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Original Articles

Original Article

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Evaluation of Thyroid Function in Type 2 Diabetes Mellitus Patients

[1]Prajna K, [2]Nandana Muralidharan, [3]Ullal Harshini Devi, [4*]Kathyayani Sathish, [4]Suchetha Kumari N

Author's Affiliation: [1]Dept. of Biochemistry, Mysore Medical College and research centre, Mysuru, Karnataka 570001, India. [2]MBBS Student, K.S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, Karnataka 575018, India. [3]Central Research Laboratory, K.S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, Karnataka 575018, India. [4]Dept. of Biochemistry, K.S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, Karnataka 575018, India.

Abstract

Background: The ability to diagnose and treat subclinical hypothyroidism in diabetic patients may greatly enhance the quality of life. The treatment of hypothyroidism helps in better control of other associated co-morbidities. Therefore, in present study association between thyroid dysfunction and Diabetes was assessed by correlating Fasting plasma glucose and Thyroid profile. *Materials and Methods:* A total of 40 subjects attending the OPD who were diagnosed for diabetes were recruited.40 normal age and sex matched participants were recruited as controls. Informed written consent was obtained from all the participants. Fasting plasma glucose was estimated by glucose oxidase method and TSH, T_3 and T_4 by enzyme-immunoassay (ELISA) method using commercially available kit. Subjects grouped as normal with TSH level0.39-6.16mIU/l, T_3 levels 52-185ng/dl and T_4 level as 5.0-15.0µg/dl. Lower T_3 and T_4 with high TSH is considered as Hypothyroidism and higher levels of T_3, T_4 with low TSH is considered as hyperthyroidism.The data was analysed using SPSS version 20. Unpaired t test and Pearson's correlation was performed to find the significant differences between the groups and their correlation. $P<0.05$ was considered as statistically significant. *Results:* The Chi-square test was used to find the association between diabetes and thyroid dysfunction showed significant association ($p<0.05$) between Diabetes and thyroid dysfunction. On comparison, showed no difference in the sugar levels according to dysfunction status($p<0.05$). *Conclusion:* A routine assessment of thyroid hormone levels in diabetics is necessary, particularly with subclinical thyroid hormones level.

Keywords: Fasting Plasma Glucose Level; Glucose Oxidase Method; TSH; T3; T4; Enzyme-linked Immunoassay.

Introduction

Diabetes mellitus is a common endocrine disorder rising in India and has reached approximately 20% in urban populations and approximately 10% in rural Population [1]. On long term it is associated with vascular complications these are responsible for increased morbidity and mortality among diabetic subjects [2]. New addition to these complications is the thyroid dysfunction which is indicated by the recent studies [3,4] The first report showing the association between diabetes and thyroid dysfunction was published in 1979 [5,6]. Since then a number of studies have estimated the prevalence of thyroid dysfunction among diabetes patients to be varying from 2.2 to 17%, the most common disorder being subclinical hypothyroidism [7,8]. However, few studies also estimated much higher prevalence of thyroid dysfunction in diabetes i.e., 31% and 46.5% respectively [9,10] also not showed any significant correlation between Fasting plasma glucose and thyroid profile parameters. Thyroid

Reprint Request: Kathyayani Sathish, Assistant Professor, Dept. of Biochemistry, K.S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, Karnataka, 575018, India.

E-Mail: suchethashetty.biochem@gmail.com

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hormones directly control insulin secretion. In hypothyroidism, there is a reduction in glucose-induced insulin secretion by beta cells, and the response of beta cells to glucose or catecholamine is increased in hyperthyroidism due to increased beta cell mass. Moreover, insulin clearance is increased in thyrotoxicosis. Diabetes may affect the thyroid function to variable extent. Diabetes mellitus appears to influence thyroid function in two sites; first second at peripheral tissue by converting T_4 to T_3. Unrecognized thyroid dysfunction not only worsens the metabolic control but also impede the management of diabetes. Studies also have suggested that type 2 diabetes mellitus patients with subclinical hypothyroidism are at risk of complications like nephropathy and cardiovascular events.The ability to diagnose and treat subclinical hypothyroidism in these patients may greatly enhance the quality of life. Hence, there is need to detect such cases where hypothyroidism contributes to morbidity and where it is the cause for poor control of the associated conditions. The treatment of hypothyroidism helps in better control of other associated co-morbidities. So, patients with diabetes need to be screened for thyroid dysfunction. Therefore, in present study association between thyroid dysfunction and Diabetes was assessed by correlating Fasting plasma glucose and Thyroid profile.

Materials and Methods

In this study, a total of 40 patients attending the outpatient department of K.S. Hegde Charitable Hospital from June 2017 to November 2017 who were diagnosed for diabetes were recruited. Diagnosis of diabetes was based on the American Diabetes association criteria. 40 normal age and sex matched participants were recruited as controls. A medical history regarding the age at diagnosis of diabetes and current medication was obtained. The study protocol was approved by the institutional ethical committee. Informed written consent was obtained from all the participants.

Blood samples were drawn after 10-12 hours fast for measurement of fasting plasma glucose (FPS) and thyroid status. All the diabetic subjects were confirmed diabetics who had Fasting plasma glucose level > 126mg/dl and others were taken as control. The study excluded very ill patients with complication of Diabetes Mellitus.

Of the 4ml blood drawn from the subjects 2ml was dispensed into fluoride oxalate bottles for plasma glucose estimation and the rest of the blood sample was discharged into plain samples bottle and allowed to clot. Serum separated from the cells was stored at -20^{0}C and thawed only when required. Plasma from fluoride tubes was also stored at- 20^{0}C until needed for use. Fasting plasma glucose was estimated by glucose oxidase method and TSH, T_3 and T_4 by enzyme-immunoassay (ELISA) method using commercially available kit. Procedure wasfollowed as per the manufactures instructions. All the analysis was done in duplicate and the average of the duplicate data was used for calculation. The data obtained was classified as raised, low, or normal thyroid hormone levels were based on the following criteria. Subjects grouped as normal with TSH level0.39-6.16mIU/l, T_3 levels 52-185ng/dl and T_4 level as 5.0-15.0µg/dl. Lower $T_3$and T_4 with high TSH is considered as Hypothyroidism and higher levels of T_3, T_4 with low TSH is considered as hyperthyroidism.

Data analysis

The data was expressed as percentage and mean ±SD. Statistical analysis was performed using software statistical package for social sciences (SPSS) version 20, unpaired t test and Pearson's correlation was performed to find the significant differences between the groups and their correlation. $p<0.05$ was considered as statistically significant.

