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INVITED EDITORIAL

Eat, drink and live merry?

G. R. Sridhar

Int J Diab Dev Ctries. 2011 ; 31:1–3

Eat doesn't need explanation; drink doesn't always refer to alcohol; and live is the environment one resides. The parodied saying contains three of the crucial elements in our efforts to contain the diabetes juggernaut. Ten years after genomics threatened to overrun everything else, the excitement abated and clinical medicine still has the moorings in which it had evolved. This is nowhere more true than in diabetes mellitus.

To tackle obesity, insulin resistance and type 2 diabetes mellitus requires prevention by increasing the energy expenditure over the energy ingested, a straightforward thermodynamic principle that cannot be so easily translated to practice. Prevention studies all over the world have shown that increased physical exercise and appropriate nutrition are of prime importance. Theoretical underpinnings and now empirical data have suggested that built environment, or the environment in which people live is a principal facilitator or inhibitor of such behaviour.

The complex of obesity and diabetes mellitus is becoming more common, the primary reasons being environmental changes, of which stress and the consumption of food are important.

The prevalence of diabetes mellitus in rural India is lower than in the urban areas; in a study involving population above 24 years, (3294 men and 3714 women) the crude prevalence of diabetes was 2.8% (men at 3.31%, and women 2.34%). However, age-specific prevalence of diabetes between the ages of 45 and 64 was 4.69% [1].

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More bothersome, India is increasingly becoming urbanized along with the rest of the world [2]. The difference is only in degree and there is no convincing evidence that we will go against the trend.

Similarly, obesity is also becoming more common; Indians have the reputation of being 'thin but fat.' It is not commonly realized that obesity is more strongly linked to diseases than living in poverty, smoking or drinking [3].

Many layers of complex control exist to maintain body weight, making sustained weight loss difficult. Over evolutionary time, forces ensured an ability to conserve energy in times of fasting and feasting, which were the norm for most of mankind's existence. Unlimited availability of food without one having to work for it is a recent phenomenon.

Two studies published in the current issue of the Journal [4] focus on the environmental influences on food habits and bring out the awareness and subsequent possibility of promoting healthy lifestyle to prevent diabetes. Both were from major cities of India, Hyderabad and New Delhi. The results are distressing as far the attitudes and behavioural patterns go. In areas adjoining a large medical centre, prediabetes was identified in more than a quarter of subjects; it was associated with family history of diabetes, history of gestational diabetes, and increased birth weight. Therefore educational programme and appropriate built environment are essential components in halting the march to full fledged diabetes. Of equal if not greater concern is that South Delhi adolescents aged between 14 and 19 consumed less of healthy food such as fruits, greens and vegetables and relied on junk foods including carbonated beverages to satiate their hunger. They often missed breakfast, which worsened the situation. The type of residence also factored as a chief correlate in logistic regression analysis.

It is being increasingly realized that availability of high calorie foods and sedentary habits form the chief underpinning of urban diabetes and obesity. Such behaviour, as the Delhi study showed, begins even at adolescence. Convenience, marketing and peer-pressure lead to increased intake of soft-drink and 'fast food.' Supermarkets and large malls increase potential access to fast foods, which could eventually spread even to rural areas. Access to food by itself is not bad if one understands that branding influences children's' taste perception and one can use branding to improve eating behaviour

Studies have shown that there is decreased energy expenditure with soft drinks than with milk. High sugar drinks do not induce satiety compared to milk, and fast foods simultaneously lead to increased calorie intake. This forms a vicious cycle leading to obesity.

The concept of built environment, defined as that including 'our homes, schools, workplaces, parks/recreation areas, business areas and roads...' [5] truly encompasses a range of physical and social elements that make up the structure of a community [6].

Just like how access to food can improve healthy eating, built environment must be developed to promote active living to prevent obesity and diabetes mellitus. Therefore modifying 'built environment' that helps physical activity is of foremost concern [7]. Recently we have shown that obesity was related to the kind of work, ability to relax, the access to afternoon nap and to psychosocial stress [8].

As elementary an advice is, asking individuals to go for walk has many potential barriers, viz the distance to cover (if the distance is too great, one tends to take a vehicle), the occurrence of steep slopes, which hinders one from walking, as well as the security issues particularly during night-time when going out to walk [9].

All these underline the principle that intervention strategies, whether applied to the environment or to a society may be targeted not just at single diseases but to the cluster of related conditions, obesity, hypertension and diabetes mellitus [10].

Population based interventions can adopt ways to disseminate information about the health benefits of proper eating, physical exercise and smoking cessation. Often the enticing advertisement for all that is unhealthy leaves no chance for healthy lifestyle to be adopted. Message of proper lifestyle can be spread by local social associations, the media or through food associations. It is also essential to make sure healthy food options are available at restaurants; food labeling is both educative and informative. In addition, face-to-face interactions about weight loss healthy diet, decreasing sodium intake and physical activity can be started. The message of healthy living gradually spreads by word of mouth.

Another important asset that is being squandered in modern society is sleep. Along with the development of urban areas, easy access to calorie dense food and ability to live by without physical exercise, the duration of sleep tended to decrease over time. A number of studies have evaluated the relation of sleep loss, appetite, obesity and diabetes mellitus. Biochemical parameters that could provoke obesity were found in association with inadequate sleep. Leptin, a well-known adipocyte signal to stop eating was shown to be lower with sleep deprivation. On the contrary, gherlin, the appetite signal was higher [11]. A variety of other factors are also believed to play a role including availability and craving for energy dense rich foods, especially for those who work night-shifts [12].

Is it really practical to avoid obesity, ranged as we are against evolution, biology and environmental pressures? Can the seemingly straightforward goal of decreased calories and increased physical exercise be put into practice? It may not be easy but it should not be impossible. Starting from basics, to lose 1 kg requires about 7,000-calorie negative balance: start with small steps of both, reduce the number of calories, and perform about 20–30 min a day of additional moderate exercise. This can be buttressed with behaviour modification techniques such as stimulus control, in which the factors leading to increased food intake are first identified and then measures are implemented to avoid or overcome the food-seeking behaviour. In addition it may help to make available low calorie foods in small amounts [13].

In addition, steps must be taken to lowering stress, or to lower the response to stress or both. Yoga is a practice that has been shown to do both [14]. If one has access to a properly trained Centre, that will help.

It is evident that there is what is called 'politics of obesity.' Improved prosperity increases obesity. Just as antibiotics by themselves did not improve the health and increase life expectancy, in recent years people did not suddenly become lazy and gorge themselves with junk foods. In a broader context, when people obtain extra income, they tend to use it to eat more and to be less active. Market economies encourage this. Gaining weight is good for business, both for the food industry and the health industry. Ultimately food is particularly big business because everyone eats [3].

Obesity has been called '..metaphor for the adverse health effects of economic and technological advancement' [15]. Even though 'obesity epidemic will not be cured in the consultation room' [16] one must at least begin somewhere. Why not start in the consultation room and watch it go out into the world!

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ORIGINAL ARTICLE

Recipe for diabetes disaster: a study of dietary behaviors among adolescent students in south Delhi, India

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Abstract : Objective: To study unhealthy dietary behaviors amongst adolescent students in South Delhi and the epidemiological correlates. Design: Cross-sectional study. Setting: Three schools and two colleges in South Delhi. Subjects: Adolescent students aged 14–19 year selected by multi-stage cluster sampling. Statistical Analysis: Proportions, Chi square test, Multivariate logistic regression. Results: Almost half of the respondents reported not having fruits, green vegetables or other vegetables daily. About 177 (32.2%) students were having 'junk food' and 28.2% were consuming carbonated soft drinks daily. More boys than girls reported unhealthy dietary practices. One in every two students (49.6%) had missed their breakfast once or more in the past 30 days. On logistic regression analysis, father's education and type of residence were found to be the two chief correlates of unhealthy dietary behaviors. Adolescents with father's education level lesser than graduation were more likely to be at risk for unhealthy dietary practices (Odds Ratio 4.5, 95% CI: 1.6–12.7). Those residing in private colonies or bungalows were more likely to be having unhealthy dietary practices, than those from government colonies (OR 2.9, 95% CI: 1.3–6.5). Conclusion: The dietary behavior of an overwhelming majority of the students was found to be unhealthy. The study points to an urgent need for measures amongst the adolescents to prevent a catastrophic burden of lifestyle diseases such as diabetes in the coming generations.

Keywords Dietary behavior · Adolescent · Students

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Introduction

Problems concerning eating and weight have become increasingly frequent reasons for the referral of young people, to medical specialists [1]. Poor diet and physical inactivity are the two important causes of death in the United States. The problem has now "immigrated" to the developing nations and is no more localized to developed countries. Physical activity and the environmental setting during adolescence serve as strong predictors of adult obesity [2].

India holds special importance by virtue of being home to the largest adolescent population and one of the fastest growing economies in the world. Diseases like obesity and diabetes have started rising in this country, especially in the urban cities and towns, as the riches grow and lifestyles change. The number of diabetic patients in India is already estimated to have crossed 50 million, and is expected to further increase by the year 2030 [3]. Even as India assumes a growing role in the world affairs, the nutritional choices that her adolescent population is making now, are likely to have an important global effect as and when the generation matures.

It has been stated that one of the profound developments in epidemiology has been the new awareness of 'behavior' as a risk factor. Much of the burden of illness can be linked to patterns of human behavior. The challenge for epidemiology is to move beyond its usual biomedical focus and address the understanding of behavior and its antecedents and consequences [4].

This study was carried out with the purpose of finding out the prevalence of various health risk behaviors among the adolescent student population in one region of Delhi. It also sought to study the association, if any, of the health risk behaviors with various socio-demographic characteristics of the subjects. In this paper, the findings related to

unhealthy dietary behavior among the adolescent students are presented.

Materials and methods

The study was a cross-sectional analysis of the subject population with the units of study being 14 to 19 year old adolescents studying in various schools and colleges in South Delhi. The study being a doctoral thesis research work was reviewed and approved by the institutional ethics committee.

The metropolitan city of Delhi is also the capital of India. It has a population of nearly 13.8 million (as per the 2001 Census of India), in an area of 1,483 square kilometers. From January 1997, the city is divided into nine districts [5]. For the purpose of the present study, two districts of Delhi—South Delhi and Southwest Delhi districts were together considered as South Delhi region.

All the schools and colleges in the South Delhi region were included in the sampling frame. A two-stage cluster sampling design was used to draw a representative sample of students in classes 9th–12th in schools; and first two years of graduation in colleges. These classes were chosen as they correspond to the desired age group of 14 to 19 year old students.

The first stage was random selection of three schools from the list of schools, using a table of random numbers for the purpose. Similarly, two colleges were randomly selected from the list of colleges. The second stage comprised of random sampling of one class each from standards 9th–12th in each selected school. In the selected colleges, two classes each were randomly chosen from the first and second years of graduation. All students in the selected classes, present on the day of the survey, were eligible to participate, allowing for anonymous and voluntary participation. At the time of data analysis, the forms of respondents who had stated their age to be either less than 14 years or more than 19 years were excluded from analysis. A written permission and consent from the principals was obtained prior to conducting the study in their school/college. Written consent was also obtained directly from the subjects who were above the age of majority.

A pre-tested, semi-open ended and self-administered questionnaire was used in the study. There were six questions regarding the consumption of various dietary items (and one question regarding having breakfast daily), to which the respondent had to indicate the frequency of consumption by ticking an option. The information thus collected on the tools, was converted into a computer-based spreadsheet using Microsoft Excel software. Statistical analysis of the data was done using cross-tabulation with the Chi-square test (Fisher's exact test was applied, wherever applicable). Multiple logistic regression was applied to analyze the relationship between unhealthy dietary behavior and various independent variables under study.

Results

The mean age of the respondents was 16.5±1.5 years. Overall, among the 550 respondents, there were 67.1% males and 32.9% females. A large majority of the respondents were Hindus (89.6%). A majority of subjects (62.4%) reported their place of residence as being a private colony or a separate bungalow. Three-fourths belonged to a nuclear family and remaining were part of a joint family. Table 1 depicts the socio-demographic profile of the study population.

Seven questions regarding various aspects of unhealthy dietary practices comprised this domain of health risk behaviors. The recall period was comparatively shorter—past 7 days only, except for one general question about missing breakfast, that mentioned a recall period of 30 days. The students were specifically instructed to include food they ate at home, at school, at restaurants, or anywhere else. Table 2 is a representation of the results observed. A large proportion (93.8%) of the students had one or more of the unhealthy dietary practices.

