Can glycated albumin assist in management of diabetes mellitus?

Jasna Lenicek Krleza, PhD.
Children’s Hospital Zagreb, Croatia
WHEN WE PREPARED PROGRAMME of THIS COURSE IDEAS?...for topic of my lecture?

My practice:
Patients with Confirmed diagnosis of diabetes and patients with no history of diabetes but with onset high glucose concentration in blood

I was READING HANDBOOK FROM 1ST COURSE
What can I talking about? We all know that glycated hemoglobin (HbA1c) is the recommended and most often used biomarker for assessing hyperglycemia in the practice, but.....

My attention was caught the small paragraph about glycation proteins which isn’t HbA1c....
And,
I decided to read out the recently published articles and present new insights into the glycated albumin and their role in management of diabetes mellitus today.

Lecture have aim to give the answer:

✘ Can glycated albumin assist in management of diabetes mellitus?

Goals of presentation: W, W and W

- **What** is Glycated Albumin (GA)?
- **Why** we need another biomarker?
  - or
  - **When** (in which situation) we can use it?
- **Which** methods can we used for determined GA today?
Reminder

Glycation,
- a non-enzymatic Maillard reaction,
- occurs when glucose molecules spontaneously react with the amine group of proteins, giving rise to stable ketoamines.

Table: several important proteins in human that can become extensively glycated under hyperglycemic conditions

Modified proteins cause chronic diseases as diabetes complications.

---

<table>
<thead>
<tr>
<th>Matrix proteins</th>
<th>Enzymes</th>
<th>Plasma proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>Cathepsin B</td>
<td>Albumin</td>
</tr>
<tr>
<td>Myelin</td>
<td>Lysozyme</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Pancreatic ribose</td>
<td>Apo A-I, II</td>
</tr>
<tr>
<td>Fibrin</td>
<td>Copper/zinc SOD</td>
<td>Apo B</td>
</tr>
<tr>
<td></td>
<td>Carbonate dehydratase</td>
<td>Apo C-I</td>
</tr>
<tr>
<td></td>
<td>β-N-acetyl hexosaminase</td>
<td>Apo E</td>
</tr>
<tr>
<td></td>
<td>Alcohol dehydrogenase</td>
<td>Haptoglobin</td>
</tr>
<tr>
<td></td>
<td>Aldose reductase</td>
<td>Ferritin</td>
</tr>
<tr>
<td></td>
<td>Aldehyde reductase</td>
<td>Transferrin</td>
</tr>
<tr>
<td></td>
<td>Sorbitol dehydrogenase</td>
<td>α1-antitrypsin</td>
</tr>
<tr>
<td></td>
<td>Na+/K+ ATPase</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Membrane proteins</th>
<th>Enzymes</th>
<th>Plasma proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell Glu transport protein</td>
<td>β-N-acetyl hexosaminase</td>
<td>Apo E</td>
</tr>
<tr>
<td>Red cell spectrin</td>
<td>Alcohol dehydrogenase</td>
<td>Haptoglobin</td>
</tr>
<tr>
<td>Red cell membrane protein</td>
<td>Aldose reductase</td>
<td>Ferritin</td>
</tr>
<tr>
<td>Endothelial plasma membrane protein</td>
<td>Aldehyde reductase</td>
<td>Transferrin</td>
</tr>
<tr>
<td></td>
<td>Sorbitol dehydrogenase</td>
<td>α1-antitrypsin</td>
</tr>
<tr>
<td></td>
<td>Na+/K+ ATPase</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intracellular proteins</th>
<th>Enzymes</th>
<th>Plasma proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>Thyroid hormone</td>
<td></td>
</tr>
<tr>
<td>Crystallin</td>
<td>Insulin</td>
<td></td>
</tr>
<tr>
<td>Tubulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calmodulin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Some of these glycated proteins can be used as biomarkers to determine the degree of glycemia in individuals with diabetes or prediabetes.

Today, HbA1c is the "gold standard" for assessing glycemia during diabetes management, and since 2009 it has been recommended by both the ADA and WHO as a diagnostic criterion for diabetes, with a diagnostic cut-off of >6.5% (48 mmol/mol).