Results

For the study, 80 subjects, were recruited. Out of these 40 were males and 40 females. Further divided into diabetic and conntrol groups having 20 subjects in each group.Their age ranged between 40-75 years with mean age 57± 8 years (Figure 1).

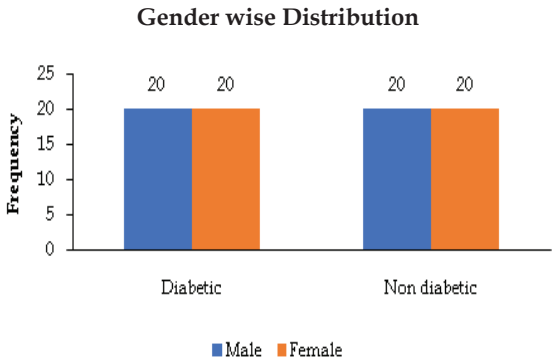

Fig. 1: Showing gender wise distribution

Thyroid hormones (TSH, T_3, T_4) levels were estimated for both the groups. Results revealed that 6 had abnormal thyroid levels. Of these 3 females had hypothyroidism and 2 had hyperthyroidism in diabetic group. In males only one had hypothyroidism in diabetic group (Figure 2). The Chi-square test was used to find the association between diabetes and thyroid dysfunction. The obtained p values are less than 0.05 and hence there was association between Diabetes and thyroid dysfunction (Table 1).

Unpaired t test was used to compare the difference in Fasting blood glucose levels. The obtained p value was less than 0.05 and hence there was a difference in the sugar levels (Table 2). It has been observed that, in control group the level of Fasting blood glucose was less (98.4±12) than the diabetic group (161±36). One-way ANOVA was used to compare Fasting blood glucose level according to the dysfunctional status. The obtained p value was > 0.05and hence there was no difference in the sugar levels according to dysfunction status (Table 3).

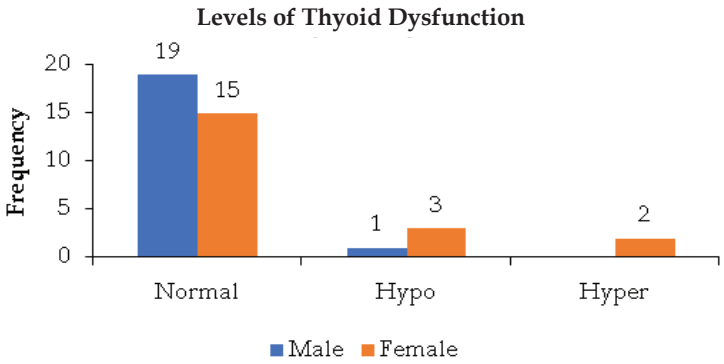

Fig 2: Showing levels of thyoid dysfunction in males and females.

Table 1: Thyroid dysfunction and thyroid hormone levels in diabetic and control group.

(n = 80)		Diabetes Frequency (%)	Control Frequency (%)	p value
Thyroid dysfunction	Hyper	13 (76.5)	4 (23.5)	0.003
	Hypo	13 (65)	7 (35)	
	Normal	14 (32.6)	29 (67.4)	
TSH(UNIT)	< 0.38	10 (76.9)	3 (23.1)	0.003
	0.39-6.16	17 (34.7)	32 (65.3)	
	> 6.17	13 (72.2)	35 (27.8)	
T3	< 51.9	9 (75)	3 (25)	0.011
	52-185	23 (39.7)	35 (60.3)	
	>185.1	8 (80)	2 (20)	
T4	<4.9	6 (85.7)	1 (14.3)	0.001
	5-15	18 (32.1)	38 (67.9)	
	>15.1	16 (94.1)	1 (5.9)	

Table 2: Comparison of fasting blood glucose among the groups.

	Mean	Standard deviation	p value
Diabetic	161	36.07	<0.001
Control	98.4	12.08	

Table 3: Comparison of Fasting blood glucose level according to the dysfunction status.

Group	Mean	Standard deviation	p value
Hyperthyroidism	170.5	2.12	0.667
Hypothyroidism	146.2	6.5	
normal	127.7	24.63	NS

Discussion

Among diabetic subjects investigated, 10% of the subjects had low levels of thyroid hormones while 5% had raised level. Non-diabetic subjects showed no thyroid dysfunction. This shows a high incidence of abnormal thyroid hormones level (high or low) in diabetic population. Pranav K Raghuvanshi [11] stated that total T_3 and total T_4 were significantly low, while serum TSH levels were higher in type 2 diabetes mellitus subjects as compared to the non-diabetic healthy subjects. Alok Mawar, K.P. Mishra et al. [12] concluded through their study that prevalence of hypothyroidism and subclinical hypothyroidism was found to be higher in type 2 diabetes mellitus subjects as compared to non-diabetic subjects. MJ Smithson [8] concluded from a study conducted amongst 11300 patients of which 223 were diabetic that the prevalence of undiagnosed thyroid disease in diabetic patients receiving community diabetes care was 5.5%.

Our finding is in agreement with Smithson [8], Suzuki et al [13]. They found altered thyroid hormones level of different magnitude in diabetic patients. Abnormal thyroid hormone level may be the outcome of various medications the diabetics was receiving. It is widely known that insulin [14] an anabolic hormone enhances the level of FT_4 while it suppresses the levels of T_3 by inhibiting hepatic conversion of T_4 to T_3. Likewise, the oral hypoglycaemic drugs such as sulfonylureas are known to suppress the levels of FT_4 and T_4 while causing raised levels of TSH. The presence of both raised and low thyroid hormone levels in diabetics in present study may be due to the modified thyroid releasing hormone (TRH) synthesis and its release [15] and may depend on the glycaemic status of the diabetics studied. Glycaemic status is influenced by insulin, which is known to modulate TRH and TSH level.

Conclusion

It was also observed that hypothyroidism was more in female subjects. Although it is possible that the magnitude and precision risk between diabetes and thyroid may be observed with a larger sample size and taking the medication into account.In conclusion routine assessment of thyroid hormone levels in diabetics is necessary, particularly with subclinical thyroid hormones level.Further studies are needed to establish the risk of thyroid dysfunction because the present study had low sample size.

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Original Article

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Single Step Purification of Soybean Isoflavones Employing Silica Gel Adsorption Chromatography

MC Lakshmi[1], MC Madhusudhan[2], HS Prakash[3], KSMS Raghavarao[4]

Author's Affiliation: [1]Oscar House, La Pouquelaye, St Helier, JE13ZD, United Kingdom [2,3]DOS in Biotechnology, Manasagangothri, University of Mysore, Mysuru 570006, India. [4]Department of Food Engineering, CSIR-Central Food Technological Research Institute (CFTRI), Mysuru 570 020, India.

