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Studies on random blood sugar (RBS) levels of diabetes mellitus individuals subjected to a biochemical method - o- toluidine method

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Abstract
According to Ayurveda diabetes mellitus (Madhumeha) is mainly Kapha dosha. Diabetes mellitus is a group of metabolic disorders with one common manifestation – hyperglycemia. India is one of the main country which is having high % of diabetic patients. According to Ayurveda diabetes mellitus (Madhumeha) can not be completely cured but can be controlled by life style habits, food management and medication. There are two types of Diabetes mellitus: Type 1 Diabetes mellitus (T1DM) and Type 2 Diabetes mellitus (T2DM). T1DM is an autoimmune condition. In people with T1DM, the damaged pancreas doesn't make Insulin. So, T1DM is known as Insulin-Dependint-Diabetes (IDDM). The most common form of Diabetes is T2DM account for 95% of Diabetes cases in adult. T2DM is also called Non-Insulin- Dependent Diabetes (NIDDM). With T2DM, the pancreas usually produces some Insulin. But either the amount produced Insulin is not enough for the body's need, or the body's cells are resistant to it. Insulin resisrance or lack of sensitivity to Insulin. A Random Blood Sugar (RBS) meas that it can be done at any time, under any conditions either fasting or after meals. The RBS test measures the amount of glucose in the blood at any given time of diabetic patient. The reference range of random blood sugar (RBS) is 80-140 mg%.
Random Blood Sugar (RBS) levels of 3 diabetic individuals was estimated by O-Toluidine method. Results of O-Toluidine method in relation to Random Blood Sugar (RBS) levels of diabetic individual - A, B and C is 225 mg %, 327 mg % and 272 mg % respectively.
RBS levels of diabetic persons B 327 mg % > C272 mg% > A 225 %.

Keywords: random blood sugar, mellitus individuals, biochemical

Introduction
Sushruta most ancient Indian ayurvedic scholar have grouped madhumeha (Diabetes mellitus) under one among the 20 prameha (Urinary disorders). Prameha are a list of urinary disorders, especially characterized by profuse urination with several abnormal qualities. Any one of the prameha if neglected ultimately it ends up in madhumeha. According to Ayurveda diabetes mellitus (Madhumeha) is mainly Kapha dosha. In the present stressful modern living the incidence of Diabetes mellitus is increasing day by day. Previously the onset of the disorder diabetes mellitus was noted at the age of 40 years and above, but due to utter negligence in health rules plenty of diabetes mellitus cases are noticing is still early age groups and especially in children also. India is one of the main country which is having high % of diabetic patients. According to Ayurveda diabetes mellitus (Madhumeha) cannot be completely cured but can be controlled by life style habits, food management and medication. Before the discovery of insulin in the early 1920s and the later development of oral hypoglycaemic agents, the major form of treatment of diabetes mellitus involved starvation, dietary manipulation and the use of plant therapies (Baily and Day, 1989 and Bailey & Flatt, 1990) [2, 3]. Diabetes mellitus today is recognized as an epidemic disease in most countries that are undergoing socioeconomic transitions [Duyff, Roberta, 2002] [4]. Population growth, aging, urbanization, low physical activity, modern life style habits and obesity are main causative factors of high prevalence of diabetes mellitus (Wild *et al*., 2004) [10]. The current scenario of diabetes mellitus is likely to worsen in coming decades. The most disturbing trend is the shift in age of onset of diabetes to a young age in the recent years (American Diabetic Association, 2006) [1]. Diabetes mellitus is a group of metabolic disorders with one common manifestation – hyperglycemia (WHO, 1980; WHO, 1985) [8, 9]. Chronic hyperglycemia causes damage to eyes, kidneys, nerves, heart and blood vessels (Mayfield, 1998) [6]. More recent studies have confirmed the antihyperglycaemic effect of Coriandrum sativum (Coriander) in streptozotocin-diabetic mice (Swanston-Flatt *et al*. 1990) [7]. Anti-diabetic agents can exert beneficial effects in the diabetic environment by improving and/ or mimicking insulin action and/or by enhancing insulin secretion (Gray & Flatt, 1997b) [5].