Almost half of the respondents reported not having fruits, green vegetables or other vegetables daily. About 177 (32.2%) students were having food items commonly termed as 'fast food' on a daily basis, and 28.2% were consuming carbonated soft drinks at least once per day. More boys than girls reported not having green vegetables/other vegetables/milk daily while having fast food and carbonated beverages on a daily basis. The most significant difference ($p=0.001$) was regarding daily consumption of carbonated beverages, with higher proportion of boys (32.2%) than girls (18.8%) having them daily. Having their breakfast was mentioned as 'never, rarely or only sometimes' in the past 30 days, by one in three (33.3%) students, proportion being significantly more among the girls ($p=0.01$). Almost one in every two students (49.6%) had missed their breakfast once or more in the past 30 days.

The unhealthy dietary behaviors were found to be significantly more among those respondents from government/ government-aided schools contrasted with those from private schools [$p=0.002$], and among students currently in school than those in college [$p=0.01$], in univariate analysis. As Table 3 shows, unhealthy dietary behaviors were more in the boys (95.7%) rather than girls (90.1%). Students belonging to government colonies had the least prevalence of unhealthy dietary behaviors. The education

Table 1 Socio-demographic profile of study population (N=550)

Socio-demographic profile	Males		Females		Total	
	N	%	N	%	N	%
Religion						
Hindu	326	88.3	166	92.2	492	89.6
Muslim	23	6.2	4	2.2	27	4.9
Others	20	5.4	10	5.6	30	5.5
Place of Residence						
Private colony/Bungalow	241	65.3	102	56.4	343	62.4
Government colony	64	17.3	63	34.8	127	23.1
Resettlement colony/Slum	26	7.0	8	4.4	34	6.2
Village	24	6.5	4	2.2	28	5.1
Other	14	3.8	4	2.2	18	3.3
Family Type						
Nuclear	278	75.3	129	71.3	407	74.0
Joint	91	24.7	52	28.7	143	26.0

level of the parents were divided into two categories for analysis—'graduate or post-graduates' as one and those with education lesser than graduation as the other category. Unhealthy dietary behaviors were found to be significantly higher among students with lesser levels of father's education [p=0.001] and mother's education [p=0.014]. In univariate statistical analysis, unhealthy dietary behaviors were not found to be significantly associated with religion, type of family (joint or nuclear), living status of parents (whether alive or dead), or number of close friends.

As shown in Table 4, father's education and type of residence were the two chief correlates of unhealthy dietary behaviors on multiple logistic regression analysis. Adolescents with father's education level lesser than graduation were a great deal more likely to be at risk for unhealthy dietary practices (Odds Ratio 4.5, 95% CI: 1.6–12.7). Those residing in private colonies or bungalows were found more likely to be having unhealthy dietary practices, than those from government colonies (OR 2.9, 95% CI: 1.3–6.5).

Discussion

Unhealthy eating behaviors have been mentioned as one of risk actions that can compromise the health in adolescence [4]. An important reason why unhealthy nutrition among adolescents needs to be targeted is that the practices regarding dietary behavior acquired at this stage of life, are likely to persist later on. Lien et al showed in a longitudinal study that individuals maintained their dietary behaviors, through adolescence and into adulthood [6]. It has been stated that obesity is perhaps the most prevalent forms of malnutrition and is becoming increasingly common the world over. Unhealthy eating habits like eating in between meals, and having excess of sugary, fat dense and refined foods have been identified as risk factors for it [7]. Another major concern globally is the coming to prominence of cardiovascular diseases as the leading cause of mortality worldwide. Behavioral risk factors for them include unhealthy diet and insufficient physical activity [7].

Table 2 Behaviors concerned with unhealthy dietary practices (in past 7 days) among the students

Unhealthy dietary behaviors	Males (N=369)		Females (N=181)		Total (N=550)		p value for difference
	n	%	n	%	n	%	
Not having fruits daily	168	45.5	85	47.0	253	46.0	0.75
Not having green vegetables daily	184	49.9	73	40.3	257	46.7	0.03
Not having other vegetables daily	190	51.5	81	44.8	271	49.3	0.14
Having fast food daily	128	34.7	49	27.1	177	32.2	0.07
Having carbonated soft drinks daily	121	32.8	34	18.8	155	28.2	0.001
Not having milk daily	150	40.7	63	34.8	213	38.7	0.19
Had breakfast never/rarely/sometimes[a]	109	29.5	74	40.9	183	33.3	0.01

[a] In past 30 days

Table 3 Association of the unhealthy dietary behaviors with various socio-demographic variables (on univariate analysis)

	Total no.	Unhealthy dietary behaviors		
		One or more n (%)	None n (%)	p value
Gender				
Male	369	353 (95.7)	16 (4.3)	0.01
Female	181	163 (90.1)	18 (9.9)	
School attended				
Government/Govt-aided	316	305 (96.5)	11 (3.5)	0.002
Private	234	211 (90.2)	23 (9.8)	
Current institute				
School	394	376 (95.4)	18 (4.6)	0.01
College	156	140 (89.7)	16 (10.3)	
Father's education				
Graduate/ Post-graduate	278	252 (90.6)	26 (9.4)	0.001
Lesser	251	245 (97.6)	06 (2.4)	
Mother's education				
Graduate/ Post-graduate	214	195 (91.1)	19 (8.9)	0.014
Lesser	156	304 (96.2)	12 (3.8)	
Type of residence				
Government colony	127	113 (89.0)	14 (11.0)	0.036
Private colony/Bungalow	343	327 (95.3)	16 (4.7)	
Resettlement colony/Slum/Village	62	059 (95.2)	03 (4.8)	

In the present study, nearly half of the adolescents were found to be not having fruits, green vegetables and other vegetables (besides green vegetables) on a daily basis. This matches the finding by Gawarikar et al [8] that only 50% of adolescent school girls consumed green vegetables daily. The same study mentions consumption of fruits as being 'very low' but the intake is not quantified. Heald had quoted that girls chose more vegetables than boys [9], a finding reflected in the present study too. The consumption of fruits was better in the present study than the findings among American school children, among whom only 26.7% reported having fruits daily in the past 7 days [10].

Nearly one in three males reported having fast food and carbonated soft drinks on a daily basis during the 7 days before the survey. The figures were a little lower but still significant among female students. High consumption of saturated fats, salt and refined carbohydrates, and low consumption of fruits and vegetables is mentioned as dietary habits posing a risk of cardiovascular disease [7]. Excessive intake of carbonated drinks has also been found to be associated with bone fractures, especially among girls [11]. The increasing consumption of 'junk food' or 'fast foods' that are energy-dense, micronutrient poor foods and beverages, and the heavy promotion of the same through advertisements in the media, has been identified as a matter of concern [7].

Table 4 Significant correlates of unhealthy dietary behaviors on multiple logistic regression

Correlates	Categories	Adjusted Odds Ratio (95% CI)	p value
Father's education	Graduate/ Post-graduate	1 (Reference)	–
	Lesser	4.5 (1.6–12.7)	0.004
Type of residence	Government colony	1 (Reference)	–
	Private colony/Bungalow	2.9 (1.3–6.5)	0.008
	Resettlement colony/Slum/ Village	1.25 (0.3–4.9)	0.75

Forward stepwise method of logistic regression was applied which gives the independent variables found significant at $p < 0.05$, adjusting for other variables in the regression model

In the current study, 38.7% respondents mentioned not having milk daily, a finding better than that among U.S. school students, in whom 55.1% had not had milk daily [10]. This can be ascribed to the value attached to milk as a food item in the Indian society. While 34.8% girls reported not having milk daily, about 21% stated not having it at all in the past week. This confirms the finding of Gawarikar et al [8] that milk consumption is low among adolescent girls, and evokes concern due to the especial importance of milk as a source of calcium for girls [11]. In a study in Bangladesh, 50.0% of the adolescent girls had not consumed milk at all in the 7-day recall period. The poorer figure can be explained by the fact that the study was conducted in rural, economically weaker areas [12].

Nearly half of the students (49.6%) had missed their breakfast once or more in the 30 days before the survey. One in three mentioned having had it 'never, rarely or only sometimes' in the same period. Chugh and Puri (2001) had found in a study of girls aged 16–18 years in a private school in Delhi, that 54% of the normal-weight girls missed

meals, though the recall period was not specified [13]. Missing breakfast was more in our study than that of Lien et al [6] in Norway, in which 33% of the subjects at age 18 years reported not having breakfast daily. Our finding that girls were more likely to miss breakfast than the boys is in line with previous international experience among adolescents [14–16]. A recent systematic review observed that eating breakfast is associated with a reduced risk of becoming overweight or obese and a reduction in the BMI in children and adolescents in Europe [17].

Previous authors have stated that diets of adolescents are characterized by frequent snacking, high energy intake, fast foods, missed meals and unorthodox meals [9]. This fact was reiterated quite emphatically in the present study. An overwhelming majority of the adolescent students (93.8%) were found to be having one or more of the unhealthy dietary practices that were considered.

On multivariate analysis, the significant correlation was found with father's education less than graduation (OR 4.5, 95% CI: 1.6–12.7) and type of residence, when other variables were adjusted for. Students from private colonies or bungalows were more likely (OR 2.9, 95% CI: 1.3–6.5) than those living in government colonies to be having unhealthy dietary behaviors. It can be hypothesized that well-educated parents are likely to be enforcing more discipline regarding the dietary practices of their children. Lesser education of the father may be indicative of a lower economic status, and lesser awareness of the importance of healthy balanced diets for the family members. Pearson et al too in a systematic review found a positive association between parental education and adolescents' healthy dietary behavior as represented by fruit and vegetable consumption [18]. A recent study in Varanasi, India too found father's education level to be a significant correlate of the nutritional status of adolescent girls [19].

The present study was limited by a few constraints. Detailed analysis regarding the reasons for the unhealthy dietary behaviours was limited by the study being a cross-sectional one. The food practices recall period was set at 7 days, which may not truly reflect actual long term dietary behavior. However, this period is believed to be the best compromise as asking students off-hand about their food intake over a longer period may create recall problems. Reputed behavioral surveys have used the same period regarding dietary practices [10].

Conclusions

The findings of the present study raise concerns as the dietary practices of an overwhelming majority of the adolescent students were found to be unhealthy. The resulting long term mortality and morbidity problem due to unhealthy dietary practices is a disaster waiting to happen. The present study points to an urgent need for preventive measures amongst this vital subset of the population to prevent a catastrophic burden of lifestyle diseases such as diabetes in the coming generations.

Conflicting interest None

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ORIGINAL ARTICLE

Infections in children with type 1 diabetes mellitus

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Abstract To identify the incidence, type, severity and risk factors of common infections in children with type 1 diabetes mellitus (DM). In this prospective observational study design, 125 children with type 1 DM (group1) and age and sex matched 125 non-diabetic children (group2) were followed up for 12 months from a tertiary care children hospital in Chennai. Infections encountered were documented in both the groups throughout the study period. Risk factors were analyzed. Among the diabetic children 46.2% had infections and the total episodes of infections were significantly high (p=0.006). Skin and soft tissue infections (p=0.03) and urinary tract infections (UTI) (p=0.002) were significantly higher in diabetic children and they were more prone to recurrent infections. Mean HbA1c was significantly higher among the diabetic children with skin infections. Children with type 1 DM are more prone to skin and soft tissue infections and UTI. Skin infections are more severe and these children have higher HbA1c levels.

Keywords Type 1 DM · Children · Infections

Type 1DM accounts for approximately two thirds of all cases of diabetes in children [1]. Adults with diabetes are prone to infections and improved glycemic control decreases the mortality and morbidity associated with severe infections [2]. However contrary to the popular belief this association is not supported by strong evidence in children. Some studies have failed to prove a clear causal relationship between hyperglycemia and infections [2]. Our concern is whether children <12 years with type 1 DM, are more prone for common infections. Current evidence does not favour the fact that infections occur in children with DM in comparison to non diabetic children [3]. Infections of the urinary tract, respiratory tract and soft tissues occur with increased frequency in the overall diabetic population [4]. Available evidence supports the concept that hyperglycemia per se or the metabolic abnormality of diabetes is sufficient to explain the impaired immune response in patients responding to infections. Impaired polymorph function, chemotaxis and killer activities have been found to be responsible for infections in diabetes. The study was undertaken to find out whether children <12 years of age with type 1 DM are at increased risk for common infections.

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Materials and methods

This was a prospective observational study conducted at a tertiary care children hospital from Chennai, which provides free medical care to children from the lower socioeconomic strata. The objectives of the study were to identify the incidence, type and severity of common infections in children with type 1 DM and to evaluate the risk factors for infections. 125 Children with diabetes mellitus attending the diabetic clinic (GROUP 1) and an equal number of age and sex matched children without diabetes mellitus were enrolled from the Integrated Child Development Centre (ICDS) and the Corporation school, Egmore, Chennai as the control group (GROUP 2). Children

with severe protein energy malnutrition, malignancies like leukemia, lymphoma, children on immunosuppressive drugs or steroids, children with renal failure, nephrotic syndrome or any known immunodeficiency states and recurrent wheeze were excluded from the study. The sample size was calculated for an expected 20% difference in incidence of infections between the two groups and found to be 112 in each group. Considering the possibility of dropouts the sample size was calculated to be 125.The study was approved by the Institutional review board. Informed consent was obtained from the caretakers of these children prior to enrollment.

