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CORRELATIVE STUDY ON FRUCTOSAMINE AND HbA_{1c} IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Glycated hemoglobin(HbA_{1c}), Fructosamine, total serum protein, albumin and hemoglobin are measured in 25 freshly diagnosed diabetic patients and 25 diabetics who are under treatment HbA_{1c} below 8%. The normal range of the HbA_{1c} is 4.7-6.5%, and Fructosamine is 285µmol/L. The results shows the level of Fructosamine and HbA_{1c} are significantly correlate with each others. The HbA_{1c} and Fructosamine are correlate at p value of <0.001 in both the cases with r = 0.639 and 0.616 for freshly diagnosed and controlled diabetics respectively. It is suggested that while the Fructosamine is not a direct substitute HbA_{1c} but it is an alternative method to determine the deterioration of the complications, it act as an intermediate measurement.

Key Words: *Diabetic Complication, Fructosamine, Glycated Heamoglobin, Ketoacidosis*

INTRODUCTION

Diabetes mellitus affects around 285 million people all around the world and is a cause of mortality and morbidity. It is characterized by hyperglycemia due to absolute or relative deficiency of insulin. Fortunately early detection and improvement therapeutic regimens allow diabetic patients to lead normal lives, and life expectancy figures have increased. Clinical chemistry plays an integral role in diagnosis, monitoring and treatment of diabetes. Glycemic control, utilizing serial measurement of glycosylated hemoglobin (HbA_{1c}), is generally recommended to limit end organ damage including cardiovascular morbidity and mortality. Fructosamine has also been used for monitoring the response to treatment and prediction of development of complications. HbA_{1c} concentration represents glucose levels over 8-12 weeks. Hemoglobin glycation occurs when glucose gets attached to the one or both N- terminal valine of beta chain to form a Schiff base. The concentration of HbA_{1c} depends upon several factors. The lifespan of the RBCs and how long HbA_{1c} is exposed to the glucose is a major determining factor. The permeability of RBC to glucose also influences the amount of glycation and explains the discordance noted in some hematological normal people with diabetes in whom HbA_{1c} appears discordant from other measures of glycaemic control. Fructosamine is a stable ketoamine formed by the glycation of serum protein through a non enzymatic mechanism involving a labile Schiff base intermediate and the Amadori rearrangement. The amount of fructosamine is increased in diabetes thus it reflects the degree of glycemic control and is useful in monitoring the effectiveness of therapy in diabetes over a period of 2-3 weeks (David, 1987). As the measurement of HbA_{1c} depends on total Hb and adequate erythropoiesis, HbA_{1c} may not be a reliable measure of glycaemic control in anemic and CRF patients and researchers have therefore explored the use of serum Fructosamine as an alternative method.

As yet the Fructosamine assay is not widely or routinely used. Hence in this review I describe protein glycation and compare and correlate it with HbA_{1c} that have been applied to assay and examine the clinical usefulness of the test.

Review of Literature

Diabetes Mellitus

Diabetes mellitus is a group of metabolic disorder in which a person has high sugar, either because the pancreas does not produce enough insulin or because cell does not respond to the insulin that is produced This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydypsia (increase thirst), and polyphagia (increase hunger).

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Diabetes was first disease described with an Egyptian manuscript from 1500BCE mentioning “too much emptying of urine”. At the same time Indian physician also identified the disease and classified it as “*Madhumeha*” or honey urine noting the urine attract ant. Type 1 and type 2 diabetes were identified as separate condition the first time by Indian physicians Sushruta and Charaka in 400- 500 BC, with Type1 associated with young and Type 2 with being overweight (Shoback *et al.*, 2011).

The effective treatment was not developed until the early part of the 20th century, when Canadian Federick Banting and Charles Herbert Best isolated and purified insulin in 1921 and 1922. This is followed by the development of long acting insulin in 1940.