Abstract

The extraction and purification of isoflavones from defatted soy flour (DSF) are attempted by leaching and adsorption chromatography. The isoflavones were extracted in methanol and processed employing silica gel column chromatography. After silica gel column chromatography 0.4 mg/ml of isoflavones content (3.9 mg/g DSF) was obtained which has 16.8 mg of total isoflavones. The process helps to the recovery of the isoflavones along with the removal of contaminants up to 90% which confirmed through HPLC.

Keywords: Concentration; Methanol; Isoflavones; Soybean; Column Chromatography.

Introduction

Soybeans (*Glycine max*) are the rich sources (1.2 - 4.2 mg/g flavonoid) of isoflavones, and are widely available, which are less expensive (Vacek et al., 2008). Isoflavones are often known as phytoestrogens, the group of plant-derived phenolic compounds which shows estrogenic activity (Wildman, 2007). These are commonly found in leguminous plants (peas, beans) and clovers (Saviranta et al., 2008). Isoflavones are known for their potential health benefits. They exhibit antioxidant activity and play an important role in preventing and treating various cancers, osteoporosis and cardiovascular diseases (Achouri et al., 2005). Isoflavones are mainly classified into four groups namely aglycons, glucosides, malonylglucosides and acetyl glucosides. The three isoflavone aglycons, namely, genistein, daidzein and glycitein, are each present in four glucosidic forms in soybeans and soy foods (Griffith and Collison, 2001; Klejdus et al., 2005; Lee et al., 2004).

Corresponding Aurhor: MC Madhusudhan, DOS in Biotechnology, Manasagangothri, University of Mysore, Mysuru 570006, India

Email: mcmsudhan@gmail.com

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Several methods and techniques have been reported for the extraction, isolation, and purification of isoflavones. They can be categorized based on several general principles (or approaches). One is based on extraction followed by precipitation. Another is based on precipitation followed by extraction or separation. The third is based on the use of chromatography or other means either before or after solvent extraction to separate or concentrate isoflavones (Zhang and Schwartz, 2005, Lakshmi *et al*., 2013).

Due to the multiple beneficial effects of soy isoflavones on human health, related products have flooded the market, with unsubstantiated claims and few regulations governing their quality or efficacy. Most products have levels of isoflavones less than their claimed contents, with some containing virtually no detectable isoflavones (Lawton *et al*., 2003). Moreover, many products only contain soy extracts with very little isoflavones (i.e., 0.2%) and abundant unknown impurities. More importantly, isoflavones in many products are in the form of glucosides, which have weaker biological activities and are more difficult to be absorbed by the body than the corresponding aglycones (Zhang *et al*., 2007). Therefore, the quality and efficacy of many isoflavone products are poor, and there is a need to develop products with higher purity and efficacy.

Most reported processes include multiple steps; some require multiple chromatography columns for the production of isoflavones. Adsorption is a potential method for purification of isoflavones with minimum processing steps, allowing the elimination of polar, nonphenolic impurities. Partitioning, using solid phase extraction (SPE), using adsorbant resin is a selective method for completely purifying isoflavones. The main objective of this study was to isolate and purify major isoflavones daidzein, genistein and their glycosylated forms (daidzin, genistin) from defatted soy flour in the single step purification method.

Materials and Methods

Materials

The soybeans *(Glycine max)* of variety JS 335 was procured from National Seed Corporation, India. They were stored at refrigerated temperature (6-8 ^{o}C) until use. Adsorbent silica gel was obtained from Sd fine-chem. Ltd, Mumbai, India. HPLC grade solvents, namely, water, ethanol, methanol, acetone and acetonitrile were purchased from Merck, Mumbai, India. All the chemicals used were of analytical grade. The isoflavone standards such as genistein, daidzein, genistin and daidzin were obtained from Sigma Chemical Co. St. Louis, MO, USA.

Methods

Extraction

Isoflavones, genistein and daidzein (aglycones), genistin and daidzin (glycosylated) were purified from defatted soy flour following the method described by Ohta *et al*., (1979) with some modifications. Finely defatted soy flour (25g) was extracted with 250 ml of 80% methanol three times at 80°C for 3 hours. The extract was filtered and the supernatant concentrated under atmospheric pressure first and then under vacuum. A brownish, syrupy liquid was obtained. This was subjected to extraction with two volumes of acetone. The acetone extract was concentrated by vacuum drying. The solid obtained was dissolved in water and subjected to solvent partition using ethylacetate. The extract was partitioned into three layers, uppermost ethylacetate layer, middle solid mass and lower aqueous layer.

Silica gel adsorption chromatography

The ethylacetate extract was subjected to adsorption chromatography on silica gel. The column (30 cm x 2.5 cm) was eluted with 50% water saturated ethylacetate, and 50% water saturated ethylacetate containing 2% ethanol with a flow rate of 1ml/min. Fraction F1 is rich genistein and daidzein, F5 is rich in genistin and F6 is rich in daidzin. Each fraction obtained was rechromatographed on silica gel using the same procedure.

HPLC

The amount of isoflavones in the extracts was analyzed using HPLC (Waters Alliance 2690, Waters, USA) equipped with a photodiode array detector (Waters, USA) and millennium chromatography manager software. A 10 µl sample was loaded onto a C18 column (SGE, 250 x 4.6 mm, 5 µm particle size, SGE, Germany). The mobile phase was composed of 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B). The elution was performed in a linear gradient of A against B. The separation was achieved using the following gradient program: 0-5 min, 85% A; 5-36 min, 71% A; 36-44 min, 65% A and 45-60 min, 85% A. The flow rate of the mobile phase was set at 0.6 ml /min and absorption was measured at 260 nm (Murphy *et al*., 1997). The temperature of the column was maintained at 25±1^{o}C. The identity and purity of isoflavones in the samples were confirmed by matching the retention times and areas with the standards.

The purity of isoflavones was determined by HPLC employing a C 18 column (Wang and Murphy, 1994) with gradient elution using acetonitrile water (15 to 35%, in 50 min, flow rate: 1ml/min and detection at 262 nm). The concentration of isoflavones was determined by the standard graph using the method as given by Coward *et al*., (1993).

Calculation of total isoflavones

The concentration of isoflavone glucosides (daidzin, genistin) in a given sample were expressed as aglycon equivalents using the following equation (AOAC, 2005; Lakshmi *et al*., 2013).

$$Ca_e = [MW_a/MW_g] \times C_g \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where Ca_e = isoflavones aglycon equivalents (µg/g); MW_a = molecular weight of aglycon; MW_g = molecular weight of glucoside; an Cg = concentration of daidzin and genistin (µg/g).

