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Relationship between Glycated Hemoglobin Levels and the Iodine uptake in Patients with Diabetes Mellitus.

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ABSTRACT

Iodination is the process of substitution or addition of iodine atoms on organic compounds. The iodine is reactive with all the three major biochemical constituents namely proteins, lipids and carbohydrates. Present study was done to know the relationship between iodine uptake and glycated hemoglobin (GlyHb) levels in patients with diabetes mellitus. The study was carried out in 50 patients with diabetes mellitus with mean GlyHb of 10.4 ± 2 gm% and 25 healthy controls with mean GlyHb level of 5.5 ± 0.5 gm%. The modified version of the colorimetric method was employed for the assay of iodine uptake and Glyco Hb levels were measured by 501 auto analyzer. The data was analyzed using SPSS version 10. Serum total iodine uptake was decreased significantly ($p < 0.001$) in the patients with diabetes mellitus compared to healthy controls. There was significant negative correlation ($r = -0.942$, $p < 0.01$) between glyHb and iodine uptake in diabetes mellitus patients. We found significant decrease in iodine uptake in diabetes patients compared as to controls. This may be due to increased glycation of proteins induced by hyperglycemia which may interfere in binding of iodine to proteins including hemoglobin. Significant negative correlation between glyHb and iodine uptake can be due to decrease in the availability of iodine binding sites due to alteration in the structure of proteins, which is possibly induced by glycation of proteins, associated with diabetes mellitus. According to our study iodine uptake is decreased in patients with diabetes mellitus which may be due to increased glycation of proteins.

Key words: Iodination, Glycation, Hyperglycemia, Diabetes mellitus.

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INTRODUCTION

Iodine is an essential component of the thyroid hormones, which are necessary for normal growth, development and metabolism during gestation, infancy and throughout life [1]. The most obvious consequence of iodine deficiency is goiter [2]. Iodine deficiency may produce conditions of oxidative stress with high TSH [3].

Iodination is the process of substitution or addition of iodine atoms on organic compounds [4]. As iodination agents, we use not only simple substance iodine but also iodine derivatives as potassium iodide, hydrogen iodide, iodine chloride etc to produce target products where the most optimum conditions for production are searched and applied [5]. Many methods for the direct iodination of aromatic compounds require acidic or basic reaction conditions and liberate strong acid [6].

The iodination of proteins has its application in chemical modification of proteins in order to identify amino acid residues required for the protein structure and function [7]. Iodination of proteins is also utilized to provide a method of increasing the sensitivity for assay procedures of proteins such as in radioimmunoassay. The radio labeled iodine is useful for studying tyrosine and histidine, the residues which incorporate iodine [8]. Application of radioactive labeling has created considerable interest in the field of biology and nuclear medicine. Labeling of proteins is carried out to study biological processes in vivo. Radio labeling is used to prepare traces for radio immune assay or radio immunotherapy. Iodination has found its application in determination of degree of carbon- carbon unsaturation of fats and oils employing titrimetric principles [9]. The lactoperoxidase catalysed iodination of lipids results in a uniform and stable labeling of neutral lipids, phospholipids, lysophosphatides, free fatty acids and triglycerols [10]. There has been a number of reports on direct aromatic iodination [11]. The carbohydrates containing primary alkyl groups being selectively iodinated within one minute to produce iodo derivatives [12]. Thus the iodine is reactive with all the three major biochemical constituents namely proteins, lipids and carbohydrates.

Present study was done to know the relationship between iodine uptake and glycosylated hemoglobin (GlyHb) levels in patients with diabetes mellitus.

MATERIALS AND METHODS

The study was carried out on 50 serum samples with mean Glyco Hb level of 10.4 ± 2 gm% and 25 serum samples with mean Glyco Hb level of 5.5 ± 0.5 gm%. The diabetic blood samples received for routine clinical investigations were collected from Clinical Biochemistry Laboratory, Kasturba Medical College, Manipal. Control blood samples were collected from adult non diabetic healthy persons. Both male and female adult diabetic cases with or without treatment were included and all pediatric cases were excluded. Serum was separated by centrifugation and used for iodination. Informed consent was taken from all subjects involved

and the study was approved by institutional review board. All other reagents used were of chemical grade.

The method employed for the assay of iodination is the modified version of the colorimetric method [13]. Normally proteins undergo denaturation when exposed to organic solvents and acidic medium. To avoid denaturation in the modified method, aqueous medium in place of organic solvent and neutral iodine reagent instead of acid was employed. The modified method was also shown to be simple, sensitive and reliable for the detection of iodine uptake by serum. Iodination of serum was carried out at aqueous medium using potassium iodate - iodide mixture as the source of iodine. All the operations were carried out at room temperature in a closed system. 500 μ l of potassium iodate-iodide solution was mixed with 4ml of 0.5N HCl in a glass stoppered tube. The test tube was kept in dark for 15min for the complete liberation of iodine. 4ml of 0.5N NaOH was added to this and mixed well. It was followed by addition of 0.2M phosphate buffer (pH 7) to make the volume up to 10ml. This reagent was used as neutral iodine reagent.