Classification

The Classification of DM is as per WHO-1985 (Lconid, 2009)

A. Clinical Class

- Insulin dependent diabetes mellitus(IDDM)
- Non – Insulin dependent diabetes mellitus(NIDDM)
 - a) Obese
 - b) Non obese
 - c) Associate with certain condition and symptoms – gestational diabetes mellitus(GDM), malnutritional related diabetes mellitus.

The other type of diabetes mellitus associated with

- a) Pancreatic diseases
- b) Disease of hormonal etiology
- c) Drug induced or chemical induced condition
- d) Abnormality of insulin or its receptors

Epidemiology

The prevalence of diabetes is rapidly rising all over the world. Globally, an estimated 285 million peoples had diabetes, with type2 making up about 90% of the cases as of 2010 (WHO Study Group, 1985). Its incidence is increasing rapidly and by 2030, this number is estimated to almost double (William).

India has more diabetic than other country in the world, according to the International Diabetes Foundation, but the recent data shows that China has even more diabetic. The diabetes effect the 50 million Indians – 7.1% of the nation’s adult and kill about 1 million Indian per annum (Wilds *et al.*, 2004).

Several community - based interventional studies have shown that early diagnosis and treatment not only delay the onset of complications (Pan *et al.*, 1997), but also more importantly, indicate that the onset of diabetes can prevented or delayed by lifestyle interventional among those identified to be risk for developing diabetes (Knowler *et al.*, 2002; William *et al.*, 2003).

Diagnostic Criteria for Diabetes Mellitus

American Diabetes Association (ADA) diagnostic criteria, 2011

A1C >6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*

FPG >126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.*

2-h plasma glucose >200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*

In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose >200 mg/dl (11.1 mmol/l).

*In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeat testing

Complications

There are mainly two types of complications:-

- A) Acute complication
- B) Chronic complication

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Acute Complication

- A) Diabetic ketoacidosis
- B) Hyperosmolar non – ketotic coma
- C) Hypoglycemia
- D) Lactic acidosis

Chronic Complication

A. Micro vascular

- Retinopathy
- Diabetic neuropathy
- Diabetic nephropathy.

B. Macro vascular

- Coronary artery disease
- Peripheral vascular disease
- Cerebro vascular disease

Risk factor: Dyslipidemia, Hypertension, Obesity, and cigarette smoking.

C. Non- Vascular complication

- Sexual problem

Male: impotence, reduce libido and ejaculatory failure.

Female: amenorrhoea (absence of menstrual cycle) galactorrhoea, infection and pregnancy.

- Gastrointestinal problem.
- Skin changes.

Fructosamine

The term “Fructosamine” refers to glycosylated protein (especially albumin), which have been abbreviate as GSA or GPA (Glycosylated serum or plasma albumin). The term “glycation” was recommended by IUPAC – IUB Joint Commissions on Biochemical Nomenclatures for any reaction linking a sugar to a protein (Roth, 1983). Johnson *et al.*, (1982) introduced Fructosamine into clinical chemistry literature in 1982 as a general term for glycosylated protein (Johnson *et al.*, 1982). Emil Fischer first synthesized the compound in 1886 named as 1- amino – 1- deoxyfructose or also called isoglucosamine (Gottchalk, 1952).

Peterson and Jones first suggested that the proteins with shorter lifetime than hemoglobin could be examined for glycation in an effort to assess diabetic control over a period of few weeks rather than months (Fischer, 1886) during the course of these investigations it was established that albumin was the main protein undergoing glycation. Glycosylated proteins are formed by a non- enzymatic reaction between glucose and protein in which unstable Schiff base are formed followed by Amadori conversion to form stable ketoamine (Peterson and Jones, 1977). As per the life span of albumin (14-20) days Fructosamine provides an index of intermediate term diabetic control as opposed to longer term for glycohemoglobin. Due to this, Fructosamine measurements are more sensitive to change in the diabetic control. This provides a mean to alert physician to improvement or deterioration in control the sugar level much earlier than HbA1c determination (Peterson and Jones, 1977).