The total isoflavones in microgram aglycon equivalents/g of sample was calculated, by summing the concentrations of daidzein, genistein and adding this total to the sum of aglycon equivalent concentrations of daidzin and genistin as indicated below.

$T_a = C_a$ (daidzein) + C_a (genistein)

$T_{ae} = C_{ae}$ (daidzin) + C_{ae} (genistin)

where T_a = sum of concentrations of aglycons and T_{ae} = sum of aglycon equivalent concentrations of glucosides.

Free Radical Scavenging Activity

The DPPH radical scavenging test was carried out as described as Blois (1958). The extracts (with different dilutions of the extract in 100% methanol, ranging from 0.05 to 0.3 mL), were mixed with 0.5 mM/L DPPH solution. The absorbance was measured at 517 nm immediately and again after 30 min to determine the amount of DPPH scavenged. The free radical scavenging activity of samples was expressed in percentage, and each sample was analyzed in triplicate. The free radical scavenging activity was calculated by using the following equation:

$$\text{Scavenging activity (\%)} = \frac{[Aa-(Ab-Ac)]}{Aa} x100$$

where Aa is the absorbance of the control solution of DPPH (without isoflavone extract), Ab is the absorbance of the mixture containing isoflavone extract as well as DPPH, and Ac is the absorbance of the blank solution without DPPH.

Table 1: The content of isoflavones

Procedure	Isoflavone content		Total Isoflavones (mg)
	mg/ml	mg/g DSF	
Extraction	0.12	1.28	1.2
after purification	0.4	3.9	16.8

** per gram of dry extract.*

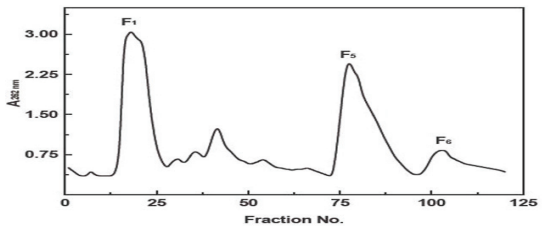

Fig. 1: Elution profile of ethyl acetate extract on silica gel column. F_1 fraction mixture of genistein and daidzein, F_5 and F_6 fraction are rich in genistin and daidzin.

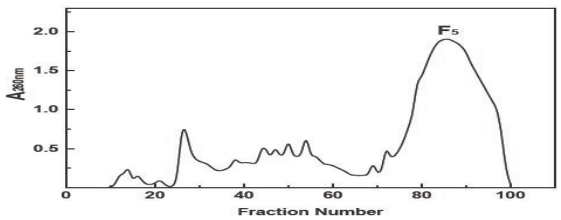

Fig. 2: Rechromatography of fraction F_5

Results and Discussion

Extraction using methanol has resulted in 0.12 mg/ml of isoflavones content (1.28 mg/g DSF) which has 1.2 mg of total isoflavones.

The solvent extract (methnolic extract) on silica gel column was resolved into three fractions F1, F5 and F6 as shown in Fig. 1. F1 fraction which was eluted with 50% water saturated ethyl acetate was found to be genistein and daidzein. F5 and F6 fractions which were eluted with 50% water saturated ethyl acetate containing 2% ethanol contained genistin and daidzin respectively. Each fraction obtained was rechromatographed using similar conditions (Fig. 2). The isoflavones genistin, daidzin, genistein, and daidzein purified from defatted soy flour, had a purity of >90% (confirmed by HPLC) as can be seen in Fig. 3.

After silica gel column chromatography it resulted in 0.4 mg/ml of isoflavones content (3.9 mg/g DSF) which has 16.8 mg of total isoflavones. The process helps to the recovery of the isoflavones along with removal of contaminants.

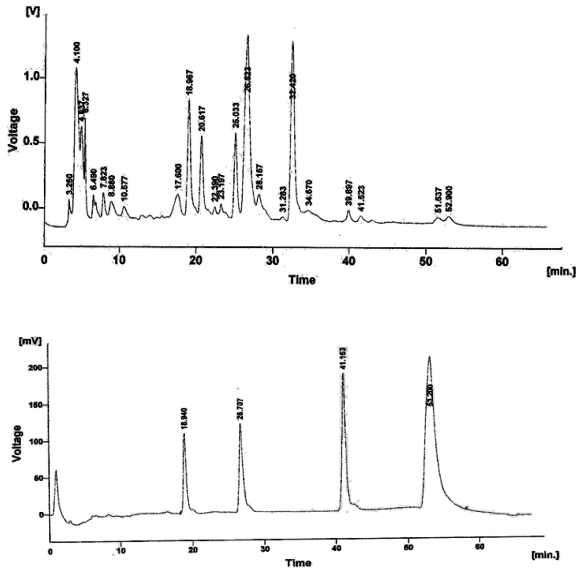

Fig. 3: Chromatograms showing crude and purified isoflavones (RT; 16.946-Daidziein, RT; 26.707-Genistein, RT; 41.163-Daidzin and RT; 53.20-Genistin)

The radical scavenging activity of all the extracts was found to increase with an increase in the concentration (from 0.05 to 0.25 mg) of isoflavones (Fig. 4). Highest antioxidant activity (65.8%) was observed at 0.25 mg concentration of isoflavones compared to other extracts and afterwards, it attained a plateau showing no further change in RSA (%) even with an increase in isoflavone concentration (0.3 mg).

Conclusions

Extraction and purification of isoflavones from defatted soy flour were carried out employing adsorption process resulted in 16.8 mg of isoflavones per gram of defatted soy flour from 1.2 mg/g (DSF) of initial extraction. The silica gel chromatography process successfully used for the purification (>90 %) and recovery of the isoflavones. The purified extract exhibited maximum radical scavenging activity of 65.8% on DPPH at 0.25 mg/g isoflavone concentration.

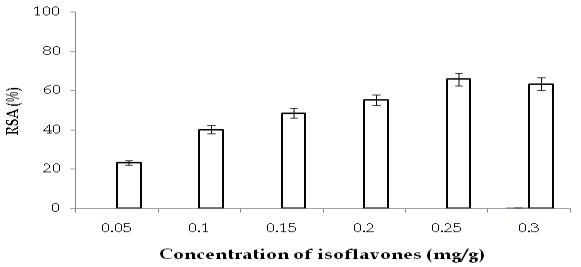

Fig. 4: Radical scavenging activities of isoflavones at different concentrations.

