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Original Research Article

Changing Trends Of Plasma Glucose And Serum Insulin Levels Among Adult Males Of Native *Mising* Population Of Assam: A Ten Year Follow Up Study

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ABSTRACT

Background: Fasting Insulin and Glucose levels are very good indicators of an individual's glucose metabolic status. Glucose metabolism among different ethnic groups may vary due to difference in genetic makeup as well as their food habits and lifestyles. A study was carried out on the "*Mising*" tribe of upper Assam a specific ethnic tribal population, on whom there is hardly any published data available.

Aim: To observe the trend of fasting Insulin, Glucose and Insulin: Glucose ratios in this unique population in 2003 and again in 2014, and to correlate the effect of changing lifestyle on them.

Methods: Fasting serum Insulin, plasma Glucose and Insulin: Glucose ratio were recorded for a group of 100 randomly selected non-diabetic males of the indigenous *Mising* tribe of upper Assam, of the age group 30-40 years. 50 among these 100 subjects could be traced again in 2014 and the same parameters were studied, after an interval of 10 years and compared with their present fasting Insulin and Glucose levels.

Result: The mean fasting plasma Glucose, serum Insulin and Insulin: Glucose ratios increased with the age of the subjects. This increase was found to be higher in subjects who had office jobs compared to the agrarian class.

Conclusion: Sedentary, so called urban lifestyle is associated with higher fasting Insulin levels, which might have a strong role in the etiopathogenesis of predominantly urban diseases like obesity, type 2 diabetes and cancers.

Key Words: Insulin, Insulin: Glucose ratio, Mising tribe.

INTRODUCTION

The Misings are an indigenous ethnic group of upper Assam have their own language and lifestyle. Very little published information is available on them. They are very hardworking, with agriculture and hunting as their mainstay activity. Obesity is not found in this population. This group was not influenced by the so-called urban easy going sedentary lifestyle and urban processed easily available food back in 2003. Over the next ten years or so, there has been a rapid change of lifestyle members of among some this community, where some of them had shifted to nearby towns and joined office work and their lives have been urbanized in several ways. In this study, we wanted to compare the fasting insulin, glucose and insulin:glucose ratios among the adult males of this community in 2003 with their values in 2014, and try to correlate the same with occupations their and changing lifestyles.

Insulin, the protein proved to have hormonal action, secreted by the islet of Langerhans of pancreas is directly related to several diseases. On one hand, ageing, poor diet, stress among other factors can deprive cells of Insulin sensitivity producing their Diabetes Mellitus, which is a group of diseases characterized by high level of glucose resulting from defect in insulin secretion, insulin action or both. The state of chronic hyperglycemia is a long disease with varied clinical term progress and manifestations and can be associated with serious complications and morbidity.

On the other hand, as a result of insulin resistance, the pancreas produces more insulin than normal producing a state of hyperinsulinemia. Insulin resistance and Hyperinsulinemia may lead to several problems, including elevated triglycerides, ^[1-4] low HDL, ^[5,6] type II diabetes ^[7-9] and obesity. ^[10-13]

Diabetes mellitus has emerged as the most prevalent metabolic and one of the major non-communicable diseases in India as well as in other developing and western countries with a 2 - 5%prevalence in most adult population.

The 'Misings' are one of the colorful Mongoloid Tribes of the North-East India. They have their own history of origin, socio-cultural life and language. They have the unique culture of making their dwellings on an elevated platform. This ethnic group from N-E region were previously known as 'Miris': they are mentioned as such in the list of schedule tribes of India under constitution order 1950. The present study was conducted among the male members of this group in Mising dominated villages of upper Assam, namely

- Natun ChiringGaon, P.O. & District Dibrugarh
- Pani Miri Gaon, P.O. Kolakhowa Chariali, District Dibrugarh

This initial study was conducted during the period June 2003 to April 2003 Department in the of Biochemistry, Assam Medical College and Hospital, Dibrugarh, Assam. As the proposed study was a prevalence study, a 10% sample from the targeted population was adequate to achieve the objective of the study. After knowing their total population, the study was carried out on 100 healthy males of the Mising tribe in the age group of 30-40 years in the two Mising dominated villages. Subjects with diabetes mellitus, any form of malignancy and first degree family history of diabetes mellitus were excluded. The eligible subjects were selected in a random process to be included in this study.

