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Original Article

Oxidative Stress in Relation to Obesity in Gujarati & Non-Gujarati Young Girls before & After Maize Diet

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Abstract

Background: Obesity is the first of the "diseases of civilization" to appear. Its prevalence is escalating at an alarming rate to epidemic proportions throughout the developed world. Environmental and behavioral changes brought about by economic development, modernization and urbanization has been linked to the rise in global obesity. Obesity may induce systemic oxidative stress. *Objectives:* The present scenario suggests that higher oxidative stress is the key factor of obesity and hence a management strategy aiming at control of lipid Peroxidation in obesity by use of Maize diet is envisaged. *Material and Methods:* This study has been conducted on 1001 Gujarati and non Gujarati girls aged between 18-30 years. They were further distributed according to age, inhabitance, socioeconomic status, dietary habits, family history and blood pressure. Every subject in each group was asked to replace the wheat chapatti for 30days; the girls were examined for oxidative stress parameter MDA before and after maize diet along with the statistical evaluation. *Results:* There was a Positive effect of maize diet on biochemical parameter of all the girls in all the subgroups Malondialdehyde level in total girls before the maize diet was 2.35 ± 0.76 nmol/ml which reduced to 1.8 ± 0.46 nmol/ml after the diet ($P < 0.001$). *Conclusions:* The oxidative stress showed improvement in normal , overweight and obese girls, most significantly on overweight and obese after taking the diet ($P<0.001$). Current dietetic practice is to recommend a healthy eating plan of reduced fat, and increased fiber intake.

Keywords: Obesity; Prevalence; Oxidative Stress; Malondialdehyde (MDA); Age; Inhabitance; Socioeconomic Class; Dietary Habits.

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Introduction

Obesity is a complex, highly heritable and heterogeneous group of disorders. Free radicals are implicated in various chronic diseases including obesity. Many recant studies have emphasized a role for reactive oxygen radicals in the development of oxidative stress and hence the inflammation in pathogenesis of diseases. Epidemiological data suggest an association of dietary intake of nutrients and fibers which are high in antioxidants and protect against the incidence of obesity. The present study was designed to investigate various risk factors and reactive oxygen species mediated changes as etiological factors in normal, overweight and obese Gujarati and Non Gujarati girls before and after maize diet. The present study emphasizes on clinical study with maize diet in 1001 Gujarati and Non Gujarati girls, aged between 18-30 years to study the oxidative stress, before and after maize diet. Out of the 1001 girls, 526 girls were Guajarati's and 475 girls were. They were further distributed according to age, inhabitance, socio economic status, dietary habits, family history and blood pressure. In the second part of the study the girls were examined for oxidative stress parameter before and after maize diet along with the statistical evaluation.

Material and Method

The present study encompasses clinical study with Maize diet in 1001 Gujarati as well as Non Gujarati girls aged between 18 to 30 years before and after maize diet. The practical work was carried out in the Department of Biochemistry, RNT Medical College, Udaipur, Department of Biochemistry, Pacific Dental College, Udaipur and Sterling Hospital Diagnostic Center, Ahmadabad, Gujarat. Normal subjects of identical age group with that of respective obese group acted as control. The subjects were divided into two groups:

Control Group

Possessing normal body weight with healthy body mass index between 18 -25 kg/m^2.

Study Group

Possessing overweight/obesity having body mass index between 25 to 30kg/m^2. Every subject in each group was asked to replace the wheat chapatti by maize chapatti and no change was made in the rest of the ingredients. They were asked to take the maize diet for thirty days. Physical and biochemical parameters were determined before and after consumption of the maize diet for thirty days, and, the effect of maize fibers was studied. The difference in the parameters was evaluated when the two communities Gujarati and Non Gujarati were compared with each other before as well as after the maize diet. The study evaluated oxidative stress in obesity and the effect of maize diet on the parameter. The parameter selected to evaluate the oxidative stress was malondialdehyde, an oxidative stress marker; along with the statistical analysis with t and p values.

Thiobarbituric acid reactive substance (TBARS)[1].

Procedure

To 0.8 ml serum, 1.2 ml of TCA-TBA HCl reagent was added, mixed and kept in boiling water bath for 10 minutes. After cooling, 2 ml of freshly prepared NaOH solution was added so as to eliminate centrifugation. The absorbance of pink color obtained was measured at 535 nm against blank which contained distilled water in place of serum.

Calculation

Molar extinction of TBARS at 535 nm 1.56X 10-5/ M/Cm.

$$\frac{V \times \text{ OD at 535 nm}}{0.156} = \frac{V \times \text{ OD at 535 nm}}{0.156}$$

= 25.6 x ΔOD at 535 n moles /serum

Normal range = 2.0-3.0 n mole/ml serum

Statistical Analysis

Data was analyzed statistically by using student t test with the help of SPSS software version 19.