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Original Article

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Comparison of Lipid Profile Levels in AMI Patients With and Without Diabetes Mellitus

Savita Deshmukh[1], Raviraj Naik[2], Ranjana Deshmukh[3]

Author's Affiliation: [1,2]Assistant Professor, Dept. of Biochemistry; Indian Institute of Medical Science & Research, Jalna, Maharashtra 431202, India. [3]Consulting Physician; Dr. Hedgewar Hospital, Aurangabad, Maharashtra 431005, India.

Abstract

Present study was aimed to assess lipid profile in diabetic and non-diabetic patients with history of myocardial infarction. *Methods*: Study conducted in the Department of Biochemistry, Indian Institute of Medical Science and Research, Warudi, Tq. Badnapur, District Jalna (M.S.)in collaboration with Hedgewar hospital during period of 2015-17. Biochemical investigations include fasting blood sugar, postprandial blood sugar and lipid profile (total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol and VLDL). *Results:* Present study shows significant increase in the levels of triglyceride, LDL-cholesterol, while cholesterol level increases non-significantly. HDL-cholesterol levels decreased significantly in patients of myocardial infarction (MI) with diabetes mellitus compared to non-diabetic patients with history of myocardial infarction. *Conclusion:* Diabetic patients with history of myocardial infarctionwere having significantly deranged lipid parameters and higher risk of dyslipidemic complications as compared to nondiabetic patients with history of myocardial infarction.

Keywords: AMI (Acute Mycocardial Infarction); HDL (High Density Lipoprotein; LDL (Low Density Lipoprotein); Diabetic & Non-Diabetic.

Introduction

Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both [1]. Hyperlipidemia and altered lipid metabolism is seen in diabetes. The chronic hyperglycemia is associated with hyperlipidemia which leads to vascular complications. Patients with type II Diabetes mellitus are on higher risk of cardiovascular disease associated with atherogenic abnormalities and dyslipidemia [2]. This dyslipidemia is characterized by increased plasma triglyceride concentration, increased cholesterol concentration increased LDL-cholesterol concentration while decreased concentration of HDL-cholesterol [3].

Reprint Request: Raviraj Rajan Naik, Assistant Professor, Dept. of Biochemistry; Indian Institute of Medical Science & Research, Jalna, Maharashtra 431202, India.
Email: raviraj_40@yahoo.com

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Cardiovascular disease is defined as "impairment of heart function due to inadequate flow of blood to the heart compared to its need caused by obstructive changes in the coronary artery disease especially MI is the leading cause of morbidity and mortality worldwide [4].

There are several risk factors for MI such as hypertension, smoking, family history of obesity. Independently of the presence or absenbce of other risk factors DM add to the risk for CVD.

Materials and Methods

This study was conducted in the Department of Biochemistry, Indian Institute of Medical Science and Research, Warudi, Tq. Badnapur, District Jalna (M.S.) and in collaboration with Hedgewar hospital, Aurangabad (M.S.) during period of 2015-17. The study was clearance from Institutional ethical committees.

Total 60 patients were included in this study. Patients were divided into two groups. Group I consists of 30 patients suffering from myocardial infarction without diabetes mellitus and Group II consists of 30 patients suffering from myocardial infarction with diabetes mellitus. The diagnosis of myocardial infarction was based on a history of prolonged chest pain, ECG changes and elevated CK-MB level within 12 hours after the onset of chest pain. The patient with other complications like liver disease, bone disease, kidney disease and inflammatory disease were excluded from the study. Biochemical investigations include fasting blood sugar (FBS), postprandial blood sugar (PPBS), CK-MB and lipid profile (total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol and VLDL) were carried out.

Statistical Analysis

Statisticalanalysis were carried out by SPSS software and p values were obtained.

Results

Data from investigations of all patients were tabulated in the MS-Excel sheet for their mean±SD and following observations were made

Table 1: Biochemical marker in myocardial patients with and without diabetes (Group I AMI without DM, Group II AMI with DM)

Parameters	Group I	Group II	'p' value
CK-MB (IU/L)	102.0±19.69	152.0±27.6	<0.001
FBS (mg%)	99.23±6.68	180.6±89.4	0.000
PPM (mg%)	130.4±34.2	224.0±114.0	0.000
Total cholesterol (mg%)	168.0±32.1	185.0±51.5	0.092
LDL-C (mg%)	111.1±32.3	131.6±31.2	0.009
HDL (mg%)	37.5±13.7	40.3±13.8	0.401
TG (mg%)	131.7±60.0	168.2±66.4	0.019
VLDL (mg%)	26.3±12.0	30.6±15.1	0.037

Above table 1 shows the levels of CK-MB (p<0.001), triglyceride (p =0.019), LDL-C (p=0.009)& VLDL(p=0.037) was significantly Increased in patients of myocardial infarction with diabetes mellitus while cholesterol level increased In patients of myocardial Infarction with diabetes mellitus and HDL cholesterol level decreased in patients of myocardial infarction with diabetes mellitus compared to non-diabetic MI patients.

Discussion

Findings of our study were similar to previous studies [6,7,9]. Cholesterol level in AMI patients with diabetes mellitus as compared to patients of AMI without diabetes mellitus.

It has been hypothesized that hyperplagia of diabetes induces increased activity of HMG-CoA reductase of the intestine resulting in increased synthesis of cholesterol leading to raised levels in plasma. Dietary cholesterol also adds upto total cholesterol by increased asorption [2,8].

LDL-C our study shows significant increase in LDL-C level in patients of AMI with diabetes mellitus than in patients of AMI without diabetes mellitus. Other previous research studies showed more or less similar findings [9,10]. Small dense LDL particles appear to arise from the intravascular processing of specific larger VLDL precursors through a series of steps, including lipolysis. Further triglyceride enrichment of the lipolytic products through the action of cholesteryl ester transfer protein together with hydrolysis of TG and phospholipids by hepatic lipase leads to increase production of small dense LDL particles [10,11]. (lipids and lipoproteins in patients with type II DM Ronald M. Krauss American Diabetes Association Diabetes Care).

In our study HDL-C level in AMI patients with diabetes mellitus is not statistically significant but it shows decreased HDL level in AMI patients with diabetes mellitus than that of in AMI patients without diabetes mellitus. The findings of our study were correlate with the previous studies [3-5,7]. Lower HDL-C in diabetes may be due to reduced lipoprotein lipase activity [2,13,14].

Our study shows significant increase in level of triglyceride in AMI patients with diabetes mellitus. Similar findings were shown by previous studies [1,3,9,15]. Hypertriglyceridemia may be due to higher rates of production of triglyceride rich VLDL by liver and to decreased removal of triglyceride by peripheral tissue, primary adipose tissue and muscle [2,15,16]. Insulin deficiency leads to high triglyceride production and subsequent high packaging in VLDL.