To 50 μ l of serum, 1.950ml of normal saline was added in a glass stoppered tube. 0.2ml of freshly prepared neutral iodine reagent was added to the test tube and kept in dark at room temperature for 30min for the uptake of iodine. The excess iodine was treated with 2ml of 0.5% starch and the contents were mixed vigorously. The blue color formed was read at 660nm after adding 3ml of distilled water. A blank without sample was also run simultaneously. Considering that under experimental condition, the optical density of the blank is equivalent to 127 μ g of iodine, the amount of iodine absorbed by the sample was calculated by the difference in optical density of the blank and test. The iodine uptake is calculated in mg/100ml using the formula,

$$(B-T) \times \text{concentration of standard} \times \text{dilution factor} \times 100/B$$

The Glyco Hb levels were measured by cobas c 501 auto analyzer.

The results were expressed as mean \pm standard deviation (SD). $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-10, Chicago, USA). Independent sample student's 't' test was used to compare mean values. Pearson correlation was applied to correlate between the parameters.

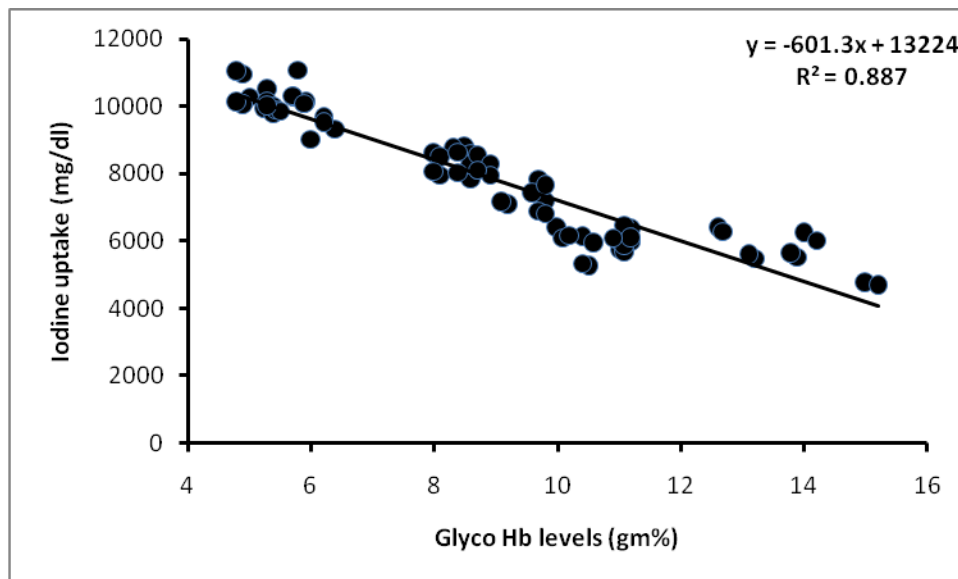
RESULTS AND DISCUSSION

Serum total iodine uptake was decreased significantly in the serum with high Glyco Hb as compared to the serum with normal Glyco Hb level ($p < 0.01$) (Table I). On applying Pearson correlation, serum total iodine uptake correlated negatively with Glyco Hb levels ($r = -0.942, p < 0.01$) (Figure I).

TABLE 1: IODINE UPTAKE AND GLYCATED HEMOGLOBIN LEVELS IN CONTROLS & CASES WITH DIABETES MELLITUS (MEAN ± SD).

	Controls (n= 25)	Cases(n= 50)
Iodine uptake (mg/dl)	10077.44±477.48	6869.30±1192.20
Glyco Hb (gm%)	5.5±0.5	10.4±2

FIGURE 1. CORRELATION BETWEEN IODINE UPTAKE AND GLYCATED HEMOGLOBIN IN CONTROLS AND DIABETES MELLITUS CASES.



The earlier studies have shown that iodine uptake by serum is due to the iodination of proteins, carbohydrates and lipids. The available reports indicate the existence of iodinated proteins in nature. These include thyroid hormones [14], Scleroproteins [15], insect cuticles [16] etc. The iodination at acidic or basic pH values enhances the attachment of the iodine atom to the sulfur atom of cysteine residues [17]. Ordinarily the mono and di iodination of tyrosyl residues are the principle modification involved in the incorporation of iodine. To a lesser extent iodohistidyl residues are also formed. The oxidizing activity of iodine converts sulfhydryl groups to disulfides and may cause some modification of tryptophan. In mild alkaline medium iodine reacts with aldehyde group [18]. There has been a number of reports on direct aromatic iodination i.e., by direct formation of a carbon–iodine bond from an iodonium species [19].The surface membrane lipids are also iodinated through an enzyme-dependent step [20]. In the present study the uptake of iodine decreased proportionately with the increase in the Glyco Hb levels. The increased Glyco Hb levels indicate the presence of high blood sugar level and low anti oxidant level. In diabetes mellitus there will be formation of reactive oxygen species because of oxidative stress and decrease in the antioxidant levels [21]. We found significant decrease in iodine uptake by serum of diabetes patients compared to health controls. This may be due to increased glycation of proteins induced by hyperglycemia which may interfere in binding of iodine to proteins including hemoglobin. Significant negative correlation between

glyHb and iodine uptake can be due to decrease in the availability of iodine binding sites due to alteration in the structure of proteins, which is possibly induced by glycation of proteins, associated with diabetes mellitus.

CONCLUSION

According to our study iodine uptake is decreased in patients with diabetes mellitus which may be possibly due to increased glycation of proteins. To conclude the iodine uptake is negatively correlated with Glyco Hb levels.

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