Results

Table 1 shows MDA in total girls (1001), Gujarati girl (526) and non Gujarati girls (475) before and after maize diet. Total girls, Gujarati and Non Gujarati girls were statistically evaluated when compared before and after consumption of maize, and also comparison was made for Gujarati v/s Non Gujarati before (BMD) as well as after the maize diet (AMD). As per ICMR recommendations the normal range of malondialdehyde is 1.05-3.2 n moles/mlIn the present study, MDA level in total girls before the maize diet was 2.35 ± 0.76nmol/ml which reduced to 1.8 ± 0.46nmol/ml after the diet ($P<0.001$). Results were almost similar for Gujarati and NonGujarati girls.

According to Age

There is no definite criterion for dividing the different age groups but presently the subjects were divided as less than 20 years and more than 20 years. Malondialdehyde followed an increasing trend with increasing age. The parameter showed significant changes when comparison was done before and after maize diet ($P<0.001$). When comparison was between Gujarati and NonGujarati of age <20 group, statistically significant change was observed for malondialdehyde ($P<0.05$) after the maize diet.

According to Inhabitance

Girls of urban category showed higher values for oxidative stress than those of rural category. Malondialdehyde for both the categories were statistically significant ($P<0.001$) (Table 1).

According to Socio Economics Class

Keeping in view that obesity is a disorder mainly of affluent class; the girls were divided as lower, middle and upper socio economic class. The oxidative stress for lower socio economic class was less as compared to that in upper socioeconomic class. When fiber diet was considered, the parameter was statistically significant in all three classes (P<0.001) (Table 1).

According to Diet

As diet plays an imp role in development of obesity, subjects were divided as vegetarian and non vegetarian. The mean values of MDA between the two groups' varied and lower values were obtained for vegetarians girls. The effect of maize was almost similar for both the groups in form of significance as (P<0.001) (Table 1).

According to Family History of Disease

Data obtained for family history of hypertension and that of diabetes were almost similar but family history of coronary Artery Disease had very high values of malondialdehyde in comparison to data of girls who were without any family history malondialdehyde (2.17 ± 0.72nmol/ml v/s 3.15 ± 0.37nmol/ml). However statistically, similar results were seen for the effect of maize diet on the parameter (P < 0.001) (Table 2a, 2b).

According to Blood Pressure

Systolic blood pressure < 120mmHg and diastolic blood pressure < 80mmHg categories showed normal oxidative stress but the high normal category showed high values. SBP < 120mmHg v/s SBP (121-130mmHg) showed mean value of malondialdehyde as 1.81 ± 0.54nmol/ml v/s 2.82 ± 0.58nmol/ml. However, effect of maize diet on all categories was almost same. Malondialdehyde was highly significant (P< 0.001) for all categories except high normal diastolic blood pressure category where it was (P < 0.05). (Table 2a, 2b)

Table 1: Oxidative stress (MDA) (nmol/ml) in Total, Gujrati and Non-Gujrati Girls before and after Maize diet in relation with inhabitance, socioeconomic class, and dietary habits

Parameters	Total Girls B vs A (n=1001)	Gujrati Girls B vs A (n=526)	Non-Gujrati Girls B vs A (n=475)	Guj vs NonGuj Girls BMD (526vs475)	Guj vs Non-Guj. Girls AMD (526vs475)
MDA (nmol/ml)	19.59[c]	13.17[c]	14.35[c]	0.83	0.69
Rural	Total Girls B vs A (n=242/1001)	Guj.Girls B vs A (n=69/242)	Non Gujrati Girls B vs A (n=173/242)	Guj. vs Non Guj BMD (69vs173)	Guj. vs Non Guj AMD (69vs173)
MDA (nmol/ml)	9.77[c]	4.9[c]	8.47[c]	0.1	0.78
Urban	Total Girls B vs A (n=759/1001)	Guj.Girls B vs A (n=457/759)	Non Guj.Girls B vs A (n=302/759)	Guj vs NonGuj BMD (457vs302)	Guj vs NonGuj AMD (457vs302)
MDA (nmol/ml)	17.01[c]	12.35[c]	11.81[c]	0.74	0
Lower class	Total Girls B vs A (n=194/1001)	Guj. Girls B vs A (n=64/194)	Non Guj. Girls B vs A (n=130/194)	Guj vs NonGuj BMD (64vs130)	Guj vs Non Guj AMD (64vs130)
MDA (nmol/ml)	8.34[c]	4.32[c]	7.18[c]	0.96	0.43
Middle class	Total Girls B vs A (n=447/1001)	Guj. Girls B vs A (n=183/447)	Non Guj Girls B vs A (n=264/447)	Guj vs NonGuj BMD (183vs264)	Guj vs NonGuj AMD (183vs264)
MDA (nmol/ml)	13.22[c]	8.04[c]	10.54[c]	0.41	1.34
Upper class	Total Girls B vs A (n=360/1001)	Guj Girls B vs A (n=279/360)	Non Guj Girls B vs A (n=81/360)	Guj vs NonGuj BMD (279vs81)	Guj vsNonGuj AMD (279vs81)
MDA (nmol/ml)	11.84[c]	9.7[c]	7.15[c]	2.05[a]	-0.57
Vegetarian	Total Girls B vs A (n=721/1001)	Guj Girls B vs A (n=404/721)	Non Guj. Girls B vs A (n=317/721)	Guj vsNonGuj BMD (404vs317)	Guj vsNonGuj AMD (404vs317)
MDA (nmol/ml)	16.21[c]	11.39[c]	11.64[c]	-0.18	1.45