Diabetes mellitus is a group of chronic metabolic conditions, all of which are characterized by elevated blood glucose levels resulting from the body's inability to produce insulin or resistance to insulin action, or both. (American Diabetes Association, 2006) [1]. This group of conditions can be subdivided manly into 2 clinically distinct types:

1. Type 1 Diabetes (T1DM), which results from autoimmune beta-cell destruction in the pancreas and is characterized by a complete lack of insulin production;
2. Type 2 Diabetes (T2DM), which develops when there is an abnormal increased resistance to the action of insulin and the body cannot produce enough insulin to overcome the resistance (American Diabetes Association, 2006) [1].

Diabetes mellitus is now approaching epidemic proportions. (Zimmet *et al*., 2001) [12]. In the United States, the prevalence and incidence of diabetes have increased dramatically during the past 2 decades (Zimmet *et al*., 2001) [12]. Diabetes can affect many different organ systems in the body and, over time, can lead to serious complications. Complications from diabetes can be classified as microvascular. Microvascular complications include nervous system damage (neuropathy), renal system damage (nephropathy) and eye damage (retinopathy). (American Diabetes Association, 2006) [1]. Macrovascular complications include cardiovascular disease, stroke, and peripheral vascular disease. Peripheral vascular disease may lead to bruises or injuries that do not heal, gangrene, and, ultimately, amputation. smoking has been shown to be an independent risk factor for diabetes **(**Will *et al*.,2001) [11]. Effective treatment was not developed until the early part of the 20th century, when Frederick Banting and Charles Herbert Best isolated and purified insulin**.** Insulin was first used as a medication to treat diabetes mellitus in Canada by Frederick Banting and Charles Herbert Best in 1922. It is on the World Health Organization's List of Essential Medicines the most effective and safe medicines needed to treat diabetes mellitus in a health system.

Methodology

Method: O-Toluidine Method

Estimation of Random Blood Sugar (Rbs) By O-Toluidine Method

AIM: To estimate the concentration of Random Blood Sugar (RBS) in the given test sample of serum by O-Toluidine method (Marks, 1959).

Principle: Glucose condenses with O-Toluidine in glacial acetic acid when heated at 100 C. The product is a blue-green N – glycosylamine.

The intensity of the colour is propotional to the concentration of glucose present in the test sample. The colour so produced is measured at 630 nm.

Material

1. O-Toluidine reagent (OTR)
2. Glucose standard
3. Distilled water
4. Serum sample
5. Glass ware and
6. Colorimeter.

O-Toluidine Method: Tables and Figures

S.no	Vol. of Serum ml	Vol. of Glucose Standard ml	Conc. of Glucose Standard ml	Vol. of OTR ml	Vol. of DW ml	OD At 630 nm
B	----	-----	----	3 ml	0.1	0
S1	---	0.1 ml	0.1 mg	3 ml	---	0.04 nm
S2	----	0.2 ml	0.2 mg	2.9 ml	----	0.08 nm
S3	----	0.3 ml	0.3 mg	2.8 ml	----	0.11 nm
S4	----	0.4 ml	0.4 mg	2.7 ml	---	0.15 nm
S5	----	0.5 ml	0.5 mg	2.6 ml	----	0.19 nm
Individual: A \Serum Test Sample	0.1 ml	----	---	3 ml	----	0.09 nm
Individual: B \Serum Test Sample	0.1 ml	-----	----	3 ml	----	0.12 nm
Individual: C \Serum Test Sample	0.1 ml	-------	------	3 ml	-----	0.10 nm

All tubes are mixed well and put them in boiling water bath at 100 C for 12 minutes. After boiling tubes are removed, cooled in water at room temparature. Then the OD of Stds and test measured against blank at 630 nm

Calculation

The concentration of glucose in the given serum sample:

$$\frac{\text{OD of test}}{\text{OD of Std}} \text{ X Conc. Of Std } \text{ X} \frac{100}{\text{vol. Of serum test}}$$

Individual: A

Concentration of Random Blood Sugar in the given serum sample of Individual: A

OD of TEST = 0.09
OD of Std = 0.08
Conc. Of Std = 0.2 mg

Vol. of serum (Test) = 0.1 ml

$$\frac{0.09}{0.08} \text{ X } 0.2 \text{ X } \frac{100}{0.1} = 225 \text{ mg \%}$$

Concentration of Random Blood Sugar (RBS) in the given serum sample of Individual: A = 225 mg%