Baker *et al.*, (1984) found Fructosamine to be more sensitive than determination of either HbA1c, 24- hours urinary glucose or fasting blood glucose in detecting deterioration of glycemic control after withdrawal of oral hypoglycemic agents in type 2 diabetes (Mosca *et al.*, 1987).

Glycosylated Hemoglobin (HbA1c)

Glycosylated or glycosylated hemoglobin is a form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolong period of time. It is formed by non- enzymatic attachment of glucose moiety to the beta chain of hemoglobin. The normal glucose produces a normal amount of the glycosylated hemoglobin. As the average amount of glucose increases the fraction of glycosylated hemoglobin also increases. This serve as a marker of average blood glucose level prior over the previous month prior to the measurement. In diabetes mellitus, higher the amount of HbA1c indicating poorer control of blood glucose level have been associated with cardiovascular diseases, nephropathy,

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retinopathy (Burtis and Ashwood, 1996). HbA1c first separated from the other form of hemoglobin by Huisman and Meyering in 1958 using chromatographic column (Baker *et al.*, 1984). Samuel Rahbar *et al.*, first described HbA1c increased in diabetes in 1969 (Larsen *et al.*, 1990). The use of HbA1c for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Anthony Cerami, Konald Koenig and coworkers (Huisman *et al.*, 1958). Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long term serum glucose regulation. The HbA1c level is proportional to average blood glucose concentration over the previous four to three months (Rahbar *et al.*, 1969). The measure of HbA1c can be unreliable in many circumstances such as blood loss (eg. After surgery, blood transfusion, anemia or high erythrocyte turnover in the presence of chronic renal disease after administration of high dose of vitamin c or erythropoietin treatment (Koenig *et al.*, 1976).

MATERIALS AND METHODS

The study was carried out in Department of Biochemistry in Sikkim Manipal Institute of Medical Sciences and Central Referral Hospital over a period of 8 month (Nov.1 2012 to June2 2013).

Total participants= 60

Freshly diagnosed= 30

Under treatment (controlled diabetic)= 30

Inclusion Criteria

- HbA1c not exceed 8%
- Interested participant who want to perform fructosamine test
- A diagnosis of diabetes as per ADA criteria 2011 and WHO criteria 2010.

Exclusion Criteria

- Patients with HbA1c above 8%.
- Any hormonal disorder
- Benign and malignancy
- Albumin less than 3.

Sample Collection

Fasting venous blood (4ml) were collected with due consent under aseptic condition in a EDTA tube for the estimation of HbA1c and Fructosamine. Blood is then use to estimate HbA1c and then centrifuge that blood at 300 rpm for 5 min. to obtain serum which were used for estimation of Fructosamine. Hemolysed and lipmic samples are not suitable for testing.

Estimation of HbA1c (Glycated Hemoglobin)

HbA_{1c} was measured by chromatographic-spectrophotometric ion exchange method

Principle

After preparing hemolysate, where the labile fraction is eliminating, hemoglobin are retained by a cationic exchange resin. Hemoglobin A_{1c} (HbA_{1c}) is specifically eluted after washing away the hemoglobin A_{1a+b} fraction (HbA1c Fact Sheet) (HbA_{1a+b}), and is quantified by direct by direct photometric reading at 415nm. The estimation of the relative concentration of HbA_{1c} is made by the measure of total hemoglobin concentration by direct photometric reading at 415nm.

Storage

Store at 15-30°C

Samples

Whole blood in a EDTA tube.

Procedure

Hemolysate preparation and labile fraction elimination

1. Bring the column and reagents to room temperature(21-26°C) (note 1)
2. Pipette into test tube

Blood	50µL
Reagent 1	200µL

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3. Shake thoroughly and let it stand at room temperature for 10-15 minutes. This hemolysate will be used in step 6 and 11.