P value: a=<0.05; b=<0.01; c=<0.001

Table 2(a): Oxidative stress in Total, Gujrati and Non-Gujrati Girls before and after Maize diet with t and P values in relation with diet, family history, and blood pressure

Non vegetarian	**Total Girls (n=280/1001)**				**Gujrati Girls (n= 122/280)**				**Non Gujrati Girls (n= 158/280)**			
	Before		**After**		**Before**		**After**		**Before**		**After**	
	Mean	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**
MDA (nmol/ml)	2.39	0.76	1.81	0.44	2.34	0.76	1.79	0.46	2.43	0.76	1.82	0.43
FH of BP	**Total Girls (n=100/1001)**				**Gujrati Girls (n=29/100)**				**Non Gujrati Girls (n= 71/100)**			
	Before		**After**		**Before**		**After**		**Before**		**After**	
	Mean	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**
MDA (nmol/ml)	2.91	0.53	2.1	0.3	3.01	0.52	2.17	0.29	2.87	0.53	2.07	0.3
FH-CAD	**Total Girls (n=68/1001)**				**Gujrati Girls (n= 22/68)**				**Non Gujrati Girls (n=46/68)**			
	Before		**After**		**Before**		**After**		**Before**		**After**	
	Mean	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**
MDA (nmol/ml)	3.15	0.37	2.22	0.21	3.27	0.12	2.29	0.11	3.09	0.43	2.18	0.24
FH-DM	**Total Girls (n= 51/1001)**				**Gujrati Girls (n= 28/51)**				**Non Gujrati Girls (n=23/51)**			
	Before		**After**		**Before**		**After**		**Before**		**After**	
	Mean	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**
MDA (nmol/ml)	2.91	0.58	2.11	0.32	2.99	0.47	2.16	0.23	2.81	0.69	2.05	0.39
SBP<120	**Total Girls (n=466/1001)**				**Gujrati Girls (n= 253/466)**				**Non Gujrati Girls (n=213/466)**			
	Before		**After**		**Before**		**After**		**Before**		**After**	
	Mean	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**
MDA (nmol/ml)	1.81	0.54	1.49	0.37	1.77	0.52	1.48	0.38	1.86	0.56	1.5	0.36
DBP<80	**Total Girls (n=543/1001)**				**Gujrati Girls (n=289/543)**				**Non Gujrati Girls (n= 254/543)**			
	Before		**After**		**Before**		**After**		**Before**		**After**	
	Mean	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**
MDA (nmol/ml)	1.92	0.62	1.56	0.41	1.92	0.64	1.57	0.44	1.92	0.6	1.55	0.38
SBP-121-139	**Total Girls (n=535/1001)**				**Gujrati Girls (n=273/535)**				**Non Gujrati Girls (n=262/535)**			
	Before		**After**		**Before**		**After**		**Before**		**After**	
	Mean	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**
MDA (nmol/ml)	2.82	0.58	2.07	0.33	2.85	0.55	2.12	0.31	2.79	0.62	2.03	0.34
DBP-81-90	**Total Girls (n= 458/1001)**				**Gujrati Girls (n=237/458)**				**Non Gujrati Girls (n=221/458)**			
	Before		**After**		**Before**		**After**		**Before**		**After**	
	Mean	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**	**Mean**	**± SD**	**Mean**	**±SD**
MDA (nmol/ml)	2.86	0.56	2.09	0.32	2.83	0.57	2.1	0.33	2.88	0.55	2.07	0.3