In some patients, levels of HbA1c are inadequate for determining the average glucose concentration.
Lack of linear correlation between HbA1c levels and average glucose concentration arises because numerous factors affect HbA1c levels, including genetics, hematological factors and the presence of certain comorbidities, such as hemoglobinopathy, certain anemias, and disorders associated with shorter erythrocyte lifespan.
<table>
<thead>
<tr>
<th>Process</th>
<th>Factors</th>
<th>Effect on HbA1c level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythropoiesis</td>
<td>iron, vitamin B12 deficiency, decreased erythropoiesis</td>
<td>increase</td>
</tr>
<tr>
<td></td>
<td>erythropoietin administration, iron, vitamin B12, reticulocytosis, chronic liver disease</td>
<td>decrease</td>
</tr>
<tr>
<td>Hemoglobin modification</td>
<td>Genetic or chemical modifications of hemoglobin (hemoglobinopathies, HbF, methemoglobin)</td>
<td>increase or decrease</td>
</tr>
<tr>
<td></td>
<td>alcoholism, chronic renal failure, decreased intra-erythrocyte pH</td>
<td>increase</td>
</tr>
<tr>
<td>Glycation</td>
<td>aspirin, vitamins C and E, certain hemoglobinopathies, increased intra-erythrocyte pH</td>
<td>decrease</td>
</tr>
<tr>
<td></td>
<td>Genetic determinants</td>
<td>increase or decrease</td>
</tr>
<tr>
<td>Erythrocyte destruction</td>
<td>increased erythrocyte life span, e.g. due to splenectomy</td>
<td>increase</td>
</tr>
<tr>
<td></td>
<td>decreased erythrocyte life span, e.g. due to hemoglobinopathies, splenomegaly, rheumatoid arthritis or drugs such as antiretrovirals, ribavirin and dapsone.</td>
<td>decrease</td>
</tr>
<tr>
<td>Assays</td>
<td>hyperbilirubinemia, carbamylated hemoglobin, alcoholism, high-dose aspirin, chronic opiate use</td>
<td>increase</td>
</tr>
<tr>
<td></td>
<td>hemoglobinopathies</td>
<td>increase or decrease</td>
</tr>
<tr>
<td></td>
<td>hypertriglyceridemia</td>
<td>decrease</td>
</tr>
</tbody>
</table>

*Adapted from WHO. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus: abbreviated report of a WHO consultation.*
1. What is glycated albumin (GA)?

- The development of new biomarkers of hyperglycemia for cases when HbA1c levels are inadequate has been the subject of intense investigation over the last 5 years.

- One candidate biomarker is albumin, which accounts for approximately 60% of serum proteins and is present in the blood at concentrations of 30-50 g/L.

- This protein is predicted to be highly susceptible to glycation because it contains numerous arginine and lysine residues near its N- and C-termini.

- It persists for 2-3 weeks once released into the circulation, making it potentially well-suited to be a biomarker that can detect short and mid-term changes.
1. What is glicated albumin (GA)?

**Albumin (HSA)**
- Most abundant protein in blood
- Produced in the liver
- 20 day lifespan
- Monomeric, 585 amino acids

**Domain:**

**Dimensions:**

**Net charge:**

**Glycation sites:**

Lys-199, Lys-281, Lys-439, Lys-525
Two reason/situation:

1. Numerous factors affect HbA1c levels

2. Life spine HbA1c in situation when frequent monitoring is required
Diabetes management, especially in early phases, requires **frequent monitoring** because significant changes can occur within 2-3 months.

Important for:

- individuals on therapy to **treat prediabetes**
- patients undergoing **new therapy** or a change in their current therapy
- individuals on **intensive insulin therapy** during early stages of diabetes
- **pregnant** women
- patients on **hemodialysis**.
In order for glycated albumin to be measured routinely in the clinic, the American Diabetes Association in 2011 called for studies to develop:

- a standardized method for its measurement
- clearly establish its clinical usefulness
- reliability for predicting diabetes-related complications

Since then, numerous studies have tried to follow these recommendations and determine:

Is the level of glycated albumin can be useful in managing diabetes?
Time differential of diabetic monitoring using blood glucose, glycated albumin and HbA1c
Recommended methods for determining glycated albumin are:

- affinity chromatography,
- ion-exchange chromatography and high-performance liquid chromatography (HPLC).