Column Preparation

4. Remove the upper cap of the column and then snap the tip off bottom.

5. Using the flat end of the pipette, push the upper disc down to the resin surface taking care not to compress it. Let the column drain completely to waste.

Separating and Reading of HbA_{1c} Fraction

6. Carefully pipette on the upper filter:

hemolysate	50µL	Let the column drain waste
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7. In order to drain any sample residue left above the upper disc, pipette

Reagent 2	200µL	Let the column drain to waste
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8. Pipette,

Reagent 2	2.0mL	Let the column drain to waste
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9. Place the column over a test tube and add

Reagent 3	4.0 mL	Collect the eluate HbA _{1c} fraction.
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10. Shake thoroughly and read the absorbance at 415nm against distilled water(HbA_{1c})

Reading of Hb_{TOTAL}

11. Pipette into a test tube

Reagent 3	12.0mL
hemolysate	50µL

12. Shake thoroughly and read absorbance at 415nm against distilled water.

Calculations

The HbA_{1c} relative concentration in the sample is calculated using the following formula:

$$\frac{A_{hbA_{1c}} \times V_{hbA_{1c}}}{A_{HbTOTAL} \times V_{Hbtotal}} \times 100 = \%HbA_{1c}$$

The volume of HbA_{1c} (V_{HbA_{1c}}) is 4 ml; the volume of Hb total (V_{Hbtotal}) is 12mL. The following formula is deducted for the calculation of the concentration:

$$\frac{A_{HbA_{1c}}}{A_{HbTOTAL}} \times \frac{3}{100} = \%HbA_{1c}$$

Linearity

- Detection limit: lower than 4%
- Linearity limit : at least 17.0%

Fructosamine

Serum Fructosamine was measured by Nitroblue Tetrazolium method (Calorimetrically).

Principle

The colorimetric test is based on the glucose capacity to reduce Nitroblue tetrazolium to formazan in an alkaline medium, when glucose is bound to protein aminic groups with a stable ketoaminic bond (Fructosamine). A purple color develops, whose intensity is proportional to the protein glycation degree and therefore to Fructosamine concentration. Measurement is done against a calibrator.

Sample

Non- hemolysed serum, heparinized or EDTA plasma.

Procedure

	B/R	S	CAL
Working reagent	1000µL	1000µL	1000µL
Calibrator	-----	-----	50µL
Sample	-----	50µL	-----
Distilled water	50µL	-----	-----

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Mix accurately and incubate at 37°C. after exactly 10 mins, read the blank reagent (A1), the sample and calibrator absorbencies against distilled water. After exactly 5 minutes of incubation, read again the blank reagent (A2), the sample and calibrator absorbance against distilled water.

Calculation

Calculate the absorbance differences for the blank reagent, the sample and calibrator : $\Delta A = A_2 - A_1$

Fructosamine($\mu\text{mol/L}$): $\frac{\Delta AC - \Delta AB/R}{\Delta AC_{cal} - \Delta AB/R} \times \text{Calibrator concentration}$

Reference value= upto 285 $\mu\text{mol/L}$

Linearity: upto 1000 μmol

Estimation of Total Protein by Biuret Method

Principle

In an alkaline medium, peptide bonds of proteins reacts with cupric ions in Biuret reagent to form violet coloured complex with an absorption maximum at 546nm(530-570nm). Intensity of the colour formed is directly proportional to the concentration of total protein in the sample.

Procedure

	Blank	Standard	Test
Working reagent	1 ml	1 ml	1 ml
Calibrator	-	20 μl	
Sample	-		
Distilled water	20 μl		20 μl

After 20mins. Read absorbance at 546nm (530-570nm).

Normal range: 6.0-8.0 g/dl.

Linearity: upto 10g/dl.

Estimation of Albumin by BCG Method

Principle

Under acidic conditions, albumin present in serum sample binds to bromocresol green to form a green coloured albumin- BCG complex, which is photometrically measured at 628nm. Intensity of the colour formed is directly proportional to albumin concentration in the sample.