Ten years later, in July 2014, only 50 of the original 100 subjects, now between 40-50 years of age, could be traced back in the mentioned villages, and they were included in the follow up study. The rest 50 have either moved out of their village in search of jobs, or were

MATERIALS AND METHODS

not available/ willing for sample collection.

A home visit was made prior to sample collection and the purpose of visit and procedure of testing was explained. Informed consent was obtained from all individual participants included in the study, in accordance with the Helsinki declaration for human studies, 1975. The name, age, height, weight and other biophysical parameters, family history suggestive of Diabetes. Thyroid disorders. Pancreatitis, Jaundice and other relevant details were recorded in a proforma developed for the purpose.

Blood pressure was measured using a mercury sphygmomanometer in comfortable sitting position in the right arm.

Sample Collection and Biochemical testing:

Early morning fasting (at least 8 hours) venous blood sample was collected with aseptic precautions (A) 2ml venous blood in polystyrene tube of 25mm x 75mm size for insulin estimation and (B) 2ml venous blood in NaF vial for glucose estimation.

Sample after collection:

In the June 2003 study, for Insulin estimation, the serum was kept at room temperature and allowed to clot. Centrifugation was done, the serum was

collected in a separate labelled vial and Insulin was estimated by Radio Imuuno Assay (RIA) in RIA laboratory, Assam Medical College and Hospital, insulin Dibrugarh, with the Radioimmunoassay (RIAK-1) kit supplied by the Board of Radiation & Isotope Technology, V. N. Purav Marg, Mumbai-400 094.

Radioimmunoassay (RIA) for estimation of serum Insulin:

The Radioimmunoassay method is based upon the competition of unlabeled insulin in the standard or samples & radio-iodinated (I¹²⁵) Insulin for the limited binding sites on a specific antibody. At the end of incubation the antibody bound and free insulin is separated by the second antibody polyethylene glycol aided separation concentration method. Insulin of samples is quantitated by measuring the radioactivity associated with the bound fraction of sample and standards.

Reconstitution of Kit Reagent:

(a) Insulin Standard (Lyophilized): 5ml assay buffer was added and mixed gently. The insulin concentration in the constituted standard is 200μ U/ml. This is standard A. 5 more standard solution were prepared as shown in table 1:

Table 1: Reco	onstitution	of Standard	l solutio	ons

Insulin Standards	В	С	D	Е	F			
Standard A (ml)	1.0	0.5	0.5	0.5	0.3			
Assay Buffer (ml)	1.0	1.0	3.5	7.5	7.7.			
Insulin Concentration (µU/ml)	100.0	50.0	25.0	12.5	7.5			

- (b) Insulin Antisera: 100ml of assay buffer was added and mixed gently over a vortex mixer.
- (c) Insulin Free Serum: 2ml of double distilled water is added

and mixed gently over a vortex mixer.

- (d) Second Antibody: 10ml of assay buffer is added and mixed gently.
- (e) I¹²⁵ Insulin: 6ml of assay buffer is added and mixed gently.

(f) Control Serum (A&B): 0.5ml of double distilled water is added and mixed gently.

Radioimmunoassay Procedure:

The first step in the RIA procedure is to reconstitute the reagent and preparation of standard as we discussed earlier. Before starting the assay, all reagents and samples should be brought to room temperature. The assay is carried out as shown in the assay flow chart. 18 numbered assay tubes for the standard curve and four tubes beginning with 19th number for each clinical sample to be assayed. Calculation:

- 1. Counter background was subtracted from all the counts to get actual account.
- 2. Average of all duplicate counts were taken.
- 3. Total count, which is the average count of tubes 1 & 2, was found out.
- 4. Average count of tube 3& 4 is called 'Blank Count' was found out:

%Blank = $\frac{\text{Blank Count}}{\text{Total Count}} \times 100$

- 5. Subtracted blank count from average of all the duplicates to get the "Corrected Average Count".
- 6. Calculated zero standard binding or B_0 as follows: = Corrected average count of standard or sample x 100 %Β₀ Total Count 7. Calculated percentage binding of B/B_0 of all standards and samples: $%B/B_0 = \frac{\text{Corrected average count of standard or sample}}{\text{Corrected average count of tubes 5 & 6}} \times 100$

- 8. Standard curve was plotted as follows: %B/B0 on the logit and µU/ml of insulin on logarithmic scale of logit-log graph sheet.
- 9. Sample value was measured from standard curve obtained the above as µUnit per ml directly.

Fable 2	: Model	Calcula	tion for Sta	ndard	Curve f	or Insuli	in Assay	by Rac	lio Immuno	Assay.
									_	

Description	Tube No.	Counts/Min. (CPM)	AVG. Counts	$\% = \frac{B}{B}$	
Background		150			0
Total	1	29729	29664		
	2	29900			
Blank	3	210	198		
	4	186			
Zero standard	5	6566	6390		
	6	6612			
7.5 μu/ml	7	6150	5921	92	
	8	6088			
12.5 µu/ml	9	5888	5704	89	
	10	5916			
25 µu/ml	11	5395	5043	79	
	12	5088			
50 µu/ml	13	4388	4292	67	
	14	4592			
100 µu/ml	15	3166	3144	49	
	16	3518			
200 µu/ml	17	2386	1929	30	
•	18	1868			

Table 3: Assay Flow Chart for Insulin Assay by Radio Immuno Assay

Tube No.	Assay Buffer (ml)	Insulin Standard (ml)	Serum Sample (ml)		Insulin Free Sample (ml)	Insulin Antiserum (ml)		[125 Insulin (ml)		Standard Antibody (ml)	PEG (ml)
1,2	-	-	-	-	-		0.1	room	-	-	nints.
3,4	0.4	-	-	0.1	-		0.1	atı	0.1	0.1	20 n
5,6	0.3	-	-	0.1	0.1	uight	0.1	ours	0.1	0.1	re for
7,8	0.2	0.1 F	-	0.1	0.1	overn	0.1	3 h	0.1	0.1	eratur
9,10	0.2	0.1 E	-	0.1	0.1	4°C	0.1	for	0.1	0.1	tempe
11,12	0.2	0.1D	-	0.1	0.1	+2 to	0.1	tubes	0.1	0.1	oom 1 nin (
13,14	0.2	0.1C	-	0.1	0.1	tubes	0.1	the	0.1	0.1	s at r for 20
15,16	0.2	0.1 B	-	0.1	0.1	ll the	0.1	all	0.1	0.1	tube 500g
17,18	0.2	0.1A	-	0.1	0.1	ates a	0.1	bates	0.1	0.1	ll the e at 1
19,20	0.3	-	0.1	0.1	0.1	ncuba	0.1	incu	0.1	0.1	ceep a ne tub
21,22	0.3	-	0.1	-	0.1	Mix gently, I	0.1	Mix gently, temperature	0.1	0.1	Vortex and k Centrifuge tł

After centrifugation, the supernatant was decanted and radio activity count was done in the precipitate. Next the standard curve was drawn to determine the patient sample value.

Interpretation:

In interpreting serum insulin concentration absolute value are not very helpful. In normal subject when glucose concentration rise insulin level also increase and when plasma glucose concentration fall insulin release in inhibited. This means that serum insulin concentration must be interpreted in the light of the simultaneously determined glucose value. Thus, a "Normal" absolute insulin (value) level may be abnormal in the face of hypoglycemia, while high absolute levels may be appropriate if the glucose concentration is high. Hence, Insulin: Glucose ratio has been considered to be more specific.

