature red blood cell death. Blood donation will result in rapid replacement of lost RBCs with newly formed red blood cells. Since these new RBCs have only existed for a short period of time, their presence will lead HbA1c to underestimate the actual average levels.

There may also be distortions resulting from blood donation which occur as long as two months before due to an abnormal synchronization of the age of the RBCs, resulting in an older than normal average blood cell life (resulting in an overestimate of actual average blood glucose levels). Conversely, higher-than-expected levels can be seen in people with a longer red blood cell lifespan, such as with Vitamin B12 or folate deficiency.

INDICATIONS

Glycated hemoglobin testing is recommended for both checking the blood sugar control in people who might be pre-diabetic and monitoring blood sugar control in patients with more elevated levels, termed diabetes mellitus. A significant proportion of people who are unaware of their elevated HbA1c level before they have blood lab work. For a single blood sample, it provides far more revealing information on glycemic behavior than a fasting blood sugar value. However, fasting blood sugar tests are crucial in making treatment decisions. The American Diabetes Association guidelines are similar to others in advising that the glycated hemoglobin test be performed at least twice a year in patients with diabetes who are meeting treatment goals (and who have stable glycemic control) and quarterly in patients with diabetes whose therapy has changed or who are not meeting glycemic goals.

Glycated hemoglobin measurement is not appropriate where there has been a change in diet or treatment within 6 weeks. Likewise, the test assumes a normal red blood cell aging process and mix of hemoglobin subtypes (predominantly HbA in normal adults). People with recent blood loss, hemolytic anemia, or genetic differences in the hemoglobin molecule (hemoglobinopathy) such as sickle-cell disease and other conditions, as well as those that have donated blood recently, are not suitable for this test.

Due to glycated hemoglobin measurement is not appropriate where there has been a change in diet or treatment within 6 weeks. The test assumes a normal red blood cell aging process and mix hemoglobin subtype. People with recent blood loss, hemolytic anemia, or genetic differences in the hemoglobin molecule (hemoglobinopathy) such as sickle-cell disease, as well as those who donated blood recently, are not suitable for this test.

Concentrations of hemoglobin A1 (HbA1) are increased, both in diabetic patients and in patients with renal failure, when measured by ion-exchange chromatography. The thiobarbituric acid method (a chemical method specific for the detection of glycation) shows that patients with renal failure have values of glycated hemoglobin similar to those observed in normal subjects, suggesting that the high values in these patients are a result of binding of something other than glucose to hemoglobin. In autoimmune hemolytic anemia, concentrations of hemoglobin A1 (HbA1) are undetectable. Administration of prednisolone (PSL) will allow the HbA1 to be detected.

CONCLUSION

Glycated Hemoglobin A1c is now standardized & traceable to IFCC methods HPLC-CE and HPLC-MS. A new unit (mmol/mol) is used as part of this standardization. The standardized test does not however test for iodine levels in the blood. Hypothyroidism or Iodine supplementation are known sources that artificially raise the A1c number. The Committee Report further states that, when HbA1c testing cannot be done, the fasting and glucose tolerance tests be done. Diagnosis of diabetes during
pregnancy continues to require fasting and glucose tolerance measurements for gestational diabetes and not the glycated hemoglobin. That HbA1c in several studies have demonstrated a positive relationship between elevated HbA1c and poor outcome in CVD, DM, inflammation and others. The purpose of writing shows that HbA1c is as a biomarker of predicting DM, CVD and inflammation.

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