Initial investigations in the diabetic children included peripheral smear, urine deposits and culture. Children with evidence of infections were excluded... Chronological age, duration of diabetes, nutritional status and anthropometric data were recorded. Children were followed up every 4 weeks for occurrence of infections. The need for hospitalization was taken as the criteria for severity of infections. All infections were appropriately investigated as per the hospital protocol. Glycemic control was assessed by HbA_{1C} levels once in 4 months. Age and sex matched controls were enrolled from the ICDS centre and the Corporation school, Egmore (as Group2). Capillary blood glucose was done to screen for diabetes in these children. Initial investigations and follow up was similar to Group 1. All infections were appropriately investigated. HbA_{1C} was not done in this group. Any documented infection based on clinical and/or lab investigations as per standard guidelines was considered as an outcome measure.

The data was analyzed using SPSS software Version 11.0 for Windows. Statistical analysis using chi square test were compared and $p<0.05$ was considered significant. The study limitation is that differentiation of viral and bacterial infections was not feasible in every child.

Results

Final analysis included 117 diabetic children and 125 non-diabetic children. Of 125 children in the diabetic group, 2 children died due to diabetic keto-acidosis and 6 were lost to follow up. Of the 117 diabetic children studied, 17.9%, 30.8% and 51.3% were in the age groups of <5 years, 6–10 and >10 years respectively. In the non-diabetic group 8.4%, 48.8% and 32.8% of children were in the age groups of <5, 6–10 and >10 respectively. The mean age of children in both the groups was 8 years. 71.8% of diabetic children and 64% of non-diabetic children were in the BMI between 5th and 95th percentile.

Out of the 117 diabetic children, 54 children (46.2%) developed infections during the study period. Out of the 125 non-diabetic children, 43 children (34.4%) developed infections during the study period. Analysis by Chi- square test, revealed no statistical difference in the number of children with infections. However, the number of episodes of infections was more in the diabetic group. A total of 91 episodes of infections were encountered among the diabetic children as against only 43 episodes of infections in the non-diabetic children. The number of episodes was found to be significantly high in the diabetic children (p value of 0.006). Frequency of infections and the number of children with infections have been tabulated (Tables 1 and 2). Among the diabetic group 17.9%(21) had more than one infection during the study period in comparison to none in the non-diabetic children. (χ^2 value: 24.6; p-value=0.00) and the number of episodes ranged from 1 to 8. Most of the recurrent infections encountered were those of skin and soft tissue infections.

In Group 1, among the episodes of skin and soft tissue infections, 49 (84.48%) were bacterial and 9 (15.52%) were fungal whereas in Group 2, of the total 21 skin infections, 19 (90.47%) were bacterial and 2(9.52%) were fungal. Impetigo (n=18) was the most common bacterial infection, followed by furunculosis and boils in 12, abscess in 8 and cellulites in 4. Of the 8 abscesses encountered, Staphylococcus aureus was isolated from 4 and klebseilla from one, other 3 did not show any isolate. Of the 6 children with injection site infection, two had abscess and four had induration, erythema and tenderness. Staphylococcal aureus was the organism encountered in the abscess. The commonest bacterial infection in Group 2 was impetigo. Four furunculosis and two abscesses were encountered. Bacterial infections were more common than fungal infections of the skin. Mucosal

Table 1 Frequency of infections in the study group

Infections	Diabetic children	Nondiabetic children	P-value*
	Range	Range	
Skin infections(Bacterial)	0–5	0–1	0.03
Fungal infections	0–2	0–1	0.02
Respiratory	0–2	0–1	0.67
Injection site	0–1	0	0.01
UTI	0–1	0	0.002
Total Infections	0–8	0–1	0.006

*Mann–Whitney U test

Table 2 Number of children with individual infections

Infections	Group1		Group2		P-value
	N	%	N	%	
Skin	30	25.6	19	15.2	0.06
Respiratory	18	15.4	22	17.6	0.73
UTI	9	7.7	–	–	0.001
Injection site	6	5.1	–	–	0.01
Fungal	9	7.7	2	1.6	0.03

candidiasis of the genitalia was encountered exclusively in diabetic children. 13.8% of infections were Candida. None of the non-diabetic children had candidal infection. 18 of the diabetic children and 22 of the non-diabetic children had respiratory infections. Among the respiratory infections, pharyngotonsillitis was common in both the groups. There was no statistically significant difference in respiratory infections between both the groups. However, none of the non-diabetic children had lower respiratory infection during the study period. Nine urinary tract infections (7.7%) were documented in diabetic children and none in the control group (0%) during the one-year follow up. E.coli, klebseilla, pseudomonas were the isolated organisms in UTI in our study. Bacterial skin infections, fungal infections, and UTI were found to be significantly, higher in the diabetic group with a p value of 0.03, 0.02 and 0.002 respectively.

Risk factor analysis for the common infections (Skin and soft tissue, respiratory and UTI) was done in the diabetic group. Age, gender, diabetic age, nutritional status, BMI, HbA1C levels were analyzed as the risk factors. Of these HbA1C levels was found to be a significantly high in those with skin and soft tissue infections (Table 3). No definite risk factor could be related to respiratory and urinary tract infections in the diabetic group.

Discussion

Diabetic patients tend to have more extensive or serious bacterial infections of the skin and soft tissue in particular, those resulting from S.aureus, Streptococcus species, and gram negative bacilli [5, 6]. They are more prone to infections of skin and soft tissue due to Polymorphonuclear dysfunction, increased rates of skin colonization with staphylococcus aureus [6]. Infections of the urinary tract, respiratory tract and soft tissues occur with increased frequency in the overall diabetic population including adults [7]. The above findings have been the same in our children with type 1 DM. E.coli, klebseilla, pseudomonas were the isolated organisms in UTI in our study. Studies by Nirmal Joshi et al. [4] reveal that E.coli is the commonest organism causing urinary tract infection. Lye et al. [8] also observed the same in their study. Each 1-mol increase in blood glucose at baseline is associated with 6–10% increased relative risk of pneumonia, UTI and skin infection after adjustment for the confounders [9]. Diabetes and infections of the skin, UTI and pneumonia are strong independent risk factors for hospitalization in adults. Diabetic children were found to have recurrent infections. Recurrent furunculosis or folliculitis as a result of Staph.aureus should prompt nasal carriage of the pathogen in the patient or family members. Intranasal mupirocin for seven days to treat carriers is useful in these situations. With reference to the fungal infections in these children, as seen in the study by Dorko et al. [10]. Candida was more common in the diabetic children in comparison to non –diabetic children (13.8% vs. 0%) in this study whereas, Dorko et al. [10] showed a difference of 31% vs 5%. The most common fungal infection is mucocutaneous candidiasis [5]. Dermatophytosis was not common in the diabetic children.

Table 3 Mean HbA_{1C} level and infections in group 1

Infections	With infections	Without infections	P value[a]
Skin	12.3±3.2	10.6±2.5	0.005
Respiratory	10.5±2.8	11.±2.8	0.32
UTI	11.8±3.	11.±2.7	0.41

[a] Two sample t-test

Severity of infections was assessed in our study by the need for hospitalization. Three children in the diabetic group were hospitalized for infections. Two for cellulites and one for multiple deep-seated abscesses. None of the non-diabetic children were hospitalized. Very recently a cohort study was done in Canada comparing patients with diabetes and matched non-diabetic subjects [11]. The risk ratio of suffering from an infectious disease or death caused by an infectious disease in diabetic patients was 1.92(1.79–2.05). Statistical analysis revealed that those with skin infections had statistically significant higher HbA1c levels in comparison with those without skin infections. Diabetes duration or the metabolic control did not reveal any association with occurrence of infection. The overall diabetic control was poor in the study population and this may explain the reason for occurrence of more infections in these children contrary to the reports from literature. However, we need further studies to explore the reasons, which make these diabetic children more prone to infections.

Conclusions

Infections occur more frequently in diabetic children. They are more prone to skin, soft tissue infections and UTI. Skin

infections are more severe and HbA1c levels are significantly high in those children with skin infections.

What is already known?

Adults with Type1 and Type 2 Diabetes are more prone for common infections.

What this study adds?

Children with Type I DM are at increased risk for skin and soft tissue infections and UTI. They are more prone to recurrent episodes of infections.

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Competing interest None

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ORIGINAL ARTICLE

Glycated hemoglobin, dyslipidemia and risk of atherosclerosis in type 1 diabetic patients

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Abstract: The aim of this study was to determine whether the dyslipidemia was associated with glycated hemoglobin (HbA1c) and to study the relationship of dyslipidemia and glycated hemoglobin with atherosclerosis as well as the gender difference in dyslipidemia. Twenty five clinically diagnosed type 1 diabetic children and adolescents in the age group of 7-18 years and 25 age and sex matched healthy children and adolescents constituted the study population. HbA1c was positively associated with total triglycerides, LDL, VLDL and HDL in diabetic cases as compared with controls. The gender differences were studied using chi-square test which showed that females were more prone to changes in lipid profiles as related to HbA1c levels. It was concluded that type 1 diabetes mellitus patients were at increased risk of premature atherosclerosis due to associated dyslipidemia that could be due to higher levels of glycated hemoglobin. Lower HDL levels, a possible risk of atherosclerosis showed inverse association with HbA1c levels, implying that elevated glycated hemoglobin was associated with multi-fold risk of atherosclerosis. Females were at increased risk of atherosclerosis than males because of higher prevalence of dyslipidemia among them.

Keywords: Glycated hemoglobin · Atherosclerosis · Diabetes mellitus · Dyslipidemia

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Introduction

The global spread of diabetes mellitus is a major factor contributing to the prediction that cardiovascular disease will become the leading cause of mortality worldwide by 2020 [1]. In addition to concomitant hypertension and dyslipidemia, increasing evidence suggests that impaired glycemic homeostasis has a direct influence on the formation and propagation of atherosclerotic plaque [2].

Premature atherosclerosis is a risk factor for cardiovascular disease, particularly coronary artery disease, seems to be related to the changes in plasma lipid profile and to poor metabolic control. Several studies have indicated that mortality and morbidity rates of coronary heart disease (CHD) were 2 to 4 times higher among patients with type 1 diabetes whose HbA_1c was higher (>10.4%) than in age-matched non diabetic subjects [3]. Thus for the purpose of devising primary prevention strategies and to achieve better control of complications such as coronary artery disease, it is necessary to study the prevalence of various lipid abnormalities and their associations with elevated levels of glycated hemoglobin in young type 1 diabetes mellitus patients.

Materials and methods

Twenty five previously diagnosed type 1 diabetic children and adolescents in the age group of 7-18 years attending the

out patient Department of Endocrinology and Diabetes at M.S. Ramaiah Medical Teaching Hospitals between September 2008 and November 2008, who were on regular insulin therapy and not on any lipid lowering drugs, constituted the study population. Twenty five age and sex matched healthy children and adolescents constituted the control group. Informed consent was taken from all patients and guardians. Ethical clearance was obtained from the institutional ethics committee. Blood samples were collected in fasting state and were analyzed for total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) using specific enzymatic methods by autoanalyzer [4]. The low density lipoprotein (LDL) was derived by Fredrickson- Friedwald's formula [LDL = (TC-HDL)-TG/5] [4]. The atherogenic index, the ratio of total cholesterol to high-density lipoprotein (TC/HDL) was calculated. Along with these parameters, the glycated hemoglobin (HbA1c) was measured by using high performance liquid chromatography (D10 Biorad kit, USA).

Dyslipidemia was defined by using ATP-III guidelines values [5] as LDL >130 mg/dl, HDL <40 mg/dl in men and <59 mg/dl in women, total cholesterol >200 mg/dl and total triglycerides >130 mg/dl.

Statistical analysis was done by using Statistical software namely SPSS 15.0. Student t test (two tailed, independent) was used to find the significance of study parameters on continuous scale between two groups (inter group analysis). Chi-square/ Fisher Exact test was used to find the significance of study parameters on categorical scale between two groups. Effect size has been computed in the present study. Pearson's correlation has been used to find the relationship between lipid parameters and HbA1c. A value of $p<0.05$ was taken to indicate statistical significance.