Procedure

	Blank	Standard	Test
BCG	1ml	1ml	1 ml
CALIBRATOR	-	10 μl	-
SAMPLE	-	-	10 μl
DISTILLED WATER	10 μl	-	-

Mix and read the absorbance at 628nm (600-650nm).

NORMAL: 3.2-5.5g/dl.

Linearity: upto 6g/dl.

Estimation of Hemoglobin by (Cyanmethemoglobin Method)

Principle

The procedure is based on the oxidation of haemoglobin and its derivatives (Except sulfhemoglobin) to methemoglobin in the presence of alkaline Potassium ferricyanide. Methemoglobin reacts with potassium ferricyanide to form cyanmethemoglobin, which has maximum absorption at 540 nm. The color intensity measured at 540 nm is proportional to the total haemoglobin concentration.

Procedure

Drabkin's solution	5000 μl
Whole blood	20 μl

Take an absorbance at 540nm against distilled water.

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RESULTS AND DISCUSSION

Results

- The result is significant at 0.001 in controlled diabetics with r value of 0.616 between HbA_{1c} and Fructosamine.
- The result is significant at 0.001 in freshly diagnosed diabetics with r value of 0.636 between HbA_{1c} and Fructosamine.
- Thus the result shows the positive correlation in between the two parameters.

Discussion

- The level of Fructosamine and HbA_{1c} are found to be statistically correlated with each other in freshly diagnosed and controlled diabetic patients in our study.
- It shows from the table 3 that increase and decrease in the mean levels of albumin and hemoglobin also effect the Fructosamine as well as HbA_{1c} concentration.
- As per our data it is noticed that whenever concentration of HbA_{1c} increased the concentration of the Fructosamine is also increase, but it shows the Fructosamine level depends upon the glycation of protein (albumin) and availability of albumin in nephropathy.
- The correlation coefficient is significant at (r= 0 .616, and 0.636) where p value is< 0.01. Braatvedt *et al.*, (1997) also found similar correlation in between HbA_{1c} and Fructosamine (r= 0.616, p < 0.01) (Van and Zijlstra, 1961).
- Mccance *et al.*, show small fluctuation in albumin level affect the Fructosamine level (ICSH Comclin Patmittee, 1978). Our study also shows the decrease in albumin level also decrease the Fructosamine level (shown in table 3).
- But *et al.*, Bruusgaard *et al.*, Rutle *et al.*, found the Pearson correlation coefficients of HbA_{1c} and Fructosamine at 0.39 their result did not show any correlation between these two parameters (Braatvedt *et al.*, 1997).
- Allgrove and Cockrill *et al.*, (1988) found correlation coefficient around 0.69 in diabetic patients. Our study also nearly correlates with their study (Pub med.gov, 1987).

	Freshly diagnosed diabetics N= 25	controlled diabetics n=25
HbA_{1c}	7.0 ±.427	6.9 ±.350
Fructosamine	329.88± 3.44	332.36 ± 2.78
Total protein	6.9 ± .505	7.01 ± .45
Albumin	3.97 ± .315	4.0 ±.21
Hemoglobin	13.08 ± 1.03	12.8 ± .848
P value	0.001	0.001
R value	0.616	0.643

Controlled diabetics		Hba1c	fructosamine
Hba1c	Pearson Correlation	1	.643**
	Sig. (2-tailed)		.001
	N	25	25
fructosamine	Pearson Correlation	.643**	1
	Sig. (2-tailed)	.001	
	N	25	25

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Correlations

	Freshly diagnosed	HbA1c	Fructosamine
HbA1c	Pearson Correlation	1	.616**
	Sig. (2-tailed)		.001
	N	25	25
Fructosamine	Pearson Correlation	.616**	1
	Sig. (2-tailed)	.001	
	N	25	25