Calculation:

Plasma Insulin/Glucose Ratio = $\frac{Serum Insulin (\mu U/ml)}{Plasma Glucose (mg/dl)}$ Normal Ratio ^[14]: 0.4 or < 0.4

Other Tests conducted with the collected samples:

A. For the 2014 study, for Insulin estimation, the serum was kept at room temperature and allowed to clot. Centrifugation was done, the

serum was collected in a separate vial and preserved at -20^{0} C at Department of Biochemistry, Assam Medical College, Dibrugarh. The sample collection of the entire study group was

done over a span of 4 days, then the collected serum were brought to Subharti Medical College, Meerut by air in portable vaccine carrier to avoid denaturation of and Insulin protein. was estimated in a ROBONIK ELISA washer and reader, using solid sandwich ELISA phase diagnostic kits (EIA 2935) from DRG Germany, on the same day. Estimation of Insulin by ELISA comparable and RIA have sensitivity and specificity.^[15]

B. For the estimation of blood glucose 2ml blood collected in NaF vials were centrifuged at 5000 RPM for 10 minutes and the plasma was used for estimation of Glucose by GOD-POD method, using Glucose estimation kit from *SPAN*, in a Semi-automatic analyzer within 6 hours of collection, at Dept of Biochemistry, Assam Medical College, Dibrugarh, for the 2003study, and in a fully automated Siemens Dimension RxL Max Clinical Chemistry Analyzer for the 2014 study.

RESULTS

The results of the survey of biochemical profiles on glucose and insulin in basal state among members of this isolated *Mising* population, are illustrated in the following tables.

The subjects pursuing any form of office job, were considered under the Service Group, while those who had farming in any form / hunting / fishing / gathering firewood from forests as their mainstay occupation, were included under the Cultivator group.

Table 4: The percentage of subjects who were working in farms or rearing animals decreased from 88 to 76%, while the percentage of subjects who switched to some form of office work increased from 12 to 24% over ten years.

Groups	Occupation of 100 subjects (2003)	%	Occupation of 50 subjects (2014)	%
Service	12	12	12	24
Cultivator	88	88	38	76



Table 5: Comparison of the fasting blood glucose of different occupational groups.

unter ent occupational groups.									
Groups	Total No of	%	FBG (2003)	FBG (2014)					
	cases		$mg/dl (\pm SD)$	mg/dl (±					
	= 50			SD)					
Service	12	2	83.83 ±7.09	107.92					
		4		±19.83					
Cultivator	38	7	82.18 ±6.58	96 ±9.23					
		6							

P<0.001(.0008) for Service group (2003 v/s 2014) P>0.05(6.135) for Cultivator group (2003 v/s 2014)

In 2003, the subjects between 30-40 years of age, did not have much difference in their fasting blood glucose, thev were from different though occupation groups. After ten years, in 2014, those who continued to be in service, were found to be having significantly higher fasting blood glucose (Table 5) and higher fasting Insulin levels (Table 6) compared to their farming counterparts. Compared to 2003, the increase in these values in 2014 is much more in the service group



than in those engaged in farming or animal rearing.



Groups	Total No of cases = 50	%	Fasting Insulin 2003 (µU/ml)	Fasting Insulin 2014 (µU/ml)
Service	12	24	15.08 (±8.93)	21.28 (±8.36)
Cultivator	38	76	9.74 (±7.12)	15.18 (±7.92)

P<0.001(.0003) for Service group (2003 v/s 2014) P>0.05(1.471) for Cultivator group (2003 v/s 2014)



Table 7: Con	nparison of Insulin: Glue	cose 1	ratios in different oc	cupational groups
Groups	Total No of cases= 50	%	Insulin: Glucose	Insulin: Glucose

	Groups	Total No of cases = 50	70	msunn: Glucose	msum: Giucos
				2003	2014
	Service	12	24	0.177 (±0.095)	0.201 (±0.083)
	Cultivator	38	76	0.119 (±0.079)	0.158 (±0.069)
1					

P>0.05 for Service group (2003 v/s 2014) P>0.05 for Cultivator group (2003 v/s 2014) The Insulin: Glucose ratio, though within the normal range (<0.4), there has been a recorded rise in 2014 compared to that in 2003, and the increment is more in the service group than among the cultivators (Table 7).