Our study showed increase in VLDL level in AMI with diabetes mellitus than in non-diabetic AMI patients but increase is not significant. Similar findings were also noted by previous studies [4,5,12,17]. Lipid metabolism in type II diabetes is modulated by series of factors among which the degree of glycemic control and the presence of insulin resistance are two most important player. One major consequence of insulin resistance of lipid metabolism is the loss of the suppressive effect

of insulin ob fat mobilization from adipose tissue. As a result there is an increase in free fatty acids flux owing to reduced suppression of lipolysis. The failure of suppress FFA in the postprandial period, due to the decreased activity of lipoprotein lipase and the rise in plasma FFA due to increased adipocyte lipolysis are key reasons behind increased VLDL.

Conclusion

Diabetic patients with history of myocardial infarctionwere having significantly deranged lipid parameters and higher risk of dyslipidemic complications as compared to nondiabetic patients with history of myocardial infarction.

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Original Article

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Study of Glycosylated Hemoglobin Levels in Iron Deficiency Anemia

Raviraj Naik[1], Sarita Dakhure[2]

Author's Affiliation: [1]Assistant Professor; Department of Biochemistry, [2]Assistant Professor; Department of Pathology; Government Medical College, Aurangabad, Maharashtra 431004, India.

Abstract

The aim of present study was to study the levels and analyze the variation in glycosylated hemoglobin (HbA1c) levels in Iron Deficiency Anemia (IDA).This study was conducted on 50 cases who were diagnosed to be having iron deficiency anemia and 50 age matched healthy controls which are not having any form of anemia. The levels of HbA1cwas significant increased in cases ofiron deficiency anemia as compared to those in the healthycontrols. There were no differences in the random glucose levels between the anemic and healthy subjects. It is not only the blood sugar levels that affects HbA1c levels but it also may be affected by other factors such as hemoglobinopathies and anemia among which most common in India is iron deficiency anemia. Hence, it very vital to be have an estimate of iron levels in the blood so as to be able to take therapeutic decision to treat diabetes based on HbA1c levels. Therefore, it is always advisable to correct any iron deficiency before any diagnostic or therapeutic decision is made.

Keywords: Glycosylated Hemoglobin; HbA1c; Iron Deficiency Anemia; Diabetes Mellitus.

Introduction

Glycosylated hemoglobin is is used in clinical practice for having an estimate of glycemic control of last 2-3 months [1]. It is not only the blood sugar levels that affects HbA1c levels but it also may be affected by other factors such as hemolytic anemias [2], hemoglobinopathies, acute and chronic blood loss [3] pregnancy [4], and uremia [5]. Vitamin B_{12}, folate. Apart from this most common cause of anemia in India which is iron deficiency anemiahave also been found to affect HbA1c levels. Iron deficiency anemia is the most common form of anemia in India [6]. One can label a subject as suffering from iron deficiency anemia if there is presence of atleast two following parameters which can also be called as indicatorssuch as serum ferritin, transferrin saturation, total iron binding capacity and peripheral blood smear picture of microcytic hypochromic anemia [7]. There had been different research studies which elaborated on relationship between iron deficiency anemiaand HbA1c.These were Brooks *et al. [8]* Sluiter*et al. [9]* and Mitchell *et al. [10]* who reported positive correlation between changes in iron levels and HbA1C variations. However, there were some researchers who contradicted with researchers mentioned above and these were Von Heyningen *et al. [11]* and Gram-Hansen *et al. [12]* But there were other studies have shown that reduced iron levels were associated with increased levels of HbA1c [8]. Inspite of iron deficiency anemia being the most common nutritional anemiathere have been many evidences of inconsistency in relation of iron and HbA1c levels [13].

Since the earlier results on the relation between HbA1c and iron deficiency anemia were inconsistent and the exact mechanism remained

Reprint Request: Sarita Dakhure, AAssistant Professor; Department of Pathology; Government Medical College, Aurangabad, Maharashtra 431004, India.
Email: raviraj_40@yahoo.com

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unclear, we have attempted to perform this study to investigate the effects of Iron Deficiency anemia on HbA1c levels.

Material and Methods

We conducted this study at GMC; Aurangabad in department of pathology during January 2016 to May 2017.5ml blood samples were obtained by venepuncture from 50 anaemic patients aging 45±5 years, and 50 age-matched healthy controls. Among the iron deficiency anemia group 18 were males and 32 were females.The study subjects were enrolled from the medicine outpatient department of our institute. All the study subjects(cases & controls) underwent following investigations; blood hemoglobin (Hb) concentration, CBC, serum ferritin and TIBC, PS, random blood glucose and glycosylated hemoglobin.The HbA1c levels were determined by sysmex HbA1c analyser. The peripheral blood smears were examined in all the patients. Estimation of blood sugar was done on fully automated transasiaautoanalyser. Patients having diabetes mellitus and any type of hemoglobinopathies were excluded from our study. All the results were presented as mean± S.D. Significant differences were evaluated using students t-test when p≤0.05.

Observation & Results

Table 1:

Sr.No	Parameters	Normal Subjects (n = 50) Mean ± S.D.	IDA subjects(n = 50) Mean ± S.D.	Significance
1.	Hb(gm/dl)	12.66 ±0.42	9.17 ±1.84	p< 0.001
2.	Serum Ferritin	0.08 ±0.76	0.06±0.01	p < 0.001
3.	TIBC	0.42 ±0.16	0.56 ±0.03	p < 0.001
4.	Glycosylated Hb	5.3 ±0.09	6.4 ±0.72	p < 0.001
5.	Random Glucose	124 ± 7.68	130± 4.71	p > 0.05

All the parameters which were tested in both the groups have been reported in table above.The peripheral blood smears showed a microcytic hypochromic picture. The HbA1c levels were significantly increased among the iron deficiency anemia patients as compared to those in the controls. There were no differences in the levels of random glucose levels between the subjects of iron deficiency anemia and the control groups (p > 0.05).