** . Correlation is significant at the 0.01 level (2-tailed).

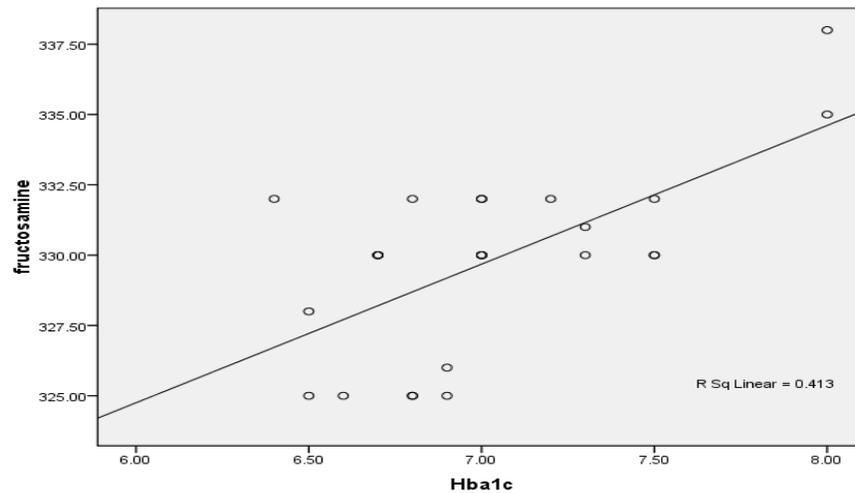


Figure 1: Shows the correlation between fructosamine and HbA1c in controlled diabetics

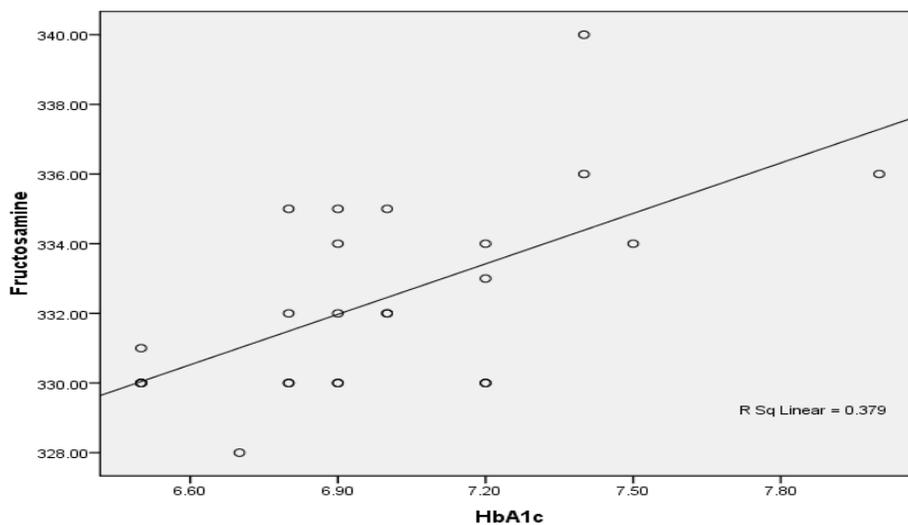


Figure 2: Shows the correlation in between fructosamine and HbA1c in freshly diagnosed diabetics

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Conclusion

Diabetes mellitus is one of the common causes of multi organ failure. It is most common in higher age group. Day by day the percentage of diabetics is increasing.

In this study patient with clinical history of diabetes and sign and symptoms has been taken as subject.

Total of 50 diabetic were studied in which 25 were freshly diagnosed and 25 were controlled diabetics (under treatment).

Sample was taken by venipuncture in an EDTA tube for estimation of Fructosamine and glycated hemoglobin.

In our study the Fructosamine and HbA_{1c} were well correlate at $p < 0.01$.

Our study shows that little fluctuation in the albumin and hemoglobin concentration effect the concentration of Fructosamine and HbA_{1c} respectively.

The study has shown that the clinical profile of patients do support previous finding where HbA_{1c} and Fructosamine were significantly correlated with each other.

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