Recent research suggests that liquid chromatography-tandem mass spectrometry (LC-MS/MS) may be the "gold standard" method for quantitative determination of glycated proteins, including albumin and all serum proteins, of which are collectively known as fructosamine.

....all these techniques are complicated and require sophisticated equipment which is not available for many clinical laboratories.
Colorimetric determination of glycated albumin

A much simpler and less expensive alternative is a colorimetric method for fructosamine determination known as the nitroblue-tetrazolium (NBT) reduction method. The method was automated soon after it was first described.

NBT reacts with various endogenous reducing substances, including thiol groups, ascorbate, and NADH—the levels of all of which can vary from sample to sample.

Interfering substances and sources of error

1. EDTA and heparin plasma—lower fructosamine results than serum samples in the NBT colorimetric assay, so the same type of sample should always be used to monitor glycemia.
2. Urate, glutathione and vitamin C lead to artificially high fructosamine results.
3. Cysteine, methyldopa, dobesilate calcium, oxytetracycline and hemolysis can cause artificially low fructosamine results (all assays).
4. Bilirubin has been shown to cause falsely elevated fructosamine results.
5. The NBT assay, like other colorimetric assays, is affected by changes in ambient temperature.
Enzymatic determination of glycated albumin

Recently a quite precise and automated enzymatic assay for determination of glycated albumin has been commercialized by Diazyme Laboratories, Asahi Kasei Pharma, and Randox Laboratories.

Principle of enzymatic assay

Proteinase K digests serum proteins into low-molecular-weight glycated protein fragments (GPF), then a specific fructosaminase™ (microbial amadoriase) catalyzes the oxidative degradation of GPF Amadori product to yield a protein fragment (PF) or amino acids and H₂O₂. The H₂O₂ released is measured by a colorimetric Trinder end-point reaction. The absorbance at 546 nm is proportional to the concentration of glycated serum proteins (GSP) or glycated albumin performed with serum or plasma on virtually all biochemical analyzers.

Multiple determinations do not require multiple blood samples or a total blood sample, as is required for HbA1c determination.

Stability tests indicate that samples for the enzymatic assay can be stored for up to 2 weeks at 2-8 ºC or up to 4 weeks frozen.
all enzymatic assay
good analytical characteristics and correlate well
with one another, as well as with HPLC-based methods.

The tests differ principally in what enzyme is used and how results are expressed: concentration (umol/L or mmol/L) or as glycated albumin fraction (%GA). Determination of %GA also involves determination of total albumin.
While the Lucica GA-L kit determines %GA, the Diazyme GlycoGap kit determines the concentration of glycated albumin in umol/L, and the Randox kit determines the concentration in mmol/L.

The Lucica GA-L kit determines albumin using a bromcresol purple (BCP) method that is more specific than the bromocresol green (BCG) method most often used to determine albumin in clinical laboratories. Each assay manufacturer provides reference intervals for glycated albumin for diabetics and non-diabetics in the appropriate concentration units or %GA.

These enzymatic tests show extremely good reproducibility and specificity, correlating closely with glycated albumin levels determined by HPLC ($r > 0.98$). Based on the performance of these enzymatic assays, which according to the manufacturers is evaluated in compliance with guideline EP5-A (CLSI),

the automated test shows the characteristics of a reference method, although it is not yet confirmed from relevant institutions.
Interference studies

the manufacturer's test insert

1. EDTA plasma samples have been internally validated to show no matrix effects in enzymatic assays; serum should be separated from cells immediately after blood collection.

2. As in the NBT colorimetric assay, cysteine, methyldopa, dobesilate calcium, oxytetracycline and hemolysis can cause artificially low GA/GSP results.