Results

Statistically significant difference in glycated hemoglobin levels were found in type 1 diabetic patients as compared to controls [Table 1]. Table 2 gives the dimensional analyses of lipid profile while Table 3 gives the categorical analyses of lipid profile. As shown in Table 2, increase in LDL levels in diabetics was statistically significant ($t=2.108$, $p=0.041$) and decrease in HDL levels in diabetics compared to controls was also statistically significant ($t=7.175$, $p=0.001$). Although total cholesterol was not statistically different between diabetics and controls ($t=0.195$, $p=0.846$), triglyceride levels were significantly higher ($t=10.927$, $p=0.001$) and consequently atherogenic index i.e. cholesterol-to-HDL ratio was also statistically different ($t=3.651$, $p=0.001$) in cases. The association between HbA1c and lipid profile were tested by Pearson's correlation analysis, HbA1c levels were not associated with total cholesterol, but they were positively associated with triglycerides ($r=0.351$) and VLDL ($r=0.352$) in the diabetic patients indicating the effects of disease in these levels, HbA1c was positively associated with HDL ($r=0.296$) as well as LDL ($r=0.311$) levels but there is statistical significant difference. HbA1c is negatively associated with atherogenic index in both control as well as diabetics but the value is significantly different. The gender difference was calculated using chi-square test [Tables 4 & 5] which shows that females are more prone to changes in lipid profile and HbA1c levels as compared to males as there is significant increase in total cholesterol, triglyceride, HDL , VLDL, LDL and atherogenic index.

Table 1 Comparison of Glycemia in controls and type 1 diabetics

Glycemic parameters	Controls	Cases	Significance
FBG (mg/dl)	77.16±5.16	235.12±123.19	$t=6.405$; $p<0.001$
PPBG (mg/dl)	95.96±9.54	274.54±144.79	$t=6.029$; $p<0.001$
HbA1c	4.18±0.57	10.00±2.34	$t=12.111$; $p<0.001$

Discussion

Diabetic patients have a 2- to 4-fold greater risk than do nondiabetic individuals of developing atherosclerosis and its complications, which include stroke, myocardial infarction, and peripheral vascular disease [6]. The major risk factor in type 1 diabetes mellitus patients is glycemic status which causes substantial changes in lipid profile often called as lipid triad in which change in level of every component (VLDL, LDL, HDL) accounts for atherogenecity [7, 8]. This is likely to underscore the observation that even in the absence of ischemic symptoms the presence of diabetes confers a prospective risk of clinical events comparable to that observed in nondiabetic survivors of myocardial infarction [9].

The present study was an attempt to provide an insight in to the major risk factors such as dyslipidemia and increased levels of glycated hemoglobin in type 1 DM patients. This study reveals 4% of the cases with hypercholesterolemia and hypertriglyceridemia. 76% of the diabetics and 20% of the controls showed decrease in HDL level, 36% of the diabetics showed increase in LDL levels. Our study reveals the maximum changes in the HDL levels.

Early detection and treatment of this condition prevents complications and further may decreases the morbidity and mortality due to coronary heart disease. In this study we observed that along with the increase in glycated hemoglobin levels there is also moderate to high risk levels of LDL,

Table 2 Comparison of lipid parameters in controls and type 1 diabetics

Lipid parameters	Controls	Type 1 diabetics	Significance	Effect size
Total cholesterol (mg/dl)	156.19±18.86	158.72±32.71	t=0.195; p=0.846	0.05(N)
Triglycerides (mg/dl)	79.95±13.28	120.68±13.07	t=10.929; <0.001**	3.04(VL)
HDL (mg/dl)	51.08±6.23	40.52±3.96	t=7.155; p<0.001**	1.99(VL)
LDL (mg/dl)	91.40±14.56	98.68±9.37	t=2.108; p=0.041*	0.59(M)
VLDL(mg/dl)	16.27±2.58	23.88±2.65	t=10.293;p<0.001**	2.86(VL)
Cholesterol/HDL ratio	3.24±0.49	3.88±0.73	t=3.651; p=0.001**	1.01(L)

N No effect, VL very large effect, M moderate effect, L large effect

* significant

** highly significant

Table 3 Comparison of levels of lipid parameters in controls and type 1 diabetics

Lipid parameters	Controls (n=25)	Type 1 diabetics (n=25)	'p' value
Total cholesterol (>200 mg/dl)	0	1(4.0%)	
Triglycerides (>150 mg/dl)	0	1(4.0%)	
HDL (<40 M and <50 Fmg/dl)	5(20.0%)	19(76.0%)	<0.001**
LDL (>100 mg/dl)	9(36.0%)	9(36.0%)	
VLDL(>35 mg/dl)	0	0	-
Cholesterol/HDL ratio >5.0	0	0	-

** highly significant

Table 4 Comparison of lipid profile in boys

Lipid parameters	Controls	Cases	Significance	Effect size
Total cholesterol (mg/dl)	145.62±21.26	156.18±43.90	t=0.858; p=0.399	0.33(S)
Triglycerides (mg/dl)	83.18±13.25	116.55±10.25	t=6.879; <0.001**	2.77(VL)
HDL (mg/dl)	50.24±6.82	39.73±3.61	t=4.616; <0.001**	1.90(VL)
LDL (mg/dl)	88.29±13.17	97.09±8.67	t=1.911; p=0.069	0.78(M-L)
VLDL(mg/dl)	16.78±2.92	23.09±1.97	t=6.127; <0.001**	2.49(VL)
Cholesterol/HDL ratio	3.15±0.45	3.69±1.09	t=1.696; p=0.103	0.64(M)

** highly significant

Table 5 Comparison of lipid profile in girls

Lipid parameters	Controls	Cases	Significance	Effect size
Total cholesterol (mg/dl)	160.20±16.07	165.79±17.07	t=0.832; p=0.414	0.33(S)
Triglycerides (mg/dl)	75.8±412.72	123.93±14.45	t=8.696; p<0.001**	3.48(VL)
HDL (mg/dl)	52.17±5.53	41.14±4.24	t=5.653; p<0.001**	2.20(VL)
LDL (mg/dl)	95.37±15.76	99.93±10.01	t=0.883; p=0.387	0.34(S)
VLDL(mg/dl)	15.61±1.99	24.50±3.01	t=8.438; p<0.001**	3.43(VL)
Cholesterol/HDL ratio	3.36±0.54	4.05±1.84	t=11.26; p<0.001**	1.72(VL)

HDL, and VLDL, which suggests relationship of dyslipidemia and glycated hemoglobin along with increased risk of atherosclerosis. The United Kingdom prospective study [UKPDS] following newly detected diabetic individuals found that increased risk of CVD was significantly associated with risk factors of increased LDL, decreased HDL and increased HbA1c when measured at baseline [10]. Good control of diabetes corrects lipid abnormalities in almost 65% of the subjects [11].

Conclusion

It is concluded that type 1 diabetes mellitus patients are at increased risk of premature atherosclerosis due to associated dyslipidemia with increased levels of glycated hemoglobin. The major risk factor which can lead to atherosclerosis as revealed in our study is a significant decrease in HDL levels which shows negative correlation with HbA1c levels, that is, an increase in glycated hemoglobin is associated with a significant decrease in HDL levels which in turn increases the risk of atherosclerosis manifold. Females are at increased risk of atherosclerosis than males because of higher prevalence of dyslipidemia among females as compared to males. Therefore to prevent cardiovascular disease associated with derangement in the lipid profile, strict glycemic control should be the first objective in these patients. It is suggested that along with glycemic control physician should focus on lipid profile as well.

Conflict of interest There is no conflict of interest.

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ORIGINAL ARTICLE

Application of data mining techniques on diabetes related proteins

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Abstract Genomic Data is growing very rapidly with the sequencing of genomes of various forms of life. To understand the overwhelming data and to obtain meaningful information, Data Mining techniques such as Principal Component Analysis and Discriminant Analysis are used for the purpose. Data Mining is basically used when the data is vast and there is need to extract the hidden knowledge in the form of useful patterns. The data set taken into consideration is protein data pertaining to diabetes mellitus obtained from a database. The task at hand was to find out in which species most of the diabetes related proteins exist. It so happened that most of these proteins were prevalent in Human Beings, House Mice and Norway Rat as they are all mammals and Human Beings have orthologs as House Mice and Norway Rat. Both these techniques prove that human beings show a variation from those of House Mice and Norway Rat which are similar in terms of the variation of protein attributes. This can also be inferred from statistical analysis by using histograms and bivariate plots. Other Data Mining Techniques such as Regression and Clustering can be used to further explore the above inference.

Keywords Comparative genomics · Mammalian genome · Principal component analysis · Discriminant analysis

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Background

Bioinformatics is an emerging field of science growing from the application of mathematics, statistics, and information technology, including computers and the theory surrounding them, to the study and analysis of very large biological, and particularly genetic, data sets. The main utility of mathematics in the field is in the creation of tools that investigators can use to analyze data. Biologists need tools for the statistical assessment of the similarity between two or more DNA or protein sequences, for finding genes in genomic DNA, and for estimating differences in how genes are expressed in different tissues. Such tools involve statistical modeling of biological systems. Bioinformatics involves the analysis of biological data. BLAST is one of the most frequently used algorithms in applied statistics, one BLAST search being made every few seconds on an average by bioinformatics researchers around the World [1].

Knowledge Discovery and Data Mining (KDD) has emerged as a rapidly growing interdisciplinary field that merges together databases, statistics, machine learning and related areas in order to extract valuable information and knowledge in large volumes of data. It has been estimated that the amount of information in the World doubles every 20 months and the size and number of databases are increasing even faster. The term data mining has been stretched beyond its limits to apply to any form of data analysis. Data Mining is the search for relationships and global patterns that exist in large databases but are 'hidden' among the vast amount of data, such as a relationship between patient data and their medical diagnosis. These relationships represent valuable knowledge about the database and the objects in the database. It is concerned with the analysis of data and the use of software techniques for finding patterns and regularities in sets of data. The idea is that it is possible to strike gold in unexpected places as the data mining software extracts patterns not previously discernable or so obvious that no one has noticed them before [2].

Methods

Bioinformatics may be considered as the study of information flow within biology and medicine. The first flow is the flow of information from DNA code to biological function. Second flow is the flow of information in the design and analysis of experiments. Studies in the first flow include methods for sequence alignment, gene finding, RNA expression, protein expression, prediction of protein 3D structure, population genetics and modeling of genetic network [3]. The second flow begins with a hypothesis (drawn by scanning molecular biology databases) followed by a plan to collect data, execution of an experiment and analysis of the results. This present work falls into the second category wherein diabetes-related protein data for the three species namely Human Being, House Mice and Norway Rat is obtained from NCBI website by finding the relevant information from www.genecards.org website and using BLAST and looking for orthologs in the website. For the bivariate plots and histograms, initially a sample of 53 proteins has been taken. For Discriminant Analysis too, the data set was same [4]. For PCA, the data set size was 213 diabetes related protein. C Software has been developed to calculate the protein attributes and the histograms and bivariate plots were drawn using statistical package [5]. Protein attributes for the PCA technique were computed using JAVA Software developed for the purpose and analysis was done using SPSS 16.0 package to detect patterns hidden in the data. Details of protein attributes are given in Appendix 1.

Results

Histograms and bivariate plots

The distribution pattern of the 53 proteins with respect to the protein attributes has been done. The bivariate plots show that Human Beings show a variation from those of rodents i.e. House Mice and Norway Rat in terms of the protein attributes.

Discriminant analysis

This is a classification technique which is used to discriminate groups effectively, they being Human Beings, House Mice and Norway Rat. Distance measure shows that Human Being is distinctly different from the others i.e. Norway Rat and House Mice while there is no such significant difference between House Mice and Norway Rat.

Principal component analysis

The 8 protein variates are grouped into 3 factors in all the three species. The three components taken together have explained more than 78% of the variation among the variables; it being similar in Rattus Norvegicus(Norway Rat) and Mus Musculus(House Mice) and different from Homo sapiens(Human Being)

Discussion

After the sequencing of the human genome, rodent genome sequencing (mouse and rat) was the next step to offer data for comparative genomics and analyze human genes embedded in the database [6]. The underlying concept was that genes with significant similarity are presumed 'to have evolved from a single ancestral gene and are part of the same gene family' [7]. Globally the mouse genome is about 14% smaller than the human Genome, possibly due to higher rate of deletion in mouse lineage[8]. By the year 2002, 687 human disease genes had clear orthologs in mouse[8]. Eighty percent of mouse proteins had strict 1:1 orthologs in human genome. It thus provides a tool to understand biological function. The rat genome was smaller than human, but larger than the mouse. Both however encode a similar number of genes. Almost all human genes known to be associated with disease have rat orthologs. About 30% of rat genome aligns only with the mouse. The use of rat and mouse for comparison with human proteins is appropriate because sufficient evolutionary distance exists between rodents and humans, which is optimal for comparative gene prediction [9].