Individual: B

Concentration of Random Blood Sugar in the given serum sample of Individual: B

OD of TEST = 0.12
OD of Std = 0.11
Conc. Of Std = 0.3 mg

Vol. of serum (Test) = 0.1 ml

$$\frac{0.12}{0.11} \text{ X } 0.3 \text{ X } \frac{100}{0.1} = 327 \text{ mg \%}$$

Concentration of Random Blood Sugar (RBS) in the given serum sample of Individual: B = 327 mg%

Individual: C
Concentration of Random Blood Sugar in the given serum sample of Individual: C

OD of TEST = 0.10
OD of Std = 0.11
Conc. Of Std = 0.3 mg

Vol. of serum (Test) = 0.1 ml

$$\frac{0.10}{0.11} \text{ X } 0.3 \text{ X } \frac{100}{0.1} = 272 \text{ mg \%}$$

Concentration of Random Blood Sugar (RBS) in the given serum sample of Individual: C = 272 mg%

Type of Individual	OD of Test nm	OD of Std nm	Conc. Of Std mg	Vol. of Serum Test ml	Conc. Of RBS in the Serum Sample mg %
Individual: A	0.09 nm	0.08 nm	0.2 mg	0.1 ml	225 mg %
Individual: B	0.12 nm	0.11 nm	0.3 mg	0.1 ml	327 mg %
Individual: C	0.10 nm	0.11 nm	0.3 nm	0.1 ml	272 mg %

Result and discussion

Result

Diabetes mellitus is characterized by Hyperglycemia due to insufficient or inefficient insulin. To maintain normal blood sugar levels insulin inhibits glycogenolysis, gluconeogenesis and promotes glycogenesis in case of healthy person. In case of diabetic patients, defeciency of insulin promotes glycogenolysis and gluconeogenesis results hyperglycemia. The fasting blood glucose level in a post-absorptive state is 70-100 mg/ dl (plasma glucose 80-120 mg/ dl). Following the ingestion of a carbohydrate meal blood glucose may rise to 120-140 mg/dl. Glucose is continuously filtered by the glomeruli, reabsorbed and returned to the blood. If the level of glucose in blood is above 160-180 mg/dl, glucose is excreted in urine (Glycosuria). This value160-180 mg/dl is referred to as renal threshold for glucose. The maximum ability of the renal tubules to reabsorb glucose per minute is known as tubular maximum for glucose (TmG). The value for TmG is 350 mg/minute. Insulin is a hypoglycemic and anti - diabetic hormone that lowers in blood glucose level. A Random Blood Sugar (RBS) meas that it can be done at any time, under any conditions either fasting or after meals. The RBS test measures the amount of glucose in the blood at any given time of diabetic patient. The reference range of random blood sugar (RBS) is 80-140 mg%.

To estimate the Random Blood Sugar (RBS) levels of diabetes mellitus individuals O-Toluidine method is used. 3 diabetic individuals are selected. Random Blood Sugar (RBS) levels of 3 diabetic individuals was estimated by O-Toluidine method as folloes:

1. Random Blood Sugar (RBS) levels of diabetic individual: A = 225 mg %
2. Random Blood Sugar (RBS) levels of diabetic individual: B = 327 mg %
3. Random Blood Sugar (RBS) levels of diabetic individual: C = 272 mg %

Results of O-Toluidine method in relation to Random Blood Sugar (RBS) levels of diabetic individual -A, B and C is 225 mg %, 327 mg % and 272 mg % respectively.

Random Blood Sugar (RBS) levels of diabetic individual B > C > A.

Random Blood Sugar (RBS) levels of diabetic individual A, B and C is 327 mg % > 272 mg % > 225 mg %.

Discussion

There are two major types of Diabetes mellitus: Type 1 Diabetes (T1DM) and Type 2 Diabetes (T2DM). Type 1 Diabetes is also called insulin-dependent diabetes (IDDM) and juvenile-onset diabetes, because it often begins in childhood. Type 1 Diabetes is an autoimmune condition. It's caused by the body attacking its own pancreas with antibodies. In people with Type 1 Diabetes, the damaged pancreas doesn't make insulin. The most common form of diabetes is type 2 Diabetes, accounting for 95% of diabetes cases in adult. Type 2 diabetes is also called Non-insulin-dependent diabetes (NIDDM) and Adult-onset diabetes. With Type 2 diabetes, the pancreas usually produces some insulin. But either the amount produced insulin is not enough for the body's needs, or the body's cells are resistant to it, Insulin resistance, or lack of sensitivity to insulin.