Table 8: Sul	Table 8: Subjects who converted to IFG & frank Diabetes in 10 years (of Total)						
Total cases	Conversion to IFG	%	Conversion to frank diabetic	%			
50	5	10	2	4			

Tab	le 9: Subjects v	who conve	erted to IFG & frank Di	iabeti	c in 10 years (among occupatio	nal grou	ips)
	Groups	Total cases	Conversion to IFG	%	Conversion to frank diabetic	%	
	Service	12	3	25	2	16	
	Cultivator	38	2	5	Nil	Nil	

None of the 100 subjects studied in 2003 were diabetic. However among 50 of them who were followed up in 2014, 5 had Impaired Fasting Glucose (3 from Service and 2 from Cultivator groups); and 2 had overt Diabetes, both from Service groups. (Table 8, 9) Thus the incidence of Impaired Fasting Glucose or overt Diabetes is more in the service group than among the cultivators.

DISCUSSION

This study is a unique work, since it is an endeavor to investigate the metabolic status of the indigenous ethnic native population of upper Assam- the simultaneously Mising tribe, by estimating Insulin and Glucose levels in the fasting state and follow it up after ten years. There are some studies done on their food habits and social setup, ^[16] but little information is available on the Insulin levels, Fasting blood glucose levels of this population.^[17] This study also throws light upon the ill effects of so-called urban easy going sedentary lifestyle, where food, water and other essentials are easily available; on metabolism of dietary carbohydrates.^{[18-} ^{20]} The population included in this study, is a typical example of hardworking people who are undergoing a rapid lifestyle change, from having farming/ hunting/ fishing as their mainstay

occupation, some are switching to office jobs, shifting to urban areas and adopting lifestyle which might involve more mental and psychological stress much less physical but activity. Probably as a consequence of this, we found a higher level of insulin hormone in these sub-groups, compared to their farming counterparts, similar to other [21-23] studies. Moreover. the socioeconomic status of cultivator group is also lower than the service group which may be another factor. Our results are similar to what the previous workers had shown that there exists an inverse relationship between socioeconomic status and the prevalence of diabetes.^[24]

Since we have excluded subjects with diabetes or with diabetic first degree relative, the fasting Glucose levels in all subjects studied in 2003 were within the normal reference range.

Ingestion and absorption of dietary Glucose triggers the release of a burst of the peptide Insulin which enables Glucose uptake by liver and muscle cells. Once glucose has been shifted inside cells for storage or energy release, insulin levels should drop sometimes to even undetectable levels. ^[25] Only a tiny amount of basal insulin is required for glucose homeostasis. ^[25] In our study, we found that among the Service group, the mean fasting Insulin

was much higher than that among the group doing mainstay farming or animal rearing, both in 2003 and in 2014. Moreover, after ten years, some subjects have developed hyperinsulinemia, 10 % developed Impaired Fasting Glucose and 4% had overt diabetes; findings are in line with those of other workers.^[19] Insulin being a strongly anabolic hormone, high levels can promote cell growth, predisposing to certain cancers. ^[26-30] Higher Insulin levels also trigger the synthesis of beta-amyloid proteins in brain and may contribute to the development of Alzheimer's disease. ^[31] Overproduction of Insulin is even a contributory factor to prostate enlargement because of its effects in promoting the overgrowth of prostate cells. ^[32] Therefore several of these so called urban diseases, may have a very intricate lifestyle based etiopathogenesis. A physically active lifestyle is an absolute must to prevent metabolic conditions like Insulin resistance, Hyperinsulinemia, Diabetes mellitus and obesity; or to delay the onset or slow the progression of degenerative diseases and cancer.

This study of Fasting Insulin and Glucose concentrations among adult males of the native Mising population of Assam is a preliminary one, carried out among a small section of *Mising* community. The study of the Fasting Insulin and Glucose levels can be further enriched by including the aspect of the change of dietary habits and urbanization of diet. Therefore it is thought that there are reasonable scope for further studies in this and other such isolated population groups, experiencing rapid lifestyle changes which would uphold new vistas for а better understanding of the etiopathogenesis and prevention of highly prevalent so

called urban non-communicable diseases like Diabetes Mellitus and Obesity.

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