Research Article

A CORRELATION STUDY OF IODINE UPTAKE AND ANTIOXIDANT STATUS IN DIABETIC PATIENTS

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ABSTRACT

Iodine is an essential component of the thyroid hormones, which are necessary for normal growth, development and metabolism during gestation, infancy and throughout life. Iodination is the process of substitution or addition of iodine atoms on organic compounds. The present study is done to find the correlation between the iodine uptake and the antioxidant levels namely protein thiols. The study was carried out on 50 cases of known diabetes mellitus with mean fasting blood glucose level of 324 mg/dl and 50 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 and antioxidant levels were measured by spectrophotometric methods. The datas were analysed using SPSS version 10. Serum total iodine uptake was decreased significantly in cases as compared to healthy controls ($p < 0.01$). The levels of protein thiols were decreased in cases when compared to healthy controls but not significantly. Serum total iodine uptake correlated positively with protein thiol levels ($r=0.602$, $p<0.01$). The results of our study indicates that there is decreased iodine uptake by diabetic serum and the iodine uptake decreases proportionately with the decrease in the antioxidant levels namely protein thiols. The cause for decreased iodine uptake may be related to the decrease in thiol groups which are utilized to neutralize the reactive oxygen species that are formed during oxidative stress.

Keywords: Iodination, antioxidants, protein thiols, diabetes mellitus.

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].

Iodination is the process of substitution or addition of iodine atoms on organic compounds[2]. 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[3]. Many methods for the direct iodination of aromatic compounds require acidic or basic reaction conditions and liberate strong acid[4].

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[5]. Iodination of proteins is also utilized to provide a method of increasing the sensitivity for assay procedures of proteins such as in radio immuno assay. The radio labeled iodine is useful for studying tyrosine and histidine, the residues which incorporate iodine[6]. 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[7]. The lactoperoxidase catalysed iodination of lipids results in a uniform and stable labeling of neutral lipids, phospholipids, lysophosphatides, free fatty acids and triglycerols[8]. There has been a number of reports on direct aromatic iodination[9]. The carbohydrates containing primary alkyl groups being selectively iodinated within one minute to produce iodo derivatives[10]. 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 correlation between the uptake of iodine and the antioxidant levels namely protein thiols and ceruloplasmin as they are the indicators of oxidative stress.

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. Special chemicals like 5 5' dithio-bis (2-nitrobenzoic acid) (DTNB), p-phenyl diammonium

dichloride (PPD) were obtained from sigma chemicals, St Louis, MO, USA. All other reagents were chemical grade.

The method employed for the assay of iodination is the modified version of the colorimetric method[11]. 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.

Serum total thiols were measured by spectrophotometric method using DTNB (DiThiobis NitroBenzoic acid) at 412 nm [12].

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). The levels of protein thiols were decreased in cases when compared to healthy controls but not significantly .On applying Pearson correlation, serum total iodine uptake correlated positively with protein thiol levels (r=0.602,p<0.01). Protein thiols correlated negatively with fasting blood glucose levels (r=-0.569, p<0.01).

Table 1: Iodine uptake, fasting blood glucose and antioxidants 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
Protein Thiols (µmoles/L)	250.92±42.25	173.96±51.39
Fasting blood glucose (mg/dl)	81.16±9.41	315.34±43.91

Figure 1: Correlation between iodine uptake and protein thiols in diabetes mellitus cases.

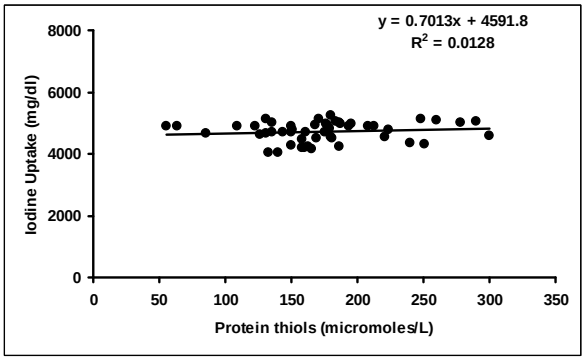
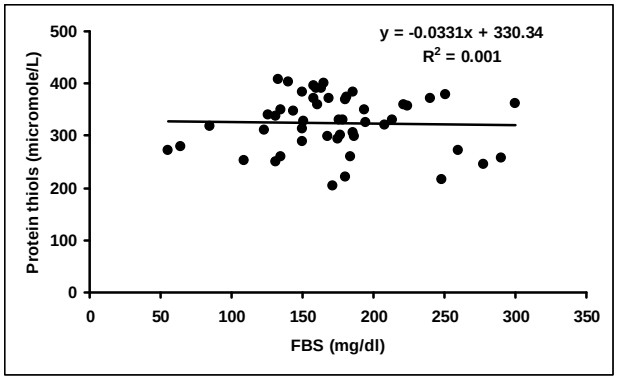


Figure 2: Correlation between fasting blood sugar levels and protein thiols in diabetes mellitus patients.



DISCUSSION

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[13], Scleroproteins[14], the proteins present in sponges[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 sulfhydril 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 the uptake of iodine decreased proportionately with the decrease in the protein thiol levels. The decrease in the protein thiol levels in cases is due to the utilization of thiol groups to neutralize the reactive oxygen species that are formed as a result of oxidative stress in diabetes mellitus. The decreased iodine uptake in cases may be related to the oxidative damage and glycation of biomolecules leading to a possible alteration in the structural conformation causing decrease in the iodine binding sites and resulting in decreased iodine uptake. The iodine uptake is inversely proportional to the blood glucose level and directly proportional to the antioxidant levels.

CONCLUSION

The iodine uptake decreases proportionately with the decrease in the antioxidant levels.

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