3. As in the NBT colorimetric assay, bilirubin has been shown to cause falsely elevated GA/GSP results.

Several common interfering substances in serum, such as ascorbic acid, glucose, triglyceride, uric acid and hemoglobin, usually show ≤ 10% interference, though the manufacturer's insert for the particular test should be consulted.
Variability of glycated albumin and limitations as a biomarker of glycemia

Disorders in albumin metabolism

- nephrotic syndrome
- Hyperthyroidism
- glucocorticoid therapy

involve elevated albumin metabolism.

lower ratios of glycated albumin to blood glucose

liver cirrhosis and hyperthyroidism

involve reduced albumin metabolism.

higher ratios of glycated albumin to blood glucose
Lower ratios of glycated albumin to glucose are also observed in obese people, smokers and in patients with hyperuricemia, hypertriglyceridemia, or alcohol-induced fatty liver disease associated with elevated levels of alanine aminotransferase.

Albumin metabolism changes rapidly in children, and the levels of both albumin, fetal Hb and glucose in infants increase rapidly with age. While these effects limit the reliability of glycated albumin as a biomarker of glycemia, they are still less severe than the significant influence of changes in fetal Hb levels on HbA1c levels.

As a result, glycated albumin, can use as an indicator of glycemic control in newborns with diabetes.
Biological variability of glycated albumin

Determination of glycated albumin in serum and plasma has become much easier since the development of automated enzymatic tests with optimal analytical performance.

<table>
<thead>
<tr>
<th></th>
<th>Enzymatic assay GA</th>
<th>Albumin</th>
<th>Fructosamine</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical CV</strong></td>
<td>1.7%</td>
<td></td>
<td>2.8%</td>
<td>2.4%</td>
</tr>
<tr>
<td><strong>Within-subject CV (CVW)</strong></td>
<td>2.1%</td>
<td>2.3%</td>
<td>2.3%</td>
<td></td>
</tr>
<tr>
<td><strong>Between-subject CV (CVG)</strong></td>
<td>10.6%</td>
<td>2.9%</td>
<td>6.3%</td>
<td></td>
</tr>
<tr>
<td>the Westgard biodatabase</td>
<td>10.3%</td>
<td>4.2%</td>
<td>5.9%</td>
<td></td>
</tr>
<tr>
<td><strong>Critical difference (CD)</strong></td>
<td>7.5%</td>
<td>9%</td>
<td>10%</td>
<td></td>
</tr>
</tbody>
</table>


These comparisons indicate a high degree of individuality.

Some authors have suggested: significant CV between-subject variation of glycated albumin levels, the critical difference (CD) should be used instead of target values for monitoring glycemia.
While the causes of this large variation are not completely clear, variation in the erythrocyte lifespan, especially in diabetics as is variation in albumin half-life due to glycation, the authors of that study strongly recommended monitoring diabetes using a combination of two or more glycemia biomarkers, in order to obtain more reliable information about glycemic state.
Although far less published results of studies on the evidence of pathological mechanisms and clinical utility of GA compared to HbA1c (approximately 32 times less),

there is growing evidence to show that the GA is a useful marker in their management of diabetes.
Finally: thanks to our first results

BIOMAX
Biomax d.o.o, Perjavička putina 5, 10090 Zagreb
Tel: +385 (0)1 34 70 173 Fax: +385 (0)1 34 70 195
email: info@biomax.hr
http://www.biomax.hr

Marko Maric, mag. pharm.
Biomax d.o.o.

DIABETES BIOMARKERS
UNIQUE MARKERS FOR IMPROVED
DIABETES MONITORING AND DIAGNOSIS

GSP/GLYCATED ALBUMIN
- Reliable short-term marker of glycemic control
- Reflects the average blood sugar of the previous two weeks
- New enzymatic method is more accurate and reliable than conventional fructosamine assays
- Rapid evaluation of effectiveness of diet, activity or medication adjustments

HbA1c
- Gold Standard for measurement of glycemic control
- The HbA1c test measures mean glycemic control
- Diazyme's IFCC certified enzymatic method offers significant advantages over other assays
- No interference from major hemoglobin variants including HbS, HbC, HbE, Carbamylated Hb, or Labile HbA1c
- Single channel assay (no need for a separate Hb channel)
- Outstanding precision and accuracy

Diazyme Laboratories
Glycated Serum Protein Assay

Diazyme Laboratories
INNOVATIONS IN CLINICAL DIAGNOSTICS
Mini project: Comparsion of Glycated Albumin and HbA1c in Type 2 Diabetic Patients on Hemodialysis.

In process
Thank you for your attention