Earlier studies have employed principal component analysis for a variety of factors in diabetes mellitus. In a study, Principal Component Analysis was used to understand correlations between the continuous variables within the clinical database, and to identify principal factors (combinations of variables) and the magnitude of HT in the combinations. In subjects with the metabolic syndrome the principal factors were dominated by blood pressure in both genders with higher loadings in men than in women [10].

Conclusion

In conclusion using Principal Component Analysis, protein attributes related to diabetes among the three species shows that the protein attributes variation in Homo Sapiens differ from the other two species(both being similar). Discriminant Analysis technique too supports this fact using a different approach. It is evident from the bivariate plots and histograms between the protein attributes across the 3 species. All the above facts confirm that the biological facts are reaffirmed by performing computational analysis using data mining techniques.

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Conflict of interest None

Appendix 1

The protein variates are:

1. Variate 1 is the length (L) of the protein in number of amino acids.
2. Variate 2 is the percent of basic amino acids in a given protein. The basic amino acids are H, K; R. percent basic is given by

$$\frac{\text{Number of basic amino acids}}{\text{Total number of amino acids}} \quad 100$$

3. Variate 3 is the percent of acidic/amide amino acids in a given protein. The acidic/amide amino acids are D, E, N, and Q. Percent acidic/amide is given by

$$\frac{\text{Number of acidic=amide amino acids}}{\text{Total number of amino acids}} \quad 100$$

4. Variate 4 is the percent of small and medium hydrophobic amino acids in a given protein. The small and medium hydrophobic amino acids are V, L, I, M. Percent hydrophobicity is given by

$$\frac{\text{Number of hydrophobic amino acids}}{\text{Total number of amino acids}} \quad 100$$

5. Variate 5 is the percent of aromatic amino acids in a given protein. The aromatic amino acids are F, Y, and W. Percent aromatic is given by

$$\frac{\text{Number of aromatic amino acids}}{\text{Total number of amino acids}} \quad 100$$

6. Variate 6 is the percent of small/polar amino acids in a given protein. The small/polar amino acids are A, G, S, T, P [32]. (Teresa K. Attwood et al. 2004). Percent small/polar is given by

$$\frac{\text{Number of small=polar amino acids}}{\text{Total number of amino acids}} \quad 100$$

7. Variate 7 is a measure of distance of a protein sequence from a fixed reference point.

   The distance is measured according to the formula:

$$\text{Distance}\eth D\text{Þ}_{\text{fixed}} \text{¼} \mathbf{p}^{\mathbf{X}\mathbf{Q}}_{\text{i¼1}} \eth O_i \quad E_i\text{Þ}^2$$

   where O_i is the observed number of amino acid of type 'i' in the concerned protein and E_i, the expected number of amino acid of type 'i' in the same protein. E_i is L/20 considering all amino acid to be uniformly distributed in the protein. We refer to this point as the fixed reference point. Dfixed is square root of sum of squares from i = 1 to 20 of difference of observed and expected number of amino acids. Here it is considered fixed as Ei=L/20 is a constant for all the amino acids.
8. Variate 8 is the distance of a protein sequence from a variable reference point. The distance Dvar, globular has the same formula as that in variate 4 but the Ei is calculated according to the formula:

   Ei ¼ fixL

   where L is the length of the concerned protein in amino acids and fi is the average frequency of occurrence of the ith amino acid in the set of proteins that are of high sequence complexity [11]. Here this is considered variable reference point since fi changes for every amino acid and hence Ei changes.

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ORIGINAL ARTICLE

Basalog® is similar to Lantus® in producing glycemic control in patients with type 1 diabetes mellitus on multiple daily insulin regimens

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Abstract This study was performed to compare the insulin glargine produced by two different manufacturers. The study determines the efficacy and safety of a new insulin glargine (Basalog®) having the same amino-acid sequence as Lantus® in patients with type 1 diabetes mellitus on multiple daily insulin regimen. After a run-in period of 4 weeks on once-daily Lantus®, patients were randomized in 1:1 ratio to receive once-daily treatment with either Basalog® (n=107) or Lantus® (n=108) for 12 weeks in this open-label multicenter study. Patients were enrolled from 15 hospitals in India. Change in HbA1c was the primary efficacy parameter; FPG, 7-point glucose profile and PPG were the secondary efficacy parameters. Hypoglycemia and immunogenicity were the main safety parameters. This was a non-inferiority study where non-inferiority could be claimed if mean difference (including 95% CI) was less than or equal to 0.5% for the primary efficacy parameter HbA1c. There was no statistically significant difference between the groups with respect to change in HbA1c (p= 0.69), FPG (p=0.25) or PPG (p=0.68). The change in HbA1c from baseline to end-point was 7.86±1.11 to 7.80± 1.24% in Basalog® treated patients and 7.76±1.17 to 7.58± 1.27% in Lantus® treated patients. Proportion of patients achieving HbA1c <7% was also comparable (40.48% in Basalog® vs 38.30% in Lantus®). The nature and frequency of adverse events, percentage of patients positive for anti-insulin glargine antibodies were similar in both groups. Forty-three (40.19%) subjects on Basalog® experienced at least one hypoglycaemic event, while 45 (41.67%) subjects on Lantus® experienced the same. Basalog® was found to have similar efficacy and safety as Lantus® in treatment of patients with type 1 diabetes mellitus.

Keywords Glargine · Basal insulin · Type 1 diabetes

Introduction

With increasing incidence and prevalence of diabetes mellitus globally, there is a huge demand of medications for treatment. The growing incidence of diabetes mellitus in developing countries is putting a tremendous stress on healthcare costs necessitating the requirement for affordable high quality medicines [1]. The best evidence-based method to reduce this disease burden is prevention of disease and its management at an early stage. The American diabetes association (ADA) and European association for the study of diabetes (EASD) in their consensus statements recommend early therapy in the form of basal insulin, underscoring the importance of tight glycemic control and benefits of early insulin therapy [2].

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Keywords: Basal insulin, Biosimilar insulin

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Insulin glargine is a long-acting recombinant human insulin analogue produced by recombinant DNA technology, marketed under trade name Lantus® in the US and Europe since 2000. Glargine combines the physiological benefits of peakless basal insulin delivery with the enhanced compliance benefits of once a day administration. Lantus® is produced by recombinant DNA technology utilizing a non-pathogenic laboratory strain of Escherichia coli (K12) as the production organism. It differs from native insulin in that the 21st amino acid residue on the A chain (asparagine) has been substituted with a glycine residue, and 2 arginine residues have been added at the end of the B chain [3]. Basalog® is a new insulin glargine developed by Biocon Limited expressed in a non-pathogenic yeast Pichia pastoris, with the same amino acid sequence as Lantus®. Extensive comparative character- ization studies using state of art methodologies and pre- clinical studies in relevant animal models were performed before proceeding for studies in human. The aim of the present clinical study was to compare the efficacy and safety of Basalog® with Lantus®.

Subjects and methods

Protocol

The current study was a multicenter randomized open-label comparison of insulin glargine (Basalog®) and insulin glargine (Lantus®) as basal insulin in treatment of patients with type 1 diabetes who were on multiple daily insulin regimens. Patients were enrolled at 15 hospitals located in large cities of India. The study was conducted from December 2007 to January 2009 (Trial Identifier: CTRI/2008/091/000226). It was conducted in a manner consistent with standards established by the Declaration of Helsinki, in conformance with Good Clinical Practice standards and fulfilling regional and local statutes and regulations. The protocol was approved by the local ethics review board at every study site, and all patients provided written informed consent prior to participation. Eligible patients were men and women 18 to 70 years of age and diagnosed with type 1 diabetes for at least 1 year (defined as, patients with a confirmed history of diabetic ketosis with the presence of GAD antibodies or by presence of a C-peptide value less than 1 ng/ml at a time when the person is hyperglycaemic). Enrolled patients had a Body Mass Index of 18 to 38 kg/m2 and were required to have an HbA1C≤10.5%. Patients were required to be on a stable insulin regimen that met the current standard of care and that included at least two daily insulin injections for at least 3 months. Patients with hepatic or renal impairment, moderate-severe non-proliferative diabetic retinopathy, proliferative diabetic retinopathy, severe peripheral vascular disease, those who were pregnant or breast feeding, and those who had two or more severe hypoglycaemic episodes within 6 months of the study were excluded. Patients with significant cardio-vascular, respiratory, gastrointestinal, neurological, psychi-atric and/or haematological disease were also excluded from the study. Patients were withdrawn from the study if there was an increase in HbA1c of ≥1% from the base line or FPG >270 mg/dl at any time during the study.

Patients were screened during a 1 to 2 week screening phase in which patients continued their current insulin treatment and were instructed on the use of the glucose meter for at-home self-monitored blood glucose assess-ment. After the screening phase, patients entered a 4 week run-in period during which their insulin regimen was optimized on the reference product Lantus®. The starting dose of Lantus® was based on the manufacturer recom-mendation as follows: patients on twice daily NPH received 80% of the total daily units as once daily insulin glargine dose and patients on once daily NPH were switched to same dose of insulin glargine given as once daily. Thereafter, insulin glargine and regular insulin doses were to be individually titrated to obtain a target fasting blood glucose of <140 mg/dl and post-prandial glucose of <160 mg/dl respectively. All patients were also shifted to a single type of regular human insulin (Insugen R®, Biocon Limited) to keep consistency in pre-meal insulins between the two arms. At the end of the run-in period patients were randomized in 1:1 ratio via a central randomization list present at the CRO facility to receive open-label subcuta-neous insulin glargine, Basalog® or Lantus®, once a day at bedtime for a 12-week treatment period. Lantus® (Aventis Pharma, Frankfurt, Germany) was supplied in vials con-taining a 10-ml solution (1 ml containing 100 IU insulin). Basalog® (Biocon Limited, Bangalore, India) was also supplied in vials containing a 10-ml solution (1 ml containing 100 IU insulin). Because of differences in vial dimensions between the two treatments, the study was designed to be open-label.

Study visits were spread over 4 weeks of run-in period and 12 weeks of treatment period. Patients made the following seven clinic visits: screening visit, run-in visit, baseline/randomization visit (week 0), and visits at weeks 2, 4, 8, and 12. There was an additional telephonic visit in week 1 for treatment review. The study end point was defined as the last available measurement on treatment. Efficacy variables included changes in HbA1c between baseline and study end point, fasting plasma glucose, 7-point self-monitored blood glucose, total daily insulin dose, body weight and proportion of patients reaching glycemic target (HbA1c <7%).

All laboratory assays were performed at a CAP (College of American Pathologists) and NABL (National Accreditation Board for Testing and Calibration Labora-

tories) certified central laboratory located in Bangalore, India. HbA1c was measured in whole blood using immunoturbidometry method. Fasting plasma glucose was measured by standard laboratory methods at the same laboratory. Self-monitoring of blood glucose was performed with calibrated glucometer Accu-Chek Active® (Roche Diagnostics).

Hypoglycemia was defined as any blood glucose reading <70 mg/dl or if patient experienced symptoms that he/she associated with hypoglycemia and that resolved with appropriate glucose treatment. Severe hypoglycemia was defined as an event with symptoms consistent with hypoglycemia in which the subject required assistance from another person or if episodes required hospitalization or resulted in unconsciousness and which were accompanied by a blood glucose level of <70 mg/dl or associated with prompt recovery after receiving oral carbohydrate, intravenous glucose, or glucagon administration.

Safety and tolerability were evaluated by physical examination, measurement of vital signs, fundoscopic examination for changes in diabetic retinopathy, recording of electrocardiograms, and safety laboratory measurements, including serum chemistry, haematology, assessment of anti-insulin glargine antibodies and urinalysis. Anti-insulin glargine antibodies were measured from blood samples collected at screening visit, randomization visit and 'end of trial' visit by a validated radio immune-precipitation assay (RIPA) at a centralized, Biocon Bioanalytical laboratory. Periodic pregnancy testing was done in women of child-bearing potential. Adverse events (AEs) were monitored throughout the study and evaluated by the investigators for level of severity, duration, outcome, and relationship to study drug.

Statistical analysis

To achieve the target of non–inferiority criterion, defined as a 0.5% difference in HbA1c, with combined standard deviation of 1.1, 226 subjects were planned to be enrolled to get 204 completed subjects considering 10% drop outs. This sample size provides a power of 90% at two-sided α level of 5%. The non-inferiority could be claimed if mean difference (including 95% CI) was less than or equal to 0.5%.

All the data were entered into SAS PheedIt (Version 3.00) and verified by repeat data entry. SAS (SAS Institute Inc.) Version 9.1 edit checks were used for consistency checks. Differences in HbA1c between treatment groups were assessed using Analysis of Covariance (ANCOVA) model with change from baseline as the dependent variable, and treatment, baseline as independent variables. Ninety-five percent confidence intervals and p–values for treatment differences were generated. All statistical tests were two-sided and performed at a significance level of $\alpha = 5\%$, unless otherwise indicated.