Glucose (normal fasting blood level 70-100 mg/dl) is the central molecule utilized as a source of energy. An adult Human body contains about 18 g free glucose. This amount is sufficient to meet the basal energy requirements of the body for 1hr. The liver has about 100 g stored glycogen. It is capable of producing about 180-220 g/24 hrs. The fasting blood glucose level in a post-absorptive state is 70-100 mg/ dl (plasma glucose 80-120 mg/ dl). Following the ingestion of a carbohydrate meal, blood glucose may rise to 120-140 mg/dl. About 160 g of glucose per day is needed by the entire. When the body is at total rest, about two-thirds of the blood glucose is utilized by the brain. The remaining one-third utilized by RBC and skeletal muscle. Kidney plays a special role in the homeostasis of blood glucose. Glucose is continuously filtered by the glomeruli, reabsorbed and returned to the blood. If the level of glucose in blood is above 160-180 mg/dl, glucose is excreted in urine (Glycosuria). This value (160-180 mg/dl) is referred to as renal threshold for glucose. The maximum ability of the renal tubules to reabsorb glucose per minute is known as tubular maximum for glucose (TmG). The value for TmG is 350 mg/mi. Diabetes mellitus is associated with two important metabolic alterations i.e., hyperglycemia and ketoacidosis.

Elevation of blood glucose concentration is the hallmark of uncontrolled diabetes. Hyperglycemia is due to reduced glucose uptake by tissues and its increased production via gluconeogenesis and glycogenolysis. When the blood glucose level goes beyond the renal threshold, glucose is excreted into urine (Glycosuria). Increased mobilization of fatty acids results in over production of ketone bodies (Acetoacetate and beta-hydroxybutyrate) which often leads to keto acidosis. Further, the volume of plasma in the body is reduced due to dehydration caused by the excretion of glucose and ketone

bodies. Diabetic ketoacidosis is dangerous may result in coma, and even death, if not treated. Administration of insulin is necessary to stimulate uptake of glucose by tissues and inhibition of ketogenesis.

Diet, exercise, Oral Hypoglycemic Drugs (OHD) and finally insulin are the management options in diabetes. A diabetic patient is adviced to follow dietary management i.e., consume low carbohydrate and fat, high protein and fiber rich diet. Refined sugars (sucrose, glucose) should be avoided. Diet control and exercise will help to a large extent obese NIDDM patients. The oral hypoglycemic drugs are broadly of 2 categories namely sulfonylureas and biguanides. The latter are less commonly used due to side effects. Sulfonylureas such as acetohexamide, tolbutamide and gibenclamide are frequently used. They promote the secretion of endogenous insulin and thus help in reducing blood glucose level. OHD Medications used to treat diabetes do so by lowering blood sugar levels. There are a number of different classes of anti-diabetic medications. Some are available by mouth, such as metformin, is generally recommended as a first line treatment for type 2 diabetes, as there is good evidence that it decreases mortality. It works by decreasing the liver's production of glucose.

Insulin is a hypoglycemic and anti - diabetic hormone that lowers in blood glucose level. The net effect is that insulin lowers blood glucose level (Hypoglycemic effect) by promoting its utilization, storage and by inhibiting its production. This relation is severely impaired in diabetes mellitus. About 40-50 IU of insulin is secreted daily by beta cells human pancreas. The normal insulin concentration in plasma is 20-30 IU. In plasma, insulin has a normal half -life of 4-5 minutes. One international unit of insulin (1 IU) is defined as the "biological equivalent" of 34.7 μg pure crystalline insulin. Insulin is used as a medication to treat high blood sugar in type 1 and type 2 diabetes. Insulin cannot be taken orally at the present time. Insulin is usually taken as subcutaneous injections by single use syringes with needles, an insulin pump, or by repeated-use insulin pens with needles.

The glucose transporters are responsible for the insulin mediated uptake of glucose by the cells. Glucose is the most important stimulus for insulin release. It is more predominant when glucose is administered orally. A rise in blood glucose level is a signal for insulin secretion. Glucose combines with a receptor and stimulates insulin release.

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