Discussion

In the present study it was observed that HbA1c concentrations were found to be higher in the subjects with iron deficiency. Our study results were consistent with one of the study reported in pastby Brooks et al. [8] who analyzed HbA1C levels in 35 non-diabetic patients having iron deficiency anemia both before and after treatment with iron. They concluded that HbA1C levels insubjects with iron deficiency anemia were significantly higher as compared to healthy controls. They also reported that HbA1C levels decreased when the subjects were treated with iron supplements. They postulated the following mechanism of increase in HbA1c levels in iron deficiency anemia subjects in which they stated that, iron deficiency resulted in alteration of the structure of the hemoglobin molecule and it also leadedto rapid glycation of the globin chain as compared to normal scenoario [8]. Another study reported by Sluiter et al. [9] attempted to justify the rising trend of Hba1c in iron deficiency anemia in which they proposed that there is linear relationship between HbA1c concentration and RBC age. They also reported that in iron deficiency anemia although the formation rate of RBC decreases but there is increase in average lifespan of circulating erythrocytes so it ultimately results in more glycation and thus levels of HbA1c rises [9]. Some other researchers also agreedwith the studies done by Brooks etal and Sluiteretal such as studies by El-Agouza et al. [14] and Cogan et al. [15] who in their respective study concluded that in iron deficiency anemia resulted in significantly increased levels of HbA1C and also reported that these increased Hba1c levels normalized after treatment with iron supplements. They tried to justify their observation by arguing that elevated HbA1c levels in iron deficiency anemia could be explained by the assumption that if serum glucose remains constant, a decrease in the hemoglobin concentration might lead to an increase in the glycated fraction. In contradiction to above mentioned studies there were some researchers who reported otherwise. Among them first study conducted was by Mitchell et al. [10] who analyzed the absolute concentration of HbA1c in each erythrocyteand concluded that HbA1c levels before and after iron treatment did not change.

They also studied research work done by Sluiter et al. and commented that erythreocyte lifespan was unlikely to be a significant factor in explaining the changes in HbA1c levels in iron deficiency anemia. As evident from the above studies, the exact mechanism through which iron deficiency anemia affects HbA1c levels still remains unclear. But we support the theories postulated by Brooks etal and Sluiteretal as we find factors mentioned above in their respective studies more logical reasoning for increased levels of HbA1c in subjects of iron deficiency anemia.

Conclusion

HbA1c levels were significantly increased in subjects having iron deficiency anemia.

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Original Article

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Assessment of Obstructive and Restrictive Impairments Among of Urban Population of Jaipur: A Survey Study

Sachin C. Narwadiya[1], Gulshan J. Karhade[2], P.M. Tumane[3]

Author Affiliation: [1]Scientist C, Vigyan Prasar, A-50, Instituional Area, Sector 62, Noida, Uttar Pradesh 201 309, India. [2]Laboratory Technician, Regional Ayurveda Research Institute for Endocrine Disorders, Indira Colony, Banipark, Jhotwara Road, Jaipur, Rajasthan 302 016, India. [3]Associate Professor, Department of Microbiology, RTM Nagpur University, Nagpur, Maharashtra 440 033, India.

Abstract

The Spirometry is the test usually used to diagnose the capacity of lungs. Spirometry which signifies meant the measurement of breath. The Spirometry is routinely performed as part of the pulmonary function tests (PFTs).It is used to measure the Lung Function with specific importance to the amount (volume) and/or speed (flow) of air that can be inhaled and exhaled. The used of this test involved in assessing breathing patterns that identify conditions such as asthma, pulmonary fibrosis, cystic fibrosis, and Chronic obstructive pulmonary disease (COPD). It is also helpful as part of a system of health surveillance, in which breathing patterns are measured over time. The present study focused on the patterns of the Obstructive and Restrictive Impairments among of urban population of Jaipur. The patients selected specifically from the visiting patients with some symptoms to the health camp. In this camp the assessment of Lung Functions was done by using the RMS Helios 401 PC based Spirometer. The parameters, as per the manual are used for the diagnosis of the Obstructive and Restrictive Impairments. The parameter assessed includes Age, Height, Weight, Gender, Smoker/ Non-Smokar, FVC Pred, FVC (M. Pred),% Pred. M.,% Pred, Lung Age (P),Lung Age (M). As per the instrument manual restrictive stage COPD as FEVI/FVC>=70%and FEV 1<80% . In the present study the COPD before the medication mentioned as pre-test is found to be 144 patients in the total numbers of 164 which accounts for 87.80%.

Keywords: Spirometry; Lungs; COPD; FVC; FEVI.

Introduction

Spirometry is the technology used for the detection of Lung Functions. The various indications for the use of spirometry are early detection of asthma and COPD, evaluation of the relationship between flow and volume, measurement of the degree of airflow obstruction and variability, severity of lung disease, assessment of response to therapy, to provides education and feedback for patients, preoperative evaluation. Lung functions tests are routinely performed for the estimation of Pulmonary Functions which include both physiological and pathological which can be responsible for alter in Lungs functions as well. Lung functions were prescribed when physician or pulmonologists observed the related symptoms and the tests include the estimation of lung volumes are forced vital capacity (FVC), forced expiratory volume in the first second (FEV1) and peak expiratory flow rate (PEFR) (Nku CO *et al.* 2006)

The peak expiratory flow rate (PEFR), residual volume (RV), functional residual capacity (FRC) and FEV1 expressed as a percentage of FVC are the various parameter used to differentiate between obstructive and restrictive impairments (Donnelly PM *et al.*1991) .

Aims and Objectives

To perform PFT in normal persons (control group)

Reprint Request: Sachin C Narwadiya, Scientist C, Vigyan Prasar, A-50,Instituional Area, Sector 62, Noida, Uttar Pradesh 201309, India.

Email: snarwadiya@gmail.com

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and having symptoms of impairments (study group)

To compare the values of PFT parameters in normal persons (control group) and having symptoms of impairments (study group)

Materials and Methods

Selection of Subjects

The study was undertaken during the Health camp organized in the urban area of Jaipur. The selection is based on the medical history taken by the Doctor and referred for the PFT.

Inclusion Criteria

Healthy subjects with

a) No previous history of upper respiratory tract infection within 3 months (Fulambarker A, *et al.*, 2004).

b) No history of asthma or bronchitis in the subjects as well in their family

c) No other clinically detected medical illness

d) No history of smoking

Exclusion Criteria

Subjects who were smokers (Fulambarker A, *et al.*, 2004), have had history of respiratory disorders or diseases like tuberculosis, congenital cardiac disorders and musculoskeletal deformity of chest wall were excluded.

On selection through the above inclusion criteria, their socio demographic data was recorded. The patients and normal persons underwent general physical examination and thorough clinical examination of respiratory system to rule out significant pre-existing pathology which may influence the study parameters.

All normal persons and patients physical characteristics like height and weight was measured and recorded. Body surface area will be calculated for each person, by the software incorporated in the spirometer. PFT was assessed in the subjects selected. The test was done on a normal survey day. Lung function parameters, i.e, FEV1, FVC, FEV1/FVC ratio were measured using the below described instrument, by the below described method.

Instrument: The Spirometer used for this study is RMS Helios Spirometer- 401.