Results

Patients

A total of 428 patients were screened for the study between 29 February 2008 and 14 July 2008. Two hundred and twenty six patients entered the run in period. There were 202 screen failures/drop outs (47.20%) in total. Majority of the screen failures (157) were due to subjects not meeting the inclusion criteria (Fig. 1). Baseline characteristics of patients are shown in Table 1. A total of 37 patients, 23 in the Basalog® group and 14 in the Lantus® group, withdrew from the study before the end of the treatment phase; however it should be noted that there were 11 withdrawals during the run-in period when patients were on Lantus®. Most of these patients either wanted to discontinue study participation or were lost to follow-up.

HbA1c

Basalog® and Lantus® had similar effects on HbA1c between baseline and study end point ($p = 6691$ by ANCOVA) (Table 2). The upper confidence limit for the difference in means was 0.469 which was within the pre-defined non–inferiority margin. On average, patients with higher baseline HbA1c had a statistically greater reduction in HbA1c during treatment, but the relative effects of Basalog® and Lantus® on HbA1c did not differ significantly as a function of baseline. Thirty four patients (40.48%) in Basalog® arm reached glycemic target of HbA1c of <7.0% whereas 36 (38.30%) patients in Lantus® arm reached the same glycemic target. There was no significant difference between the study groups ($p = 0.7665$) with respect to proportion of subjects achieving glycemic goal.

Fasting glucose

Changes from baseline in fasting plasma glucose (laboratory value) and fasting blood glucose (SMBG) to end point are expressed as means ± SD values in Table 2.

Seven-point self monitored glucose profile

Overall glucose values at all the time points were comparable at the baseline and decreased in both groups to similar extent (data not shown).

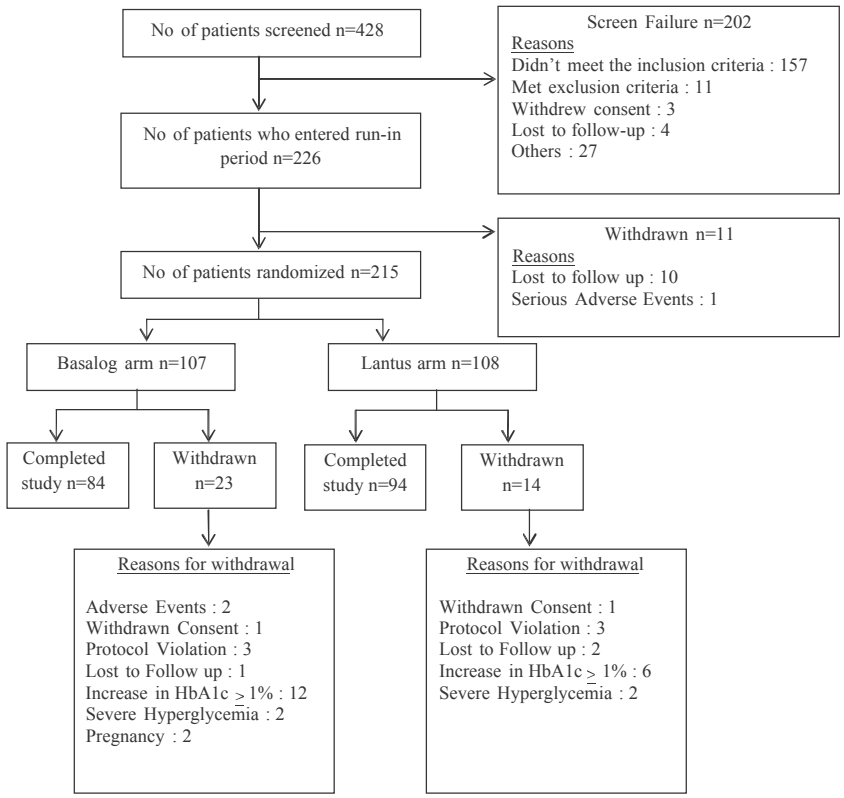

Fig. 1 Summary of patient disposition

Insulin dose

In both arms, there was a minimal reduction in insulin glargine dose (Fig. 2).

Body weight

The change in the body weight from baseline to the end of trial was not significantly different between the study treatment groups. Overall, the mean weight gain in Basalog® arm was slightly higher compared to Lantus® arm (0.71 Kg Vs 0.20 Kg; p=0.2018).

Hypoglycemia

Hypoglycemia was the most common adverse event in both the treatment groups. Out of the 147 hypoglycaemic events in Basalog® arm, 144 (97.96%) were mild and 3 (2.04%) were moderate in severity whereas in Lantus® arm, out of 181 events, 176 (97.24%) were mild and 5 (2.76%) were

Table 1 Baseline demography and characteristics[a†]

	Basalog®	Lantus®
n	107	108
M/F	57 (53.27%)/50 (46.73%)	60 (56.07%)/48 (44.86%)
Age (years)	31.45 (11.76)	28.32 (10.28)
Duration of Diabetes (years)	12.34 (9.16)	10.03 (6.55)
Weight (Kg)	56.87 (9.89)	56.62 (10.27)
BMI (Kg/m^2)	21.96 (2.84)	21.61 (3.10)
HbA1c (%)	7.99 (1.21)	7.83 (1.31)
C-peptide (ng/ml)	0.23 (0.38)	0.27 (0.51)
FPG (mg/dl)	145.00 (63.91)	146.52 (70.26)
PPG (mg/dl)	194.44 (100.55)	194.94 (98.38)

[a] Data are n, n (%), or mean (SD);
[†] P value not significant for all characteristics

Table 2 Baseline–to–end point changes in HbA1c, fasting plasma
g lucose (laboratory value), and fasting blood glucose (SMBG)[a]

Efficacy parameter	Basalog®	Lantus®	P value[†]
HbA1c (%)			
Baseline	7.86 (1.11)	7.76 (1.17)	0.5694
End point	7.80 (1.24)	7.58 (1.27)	0.2453
FPG (laboratory) (mg/dL)			
Baseline	140.85 (62.00)	141.39 (67.21)	0.9551
End point	157.85 (78.71)	139.81 (65.03)	0.0961
FPG (SMBG) (mg/dL)			
Baseline	137.42 (62.27)	134.29 (52.32)	0.6909
End point	130.25 (51.48)	122.20 (41.38)	0.2081

[a] Data are Mean (SD); [†] P value using two sample t-test

moderate. While 43 (40.19%) subjects in the Basalog® arm experienced at least one hypoglycaemic event, 45 (41.67%) subjects experienced the same in Lantus® arm. When derived for per patient per year there were 6.86 hypoglycaemic events in the Basalog® group compared to 7.56 events in Lantus® arm.

Immunogenicity

At screening visit, 76 out of the 226 subjects (33.63%) tested positive for anti-insulin glargine antibodies. Thirty-three percent positive patients to anti-insulin glargine antibodies at screening could be explained by the cross-reactivity (>80%) of the method used for detection of anti-insulin glargine antibodies to anti-insulin antibodies. There was an increase in percentage of patients who were positive for anti-insulin glargine antibodies after the run–in period i. e. at baseline visit, out of the 215 randomized subjects, 91 subjects (42.33%) tested positive. When the subjects at baseline visit were classified according to the study treatment arms they were randomized to, both the groups were comparable (p=0.9365). Among the patients who completed the study at the 'end of trial' visit, 32 subjects (38.10%) in the Basalog® arm and 27 subjects (28.72%) in Lantus® arm tested positive for anti-insulin glargine antibodies (p=0.1848). A decrease in percentage of patients testing positive for anti-glargine antibody was observed from baseline to end of the study in both the groups.

Adverse events

The nature and frequency of adverse events in both treatments groups were similar. During the post–randomization treatment period 18 patients (16.82%) in the Basalog® arm developed at least one adverse event compared to 12 patients (11.11%) in Lantus® arm. Pyrexia was the most common non-hypoglycaemic adverse event with three events in each study arm. There were three injection site reactions reported in the Basalog® arm. Two were considered possibly related to the study drug and one was considered probably related to study drug. Retinal adverse events reported in this study were also comparable between the study arms. The abnormalities in the laboratory parameters were comparable and all of them were considered not clinically significant by the investigator. Two subjects in the Basalog® arm were discontinued from the study because of adverse events, one due to abscess in the limb and other due to skin lesions. General examination and vital signs did not show any significant change from baseline to end of trial. Three ECG abnormalities developed during the study period in the Basalog® arm which was considered clinically not significant by the investigator. There were four SAEs during the study. Two SAEs were reported during the run–in period; both were severe hypoglycemia requiring hospitalization. Two SAEs were reported during the treatment period, one in each study treatment arm (menorrhagia and abscess limb). None of these two SAEs was considered by the investigators to be

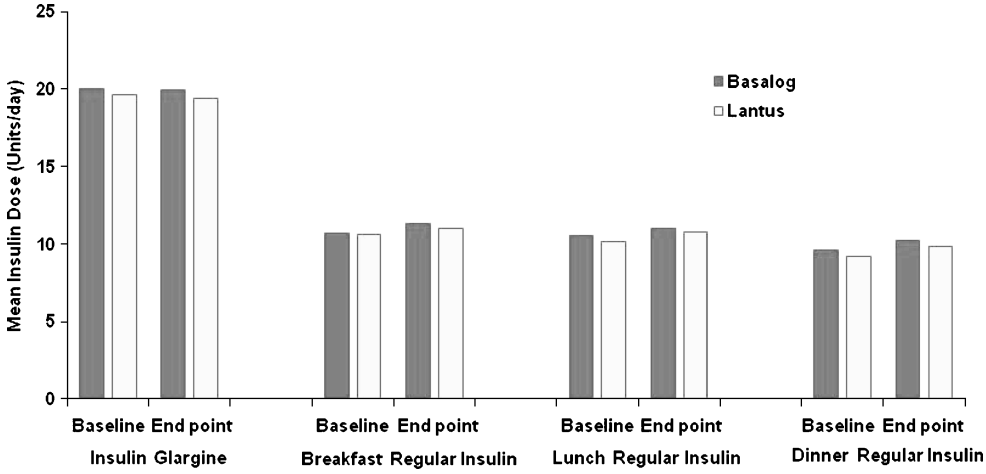

Fig. 2 Mean daily dose of basal and bolus insulin at baseline and end points

related to study medication. Two patients were detected to be pregnant during the study. One patient opted for elective abortion and other patient decided to continue with the pregnancy, outcome of which was uneventful.

Discussion

Analysis of the present study results showed no significant difference in efficacy and safety of Basalog® and Lantus®. HbA1c results established non-inferiority of Basalog® to Lantus®. The small reduction in the HbA1c over the treatment period observed in both the groups could be explained by the good glycemic control of patients at baseline for both the treatment groups (HbA1c at baseline
~7.78%). Similar small reductions have been observed in another study with Lantus® in patients with type 1 diabetes mellitus [4]. In both the arms there was a minimal reduction in insulin glargine dose which is also consistent with other published work on Lantus® [4]. The analysis of secondary efficacy parameters fasting plasma glucose and capillary blood glucose, 7-point glucose profiles at baseline and at the end of trial for both the treatment groups were comparable. The proportion of patients who achieved target HbA1c (<7%) was also comparable between the groups. The change in the body weight from baseline to end of trial was not significantly different between the study treatments. The nature and frequency of adverse events in both treatments were similar. The incidence of hypoglycaemic events reported for both treatment groups were comparable and is consistent with other published work on Lantus® [5]. The overall incidence of injection site reactions in this study (2.80%) was less than the incidence of injection site reactions (6.1 to 15.2%) reported in earlier studies with insulin glargine [6]. Percentage of patients who were positive for anti-glargine antibodies declined from baseline to end of trial visit in both study arms. A similar decline has been observed in other studies [4, 7, 8]. There was no significant difference between the two treatments with respect to anti-glargine antibodies. This is also supported by the lack of a significant difference in mean daily insulin doses between the study treatment arms.

