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A COMPARITIVE STUDY ON IODINATION OF NORMAL AND DIABETIC SERUM

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ABSTRACT

Objectives: It is a well known fact that iodine is a very important trace element for normal growth and metabolism. Iodination of biomolecules has many important functions in the field of research, assay procedures, investigation and diagnosis of diseases. The present study is done to compare the iodine uptake by the serum of diabetes mellitus cases and healthy controls. **Methods:** The study was carried out on 50 cases of known diabetes mellitus with mean fasting blood glucose level of 324 mg/dl and 25 healthy controls with mean fasting blood glucose level of 78 mg/dl. The modified version of the colorimetric method was employed for the assay of iodine uptake. The data's were analyzed using SPSS version 10. **Results:** Serum total iodine uptake was decreased significantly in cases as compared to healthy controls ($p < 0.01$). **Conclusion:** The results of our study indicate that there is decreased iodine uptake by diabetic serum. The cause for decreased iodine uptake may be related to high blood sugar level which possibly may be causing some alteration in the structure of biomolecules by glycation leading to decrease in the binding sites of iodine.

Key words: Iodination, diabetes mellitus, glycation, fasting blood glucose.

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 main source of iodine for breastfeeding infants is the iodine found in human milk [2]. The infant brain develops rapidly, especially from birth until the end of the second year, and thyroid hormone is essential for normal brain development [3]. The most obvious consequence of iodine deficiency is goiter. This adaptive response, mediated principally by TSH, attempts to cope with a shortage of the raw material (iodine) needed for hormone synthesis[4]. Iodine deficiency may produce conditions of oxidative stress with high TSH [5].

Iodination is the process of substitution or addition of iodine atoms on organic compounds[6]. 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[7]. Many methods for the direct iodination of aromatic compounds require acidic or basic reaction conditions and liberate strong acid[8].

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[9]. Iodination of proteins is also utilized to provide a method of increasing the sensitivity for assay procedures of proteins such as in radioimmuno assay. The radio labeled iodine is useful for studying tyrosine and histidine, the residues which incorporate iodine[10]. 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[11]. The lactoperoxidase catalysed iodination of lipids results in a uniform and stable labeling of neutral lipids, phospholipids, lysophosphatides, free fatty acids and triglycerols[12].There has been a number of reports on direct aromatic iodination[13]. The carbohydrates containing primary alkyl groups being selectively iodinated within one minute to produce iodo derivatives[14]. Thus the iodine is reactive with all the three major biochemical constituents namely proteins, lipids and carbohydrates.

The present study is done mainly to know whether there is any change in the uptake of iodine by the serum in diabetes mellitus cases and healthy controls.

MATERIALS AND METHODS

The study was carried out on 50 cases of known diabetes mellitus with mean fasting blood glucose level of 324 mg/dl and 25 healthy controls with mean fasting blood glucose level of 78 mg/dl. 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 the reagents were of chemical grade.

The method employed for the assay of iodination is the modified version of the colorimetric method[15]. 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. 500µl 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 50µl 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 127µg 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) × concentration of standard × dilution factor × 100/B

The blood glucose levels were estimated by Cobas auto analyzer using glucose oxidase method. The results were expressed as mean± 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

Serum total iodine uptake decreased significantly in cases as compared to healthy controls (p <0.01). There was significant increase in fasting blood glucose levels in cases when compared to healthy controls (p<0.01).On applying Pearson correlation, serum total iodine uptake correlated negatively with fasting blood glucose levels (r= -0.979, p <0.01), the results shown in Table-1 and Figure-1..

Table 1: Iodine uptake, fasting blood glucose in controls & cases with diabetes mellitus (mean ± SD).

	Controls (n= 25)	Cases (n= 50)
Iodine uptake (mg/dl)	7115.44±168.34	4713±318.12
Fasting blood glucose (mg/dl)	78.16±8.46	324.58±53.33

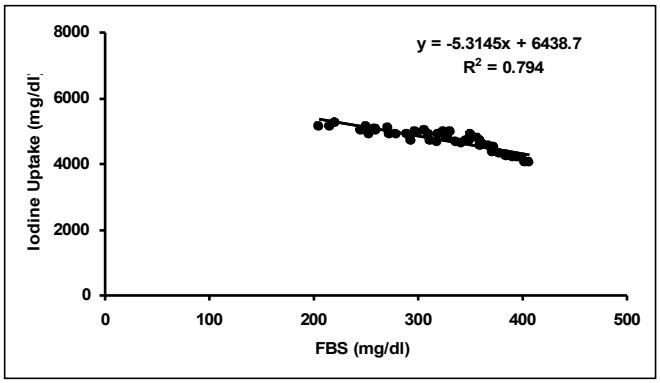

Figure 1. Correlation between iodine uptake and fasting blood sugar levels in diabetes mellitus cases

DISCUSSION AND CONCLUSION

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[16], Scleroproteins[17], the proteins present in sponges[18], insect cuticles[19] etc. Periodate is a specialized oxidant representing halogens that react with hydroxyl group, carbonyl group or amine group on adjacent carbons[20]. The iodination at acidic or basic pH values enhances the attachment of the iodine atom to the sulfur atom of cysteine residues where as iodination at neutral pH occurs predominantly on the aromatic ring of tyrosine residues. Since the cysteine- iodine bond is extremely labile even at neutral pH, cysteine residues do not represent a useful labeling site. At neutral pH values where iodination of tyrosine residues is favoured a relatively stable carbon- iodine bond is formed[21]. Formation of covalent bonds between iodine atoms at the ortho positions to the hydroxyl groups of tyrosyl moieties yielding iodotyrosines[10]. Ordinarily the mono and diiodination 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 sulfhydril groups to disulfides and may cause some modification of tryptophan. Iodination of protein molecules can be carried out under a variety of chemical and enzymatic reaction conditions[22].In mild alkaline medium iodine reacts with aldehyde group[23]. 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[24].The surface membrane lipids are also iodinated through an enzyme-dependent step[25]. In the present study it was observed that the uptake of iodine by the serum of diabetes cases is decreased when compared to healthy controls. It's a well known fact that in diabetes mellitus there is an increase in the blood glucose level. The increased blood glucose level causes glycation of proteins and lipids which may possibly alter the conformation of biomolecules. The change in the structural conformation of biomolecules may decrease the iodine binding sites leading to decreased iodine uptake in diabetic cases. The iodine uptake is inversely proportional to the blood glucose level.

Conclusion

The decrease in iodine uptake in diabetes mellitus may be related to the glycation and alteration in the structural conformation of biomolecules leading to decrease in the iodine binding sites.

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