Procedure of Recording

Pulmonary function test (PFT) parameters, viz. Inspiratory reserve volume (IRV), Expiratory reserve volume (ERV), Forced vital capacity (FVC), Forced expiratory volume in the first second (FEV1) and FEV1/FVC ratios were recorded using computerized spirometer- RMS Helios 401.

The persons were asked to perform the PFT at least three times to observe FVC, FEV1, FEV1/FVC%. After appropriate coaching, the best of three technically acceptable attempts were recorded and the best of the three results were considered for analysis. Subjects were instructed to practice the maneuver before being attached to the instrument. To achieve good results before the test, the subjects were familiarized with the machine and the detail instructions and demonstration up to the satisfaction were done (Vijayan VK *et al.* 1990). The persons were asked to loosen tight clothing and were seated comfortably erect with feet firmly on the floor (the most comfortable position, though standing gives similar results in adults). A nose clip was applied to the person's nose. Then, the person was asked to breathe in fully.

The following precautions were observed while doing the test

- Seal his lips around the disposable mouth piece.
- Blast air out 'As fast as far as he can' until the lungs are completely empty.
- Breathe in again as forcibly and fully as possible.
- Inspiration should be full and unhurried and expiration once begins should be continued without a pause.

The best report out of the several blows ranging from 3 to 4 was selected. The FEV1 between the highest and second highest result value was considered.

The largest of three FVC and FEV1 values were accepted even if the two volumes do not come from the same curve. The ratio of FEV1 to FVC were expressed as a percentage (Vijayan VK *et al.* 2000) .

The largest volume was quoted. The following guidelines were used for the manoeuvre performance.

- Minimum of 3 acceptable blows.
- Rapid start is essential.
- A minimum exhalation time of 6 seconds

- Spirometer temperature being 17 to 40®C.
- Take largest FEV1 even if not from the same curve as the best FVC.
- Smooth, rapid take off with no hesitation, cough, leak, tongue obstruction, glottis closure, etc.
- Reproducibility: the highest and the second highest FEV1 should agree to within 0.2 L.

Spirometer was calibrated periodically with an accurate 3 liters syringe. The persons were asked to take a deep breath until he/she breathes in up to total lung capacity (TLC) and close the lips around the mouth piece, and to breath out as fast as possible (up to residual volume), and finally breathe it all in again as fast as possible to TLC.

The values for Age, Height, Weight, Gender, Smoker/ Non-Smoker, FVC Pred, FVC (M. Pred), FVC%, Lung Age (P),Lung Ag e(M) for each person thus obtained was entered in the proforma and tabulated. Suitable statistical methods were applied using Microsoft Excel to analyze the data, such as, mean, standard deviation.

Results

Table 1: Showing the Age Group classification and patients with Restrictive Impairments

Age Group	Numbers	Percent of total persons under study	Number of normal persons	Number of Restrictive Impairments	Percent of Restrictive Impairments
11-20	11	6.14	02	09	81.81
21-30	61	34.07	09	52	85.24
31-40	25	13.96	02	23	92.00
41-50	43	24.02	02	41	95.34
51-60	16	8.93	03	13	81.25
61-70	8	4.46	02	06	75.00
Control	15	8.37	15	00	00

Table 2:- Showing the Means of Age, BMI and smoking details

Age	Height Mean±SD	Weight Mean±SD	Gender Mean±SD M*	F*	Body Mass Index Mean±SD	Smoker/ Non-Smokar Mean±SD
35.1± 11.7	1.626± 0.092	1.626± 0.092	113	66	23.54± 4.66	Nil

*M-Male and F-Female

Table 3:- showing the Mean PFT parameters of FVC, FEV and Lung Age.

FVC -M Mean±SD	FVC (Pred) Mean±SD	% FVC Mean±SD	FEV-1(M) Mean±SD	FEV-1(P) Mean±SD	Lung Age(P) Mean±SD	Lung Age(M) Mean±SD
2.46±0.76	4.10± 2.82	63±13	2.62±0.94	3.29±0.65	35±12	48±20

Discussion

The results obtained are suggestive of the group of persons under study are suffering from restrictive impairment as per the manual restrictive stage COPD as FEVI/FVC>=70% and FEV1<80% was considered for evaluation. The average age groups of the persons under this study is 35 which indicate the subjects were not too old and in middle age. The BMI suggests that 23.54 suggestive of the group of persons were not in the category of obese. The Lung age seems to be the 10+ years older than that of the average age predicted for the Lungs i.e 35 indicating the affected lungs. The Forced Vital Capacity was just lesser than the normal of the whole group when compared with the predicted one indicating that major numbers of persons under study were suffering from COPD. The group has maximum percentage of the 31-40 years age group. The quiet young aged groups too have more Lung age as measured by the instrument Helios 401. All the subjects under the present study and control are non-smoker thus occurrences of Restrictive

Fig. 1: The Helios 401 Spirometer

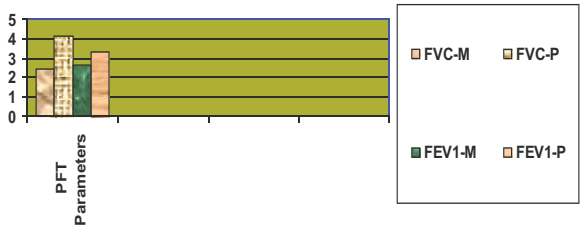

Fig. 2: PFT Parameters FVC and FEV1-P and M

Impairments through cause of smoking can not be considerable. In the age groups of 31-40 and 41-50 were the Percent of Restrictive Impairments were quite high i.e 92 and 95 percentage respectively. In a restrictive lung disease, the size of the lung is reduced, which increases the stiffness of the lung and limits its expansion. In these cases, a greater pressure (P) than normal is required to give the same increase in volume (V). Common causes of decreased lung compliance are pulmonary fibrosis, pneumonia and pulmonary edema. (http://www.ugr.es)

Conclusion

The Lungs related diseases like Restrictive Impairment slowly increasing in the society. It is depending on the quality of air inhaled, working profile, diet, exercise and many more factors. The present study showcased that when the population under study has normal levels of the BMI they suffered from older lungs and decreased FVC and FEV1. The study also showed that the FVC readings are more on abnormal side as compared to that of the FEV1. The Figure 2 depicted that the Predicted FEV1 and FVC are higher and the measured is lower. This shows that the majority of the subjects under study suffering from COPD. The dust level of the area where population residing can be a factor to be taken into consideration as one of the recent news also shown that Air Quality Index (AQI) measured in Jaipur was at 383 (very poor), a notch below the highest category of `severe' which is far above than the desired level of 100 AQI. The desired level of AQI is 100. During this hazardous situation the peoples became more prone to heart or lung diseases. (https://timesofindia.indiatimes.com, 2018)

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