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ORIGINAL ARTICLE

Hypoglycemic activity of leaves of Acanthus montanus T.Anderson (Acanthaceae) in rats

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Int J Diab Dev Ctries. 2011. 31:32-36

Abstract: Hypoglycemic activity of Acanthus montanus, used locally in Nigeria to "cure" diabetes, was evaluated. Phytochemical tests and acute toxicity tests were carried out on the methanolic extract. Using normoglycemic and alloxanized (alloxan monohydrate 70 mg/kg IV body weight) rats, pre-treatment was done with 400 mg/kg of the extract and glibenclamide 10 mg/kg as reference drug orally. Blood glucose levels of the rats were measured at various time intervals (0, 1, 2, 3, 4, 6 h). In addition, a dose dependency test was also done with alloxanized rats using 100 and 200 mg/kg of the extracts. Glucose levels were analyzed statistically using ANOVA and Dunnett's tests. The extract revealed abundance of alkaloids, terpenoids, glycosides and flavonoids. Acute toxicity tests showed an oral LD_{50} of 4,800 mg/kg. With the exception of glibenclamide 10 mg/kg (glucose reduction to 44 mg/dl, $p<0.01$) there was no significant reduction in glucose level in normoglycemic rats in all the treatments at 6 h. In alloxanized rats, there was significant reduction of glucose level by the extract and glibenclamide compared to control 2 h after treatment. Mean percentage reduction of glucose showed a time dependent increase in glucose reduction by the extract with a maximum reduction of 67.1%, $P<0.01$, at 6 h. A dose dependent glucose reduction was seen with increasing doses of the extract till the 6 h time after treatment. This study suggests that the methanolic extract of Acanthus montanus T.A possesses a hypoglycemic effect.

Keywords Acanthus montanus · Alloxan-induced diabetic rats · Hypoglycemic activity · Normoglycemic

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Introduction

Research into the possible discovery of a treatment for diabetes mellitus continues to elude experts as available treatment options have only the ability to manage the disease and reduce the incidence of complications. There has been a pressing need to discover effective treatment for this disease and current interests are being geared towards herbal medicines which according to WHO accounts for 80% of the world's primary health care needs [1]. Folkloric medicine is believed to offer novel treatments which may be devoid of side effects seen in the long term treatment with current synthetic hypoglycemics [2]. Tropical herbs with reported hypoglycemic effect in experimental animals include many compounds [3–5].

Acanthus montanus (Nees) T. Anderson (Acanthaceae) also known as "Bear's breeches" is a small shrub with sparse branches and soft stem. It is widely distributed in some parts of Europe and Africa. It is popularly called "Elele-nyijuo" and "Agameru" in southern Nigeria where it has various traditional medicinal uses [6]. Pharmacological reports shows its leaves possess antimicrobial and immunologic [7], anti-inflammatory and antipyretic [8], and spasmolytic activities [9]. Natives of Nsukka (Nigeria)

Table 1 Phytochemical constit- uents of leaves of Acanthus monthanus

Analyte	Inference
Alkaloids	+++
Proteins	−
Resins	−
Steroids	+++
Terpenoids	+++
Tannins	+
Flavonoids	+++
Glycosides	+++
Saponins	+
Carbohydrates	++
Fats and oil	−

+=present, −=absent

believe this plant possesses a diabetes healing property and is used to manage diabetes mellitus and its complications. The decoction of the dried leaves is usually taken twice a day for several weeks and it is believed to "normalize" blood glucose level for a "long time". There has been no scientific proof to this claim and hence for this study was done to evaluate the antidiabetic property of the leaves of Acanthus montanus in experimental rats.

Methods

Plant material

One kilogram of the aerial parts (shoot) of Acanthus montanus plant was collected during mid May from the outskirts of Nsukka town which harbours the University. The plant was identified at the Bioresources and Development Center (BDCP) Nsukka and a sample specimen deposited at the Department of Pharmacognosy, University of Nigeria, Nsukka. The plant part was cleaned, leaves peeled and shade dried in a well ventilated room for 5 days.

Preparation of extract

The dried leaves were pulverized (320 g) and subjected to a soxhlet extraction using 2 L analytical methanol (Sigma-Aldrich, Steinheim, Germany). The extract was left to concentrate in a well ventilated room at room temperature. The resultant paste (MEAM) was weighed and stored in a refrigerator.

Animals used

Adult wistar albino rats (weighing 100–150 g) of male sex and mice (20–28 g) of both sexes were used. These animals were obtained from the animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. They were housed in steel cages, placed on standard pellet feed (Nigerfeed, Nigeria) and were given free access to clean water. They were kept in well ventilated rooms with a 12/12 h light/dark conditions and ambient room temperature. Animals were procured 2 weeks before the experiments to acclimatize with the laboratory environment. Animal experiments were done in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23, revised 1985).

Preliminary tests

Phytochemical tests

This test was done to show the presence or absence of secondary metabolites according to methods described by Harbourne [10]

Acute toxicity

This test was to ascertain safety of the plant in mice and was done according to method described by Lorke [11] with a little modification.

Pharmacological activity

Determination of glucose level

The tail of each rat was used and was cleaned before blood collection. Blood was withdrawn from the tail vein and

Table 2 Effect of methanolic extract of Acanthus monthanus (MEAM) and Glibenclamide on blood glucose level of normoglycemic rats

Drug treatment	Blood Glucose Level (mg/dl)						% Glucose reduction after 6 h
	0 h	1 h	2 h	3 h	4 h	6 h	
MEAM 400 mg/kg	79.7±4.8	53.8±7.3	87.2±10.4	76.2±5.3	78.0±5.7	72.5±3.3	9.03
Glibenclamide 10 mg/kg	94.7±6.4	64.7±7.3	53.3±3.9	73.7±2.1	64.2±6.3	44.4±6.6*	53.12
3% Tween 80 2 ml/kg	78.0±8.9	87.5±1.6	84.0±3.0	55.2±3.6	72.0±4.2	76.7±9.3	1.67

Values are mean±SEM, n=3, *represents significance at $P<0.01$ for glucose level any time vs. 0 h (Dunnett's test)

Table 3 Effect of methanolic extract of Acanthus monthanus MEAM and Glibenclamide on blood glucose level of alloxan-induced hyperglycemic rats

Drug treatment	Blood Glucose Level (mg/dl)						% Glucose reduction after 6 h
	0 h	1 h	2 h	3 h	4 h	6 h	
MEAM 400 mg/kg	230.2±3.2	198.7±5.2	157.5±4.27*	130.3±32.0*	100.5±29.6*	75.8±2.6*	67.1
Glibenclamide 10 mg/kg	232.5±5.7	226.2±5.3	160.2±17.9*	127.3±25.3*	96.5±6.7*	69.7±5.7*	70.0
3% Tween 80 2 ml/kg	228.2±4.5	218.0±5.9	203.5±5.9	220.3±4.3	212.8±10.5	199.3±8.2	12.7

Values are mean±SEM, n=3, *represents significance at P<0.01 for glucose level any time vs. 0 h (Dunnett's test)

glucose level checked with a single touch glucometer (Roche, Germany). The procedure was repeated twice and the average value taken.

Effect of extract on normoglycemic rats

Nine normal rats were fasted for 12 hours but given water ad libitum during the experiment. At the end of the fasting period, (at time zero) blood was withdrawn from each rat and blood glucose checked as described above. The animals were divided into three groups of three rats each and group I received 400 mg/kg body weight MEAM intraperitoneally. Groups II and III received 10 mg/kg of glibenclamide (Glanil®) and 2 ml/kg of 3% Tween 20 i.p. respectively. Blood samples were drawn from the animals at 1, 2, 3, 4 and 6 hours and blood glucose level was checked.

Effect of extract on alloxan-induced diabetic rats

Diabetes was induced in overnight fasted rats by a single injection of alloxan monohydrate 70 mg/kg intravenously through the tail vein. The rats were placed on glucose, standard feed and water for 4 days and blood glucose level monitored. Nine diabetic (blood glucose levels >200 mg/dl and frequent polydipsia) rats were fasted for 12 h and divided into three groups of three rats each. Group I, II and III received same treatment as described above in normoglycemic rats. Blood samples were drawn and blood glucose level measured as above.

Dose dependency tests

Nine alloxan-induced diabetic rats were fasted for 12 h and divided into three groups and received 100, 200 and 400 mg/kg of MEAM intra-peritonially respectively and blood tested for blood glucose.

Statistical analysis

The SPSS version 13 (Chicago, IL) was used for descriptive and inferential analysis. All glucose levels were presented as mean±standard deviation (SD). Differences in mean glucose levels were analyzed using the ANOVA followed by a post hoc of Dunnett's T test. Statistical significance was put at P values<0.01.

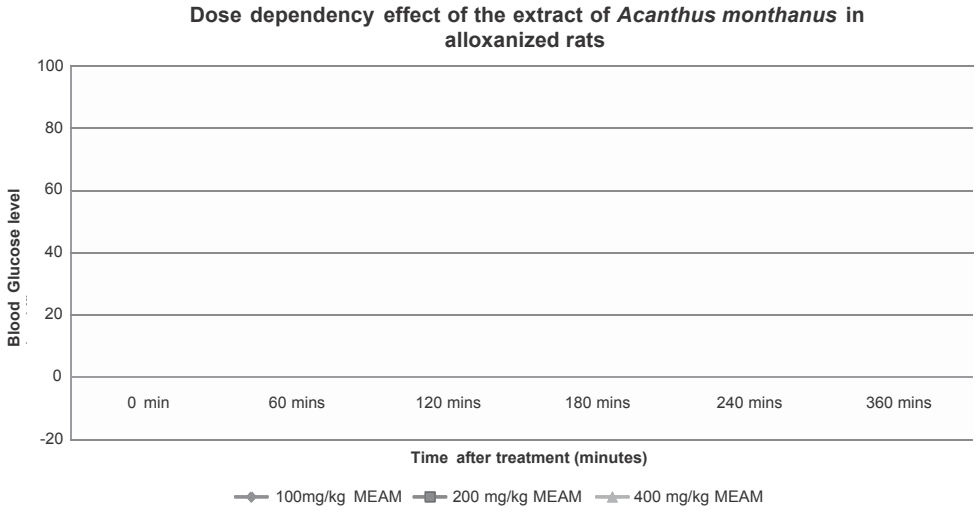

Fig. 1 Dose dependency of hypoglycemic effect of methanolic extract of Acanthus montanus in alloxanized rats (n=3 per dose)

Results

Phytochemical screening showed abundant presence of alkaloids, glycosides, terpeniods, flavonoids and tannins (see Table 1). The test of toxicity (LD_{50}) showed the lethal dose of the extract at a dose of 4800 mg/kg in mice.

In the test of blood glucose reduction in normoglycemic rats, results are displayed in Table 2. There were no significant reductions in blood glucose with the extract (MEAM) at any time but glibenclamide 10 mg/kg reduced blood glucose (44 mg/dl) significantly 6 h after treatment compared to zero hour.

Effect of MEAM on blood glucose of alloxanized rats is shown in Table 3. There were significant reductions of blood glucose by extract and glibenclamide with a faster onset of action seen with the extract 60 min after treatment. Significant glucose reduction was produced in both extract and Glibenclamide treatments 2 h after treatment. There was a 67% reduction in glucose level for the extract 6 h after treatment which was significant compared with control.

Dose dependency effect of the extract in alloxanized rats is displayed on Fig. 1. There was rapid glucose reduction (26.57% and 30.70%) within the first 1 h by 200 mg/kg and 400 mg/kg of the extract. All doses of the extract reduced sugar levels by similar percentages at 6 h post administration.

Discussion

This study evaluated the hypoglycemic activity of the leaves of Acanthus montanus in normoglycemic and hyperglycemic rats to validate its folkloric use. Treatment of the normal fasted rats with the extract did not produce any significant glucose reduction even after 6 h of treatment which may suggest that it has no insulin stimulatory effect; else a hypoglycemic effect will be seen. In contrast, glibenclamide, an insulin secretagogue produced a potent and significant glucose reduction by stimulating insulin release from pancreatic beta cells. In the hyperglycemic (alloxanized) rats, the extract showed potent glucose reduction comparable to glibenclamide after the second hour after treatment. Interestingly, since the extract had no significant hypoglycemic effect on normoglycemic rats but potent glucose reducing effect on hyperglycemic rats, this may further corroborate its mechanisms of hypoglycemia to be different from those produced by oral secretagogues. The extract also produced a sustained reduction in blood glucose levels as seen in glibenclamide till the 6th hr. An improvement of the extra-pancreatic peripheral utilization of glucose or a protection of the pancreatic cells against apoptosis may be suggested as the mechanism of glucose reduction by extract, though insulin levels were not measured. These mechanisms of blood glucose reduction have been exhibited by standard antidiabetic drugs-biguanides, which do not stimulate insulin secretion but act by reducing hepatic glucose reduction and also enhance insulin sensitivity in the tissues. However, it will be difficult to elucidate the exact mechanism of action of the Acanthus extract with the available information as site specific and biochemical analyses needed for such were not performed in this study.

The effective use of alloxan in induction of diabetes in rats is well documented and it can induce chemical diabetes by a direct cytotoxic destruction of the islet cells of Langerhans in the pancreas [12, 13] and thus impede the secretion of insulin. This insulin deficiency will lead to marked increase in blood glucose level [14] and this was clearly obtained in the rats used. However, since the destruction of beta cells may not be complete, especially in mild diabetes (blood glucose level < 300 mg%) as seen in this study, the extract may aid recovery of the cells or may sensitize surviving beta cells.

The dose-related glucose reduction produced by the increasing doses of the extract is interesting because 6 hours after treatment, increased dose did not produce any proportional increase in glucose reduction. This further corroborates its effect differing from insulin and insulin secretagogue-like drugs that produce increasing glucose reduction (and eventual fatal hypoglycemia) with increased dose [2]. Though this study was done in mild alloxan induced diabetes, results may differ in intense alloxan induced diabetes as seen in other plants [15, 16]. Flavonoids, alkaloids and tanins were isolated from the plant extract and may have been responsible for this antidiabetic effect produced by this plant as have been reported in other works [17–19].

In conclusion this preliminary study for the first time confirms the folkloric claim of the antidiabetic action of the leaves of Acanthus montanus but further research will be undertaken to ascertain its exact mechanism of action and the implicating phytochemical constituent.

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Conflicts of interest No support such as grant, equipment, or drugs was received by any of the authors.

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ORIGINAL ARTICLE

Is glucosamine-chondroitin risky to normoglycemic individuals with family history of diabetes mellitus

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Abstract Many studies have examined the therapeutic effects of glucosamine and chondroitin in relieving joint pains, but they did not determine any abnormal pharmacodynamic interactions. To determine the relationship between glucosamine-chondroitin supplement and occurrence of hyperglycemia in normoglycemic patients with family history of diabetes mellitus. 84.9% normoglycemic patients with family history of diabetes became hyperglycemic after 8 weeks course of glucosamine-chondroitin supplement ingestion. Patients who had diabetic female relatives were at risk and they represented 92.3%. When hypoglycemic agents were used, hyperglycemic patients with diabetic male relatives rapidly became normoglycemic at the end of 2nd post treatment week and they represented 83.3% of hyperglycemic patients. Only 2.5% resisted treatment. Glucosamine-chondroitin supplement, although has few side effects, it should be used with great caution in normoglycemic patients with family history of diabetes mellitus and especially those who had diabetic female relatives.

Keywords Diazoxide · Fasting blood sugar · Glycosylated hemoglobin · Hyperglycemia · Thiazides

Introduction

Cartilage may expose to a slow progressive and degenerative wear-and-tear process during life time. The degenerative cartilage treatment [1] is based on a theory that the increase in the rate of formation of cartilaginous building blocks (glycosaminoglycans) depends on glucosamine as precursor [2] and chondroitin for its resiliency [3]. Glucosamine-chondroitin supplement may cause elevated blood glucose level in some diabetic patients, but no studies showed hyperglycemia in normoglycemic people as we tried to find in our study. It is important to declare that many drugs like diazoxide, thiazides, adrenal corticoids and oral contraceptives may produce hyperglycemia [4]. HbA_{1c} (glycosylated hemoglobin) methods reflect the blood glucose level over the previous four weeks to three months [5] and the method of Michigan Diabetes Research & Training Center accepts it as the method of assessing blood glucose control in diabetic people [6]. In general, its range in healthy persons is below 6.5% [7–9]. We used this test in addition to fasting blood glucose test to find any relation between glucosamine-chondroitin and hyperglycemia in normoglycemic individuals who had a positive family history of diabetes mellitus.

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Patients and methods

Ninety three osteoarthritic adult patients, 59 males and 34 females, ranging in age from 19 to 34 years (mean± SD 23.6±0.1) participated in this prospective and open study. Approval to conduct it, was granted by the ethical committee in the college of medicine. They were recruited from the outpatient clinic of department of rheumatology of Al-Kadhemia teaching hospital from May 2008 to June 2009. Full clinical examination including fasting blood glucose and HbA_{1c} tests was done, then the nature of the trial was explained to each patient and the consent was obtained.

Table 1 Effect of Glucosamine-chondroitin in producing hyperglycemia

Duration of treatment with glucosamine-chondroitin (in weeks)	Participated patients	No. of Patients according to blood glucose levels		P. value
	No. (%)	Hyperglycemic	Normoglycemic	
2	93 (100)	8 (8.6)	85 (91.4)	0.031
4	93 (100)	60 (64.5)	33 (35.5)	
8	93 (100)	79 (84.9)	14 (15.1)	

Table 2 Effect of Glucosamine-chondroitin in producing hyperglycemia as related to the gender of the relatives with positive family history of diabetes

Relative sex group	Participated patients	No. of Patients according to blood glucose levels		P. value
	No. (%)	Hyperglycemic	Normoglycemic	
Female	39 (100)	36 (92.3)	3 (7.7)	0.031
Male	31 (100)	24 (77.4)	7 (22.6)	
Female and Male	23 (100)	19 (82.6)	4 (17.4)	
Total	93 (100)	79 (84.9)	14 (15.1)	

Table 3 Effect of sulfonylureas in controlling hyperglycemia

Duration of treatment with sulfonylureas (in weeks)	Hyperglycemic patients	No. of Patients according to blood glucose Levels during treatment	
	No. (%)	Hyperglycemic	Normoglycemic
2	79 (100)	48 (60.8)	31 (39.2)
4	79 (100)	25 (31.6)	54 (68.6)
8	79 (100)	2 (2.5)	77 (97.5)

Table 4 Effect of sulfonylureas in controlling hyperglycemia as related to the gender of the relatives with positive family history of diabetes

Relative gender	Hyperglycemic patients before treatment	No. of Patients according to blood glucose levels after treatment	
	No. (%)	Hyperglycemic	Normoglycemic
Female	36 (100)	2 (5.5)	34 (94.5)
Male	24 (100)	0 (0.0)	24 (100)
Female and Male	19 (100)	0 (0.0)	19 (100)
Total	79 (100)	2 (2.5)	77 (97.5)

Thirty-nine patients had diabetic female relatives [sister, mother or grandmother], 31 patients had diabetic male relatives [brother, father or grandfather], and 23 patients had both female and male relatives with diabetes.

They were given a combination of glucosamine at a dose of 1500 mg/ day and chondroitin at a dose of 1200 mg/day in three divided doses for three month period (13 weeks). The patients were told to have hospital visits for follow up at the end of 2nd, 4th, 8th and 13th week of treatment. Any patient found hyperglycemic after 8 weeks, would be informed to stop drug intake and started on hypoglycemic agents (sulfonylureas) for further 8 weeks during which FBG was done consecutively while HbA_{1c} test was done at 8th post-treatment week to check glycemic status [10]. The still normoglycemic patients continued receiving the supplement till the end of the 13th week. Statistical analyses were done using SPSS version and chi-square test. The results considered statistically significant if the P value was <0.05.

Results

84.9% (79 out of 93 patients with positive family history of diabetes) became hyperglycemic (HbA1c>7%, FBS> 140 mg/dL) after using glucosamine-chondroitin in 8 weeks period, while 15.1% (14 out of 93 patients) remained normoglycemic (HbA1c<7%, FBS<140 mg/dL) for the same period. At second post treatment week, hyperglycemic patients represented 8.6% (8 out of 93 patients) while at the end of 4th week, they represented 64.5% (60 out of 93 patients). There was a statistically significant difference between hyperglycemic and normoglycemic patients with a family history of diabetes mellitus (Table 1).

As regards to the gender of the relatives sex, the hyperglycemic patients with diabetic female relatives represented 92.3% (36 out of 39 patients), those patients with diabetic male relatives represented 77.4% (24 out of 31 patients), while the hyperglycemic patients with both diabetic female and male relatives represented 82.6% (19 out of 23 patients) (Table 2).

During treatment of hyperglycemia, 83.3% (20 out of 24 patients with diabetic male relatives) became normoglycemic at the end of second post-treatment week and 100% (24 out of 24 patients) at the end of 4th post-treatment week which was confirmed by HbA1c test. 27.8% (10 out of 36 patients with diabetic female relatives) became normoglycemic at the end of second post-treatment week and 63.9% (23 out of 36 patients) at the end of fourth post-treatment week, while they were 94.4% (34 out of 36 patients) at the end of eighth post-treatment week. Only 5.6% (2 out of 36 patients) remained hyperglycemic. Those hyperglycemic patients with both sex diabetic relatives who became normoglycemic at the end of second post-treatment week, represented 57.9% (11 out of 19 patients), while they represented 89.5% (17 out of 19 patients) and 100% (19 out of 19 patients) at the end of fourth and eighth post-treatment week respectively. The total hyperglycemic patients who became normoglycemic at the end of eighth post treatment week represented 97.5% (77 out of 79 patients) and only 2.5% (2 out of 79 patients) were irreversible on treatment (Table 3 and 4).

Discussion

Only few adverse effects including elevation of blood glucose concentration in diabetics had been reported during the use Glucosamine-chondroitin in the treatment of joint problems [11]. Our study focuses on those normoglycemic patients with family history of diabetes who complained of joint pains due to different causes and they took this supplement. Along eight weeks of drug intake, 84.9% (79 out of 93 patients) became hyperglycemic. It is found that patients who had diabetic female relatives became strongly hyperglycemic (92.3%) as compared to 77.4% and 82.6% of patients who had diabetic male relatives and who had diabetic both sex relatives respectively. When Glucosamine-chondroitin intake was stopped and started sulfonylureas for another 8 weeks period to control hyperglycemia, it is found that patients with diabetic male relatives became rapidly normoglycemic at the end of 2nd post treatment week and they represented 83.3% as compared to 27.8% and 57.9% of patients who had diabetic female relatives and who had diabetic both sex relatives respectively for the same period. It also found that the hyperglycemic patients with diabetic female relatives returned normoglycemic very slowly and 2.5% resists sulphanylureas and remained hyperglycemic at the end of 8 weeks of treatment.

Conclusion

Glucosamine-chondroitin supplement which is given to relieve joint pains should be used with great caution in normoglycemic patients with a family history of diabetes mellitus and especially those who had diabetic female relatives.

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LETTER TO EDITOR

Persistent organic pollutants and diabetes mellitus

Adnan Z. Bootwala, Tehsin A. Petiwala, P. G. Raman

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Sir,

Persistent Organic Pollutants (POPs) are carbon containing halogenated chemical substances which are characterized by high lipid solubility. They persist in the environment and accumulate and spread through animal fat. Twelve POPs which are chlorine containing organic compounds have been chosen as priority pollutants by the United Nations Environment Programme (UNEP) for their impact on human health and environment:

Pesticides: Aldrin, Dieldrin, Endrin, Chlordane, DDT, Heptachlor, Mirex, Toxaphene, Hexachlorobenzene (HCB)

Industrial Chemical Products: Polychlorinated biphenyls (PCBs), Hexachlorobenzene (HCB)

Unwanted By-products: Polychlorinated dibenzo-p-dioxins (PCDDs), Polychlorinated dibenzofurans (PCDFs), Polychlorinated biphenyls (PCBs), Hexachlorobenzene (HCB)

Chronic exposure to sub-lethal concentrations of POPs over prolonged peroids can cause Immune dysfunction, dermatological effects like chloracne, neurodevelopmental and neurobehavioural effects, reproductive anomalies [1], endocrine problems, rheumatologic disorders like arthritis,carcinogenesis.

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The various mechanisms implicated in these POPs, in causing diabetes are as follows:

INSULIN RESISTANCE: Organochlorine pesticides i.e. oxychlordane (metabolites of chlordane) and trans- nonachlor (impurity of chlordane) are strongly associated with insulin resistance among nondiabetic subjects [2]. Individuals with elevated levels of POPs (DDT, dioxins, PCBs and Chlordane, among others) in their body were found to be up to 38 times more likely to be insulin resistant [3] than individuals with low levels of these pollutants.

OBESITY: Because POPs are lipophilic, they accumulate in the fat thus indicating that obesity is only a vehicle for the accumulation of POPs in the body which eventually results in diabetes [4].

DECREASED INSULIN PRODUCTION: Pancreatic β-cells contain muscarinic acetylcholine receptors, which are involved in the glucose-dependent production of insulin. Organophosphate insecticides inhibit acetylcholinesterase, which causes increased accumulation of acetylcholine, potentially leading to overstimulation and eventual down-regulation of pancreatic β-cell receptors and reducing insulin production [5].

DISRUPTED GLUCOSE HOMEOSTASIS: Dioxin, a frequent contaminant of herbicides involves an estrogen-dependent peroxisome proliferators–activated receptor (PPAR) pathway and thereby upregulate insulin-like growth factor binding protein-1 (IGFBP-1) in MCF-7 cells. Studies have suggested that exposure to these contaminated herbicides increased the risk of diabetes by disrupting the glucose and insulin homeostasis.

The exposure to these POPs can be prevented by banning the use of pesticides, reducing the saturated fats in the body by following a non-fat or low-fat diet.

References

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