Table 2(b)

Non vegetarian	**Total Girls B vs A (n=280/1001)**	**Guj Girls B vs A (n=122/280)**	**Non Guj Girls B vs A (n=158/280)**	**GujvsNonGuj BMD (122vs158)**	**GujvsNonGuj AMD (122vs158)**
MDA (nmol/ml)	11.05[c]	6.87[c]	8.69[c]	-0.98	-0.56
FH of BP	Total Girls B vs A (n=100/1001)	Guj. Girls B vs A (n=29/100)	Non Guj. Girls B vs A (n=71/100)	Gu vs Non Guj BMD (29vs71)	Guj vs Non Guj AMD (29vs71)

MDA (nmol/ml)	13.28^{c}	7.58^{c}	11.06^{c}	1.21	1.55
FH-CAD	Total Girls B vs A (n=68/1001)	Guj. Girls B vs A (n=22/68)	Non Guj. Girls B vs A (n=46/68)	Guj vs Non Guj BMD (22vs46)	Guj vs Non Guj AMD (22vs46)
MDA (nmol/ml)	18.1^{c}	28.17^{c}	12.63^{c}	2.63^{b}	2.59^{a}
FH-DM	Total Girls B vs A (n=51/1001)	Guj. Girls B vs A (n=28/51)	Non Guj. Girls B vs A (n=23/51)	Guj vs Non Guj BMD (28vs23)	Guj vs Non Guj AMD (28vs23)
MDA (nmol/ml)	8.62^{c}	8.48^{c}	4.63^{c}	1.06	1.19
SBP<120	Total Girls B vs A (n=466/1001)	Guj Girls B vs A (n=253/466)	Non Guj Girls B vs A (n=213/466)	Guj vs Non Guj BMD (253vs213)	Guj vs Non Guj AMD (253vs213)
MDA (nmol/ml)	10.46^{c}	7.05^{c}	7.8^{c}	-1.79	-0.58
DBP<80	Total Girls B vs A (n=543/1001)	Guj. Girls B vs A (n=289/543)	Non Guj Girls B vs A (n=254/543)	Guj vs Non Guj BMD (289vs254)	Guj vs Non Guj AMD (289vs254)
MDA (nmol/ml)	11.16^{c}	7.54^{c}	8.34^{c}	0	0.57
SBP-121-139	Total Girls B vs A (n=535/1001)	Guj Girls B vs A (n=273/535)	Non Guj Girls B vs A (n=262/535)	Guj vs Non Guj BMD (273vs262)	Guj vs Non Guj AMD (273vs262)
MDA (nmol/ml)	25.77^{c}	19.28^{c}	17.43^{c}	1.18	3.20^{b}
DBP-81-90	Total Girls B vs A (n=458/1001)	Guj Girls B vs A (n=237/458)	Non Guj Girls B vs A (n=221/458)	Guj vs Non Guj BMD (237vs221)	Guj vs Non Guj AMD (237vs221)
MDA (nmol/ml)	25.69^{c}	17.14^{c}	19.36^{c}	-0.96	1.02

P value : a=<0.05; b=<0.01; c=<0.001

Discussion

According to Reddy KK et al [2] severe obesity is associated with lipid peroxidation. Obesity increases the mechanical and metabolic loads on the myocardium, thus increasing myocardial oxygen consumption. A negative consequence of this is the production of reactive oxygen species such as superoxide, hydroxyl radical, and hydrogen peroxides from the increased mitochondrial respiration. If the production of these oxygen species exceeds the antioxidant capacity of the cell, oxidative stress resulting in lipid peroxidation may occur. The second mechanism by which obesity can independently cause lipid per oxidation is by progressive and cumulative cell injury resulting from pressure of large body mass. Cell injury causes the release of cytokines specially tumor necrosis factor alpha, which generates reactive oxygen species from the tissues which in turn cause lipid peroxidation. A third possible mechanism is through diet. Nutritional obesity which is the predominant form in our study subjects implies the consumption of hyperlipidemic diets which may be involved in oxygen metabolism. Double bonds in the fatty acid molecules are vulnerable to oxidation reactions and consequently may cause lipid peroxidation [3].

Studies indicate that serum lipid peroxide levels tend to increase with age. During aging, oxidative stress and free radical load increase and their scavenging activity get decreased. Almost all biomarkers of oxidative stress have been found to accumulate with age. P-MDA showed higher values although in acceptable range for girls with age more than 20 years in comparison to that in age category of less than 20 years (2.39±0.77 nmol/ml v/s 2.31±0.74 nmol/ml); The increase in levels of MDA indicates that lipid peroxidation was increased because of obesity. This is in accordance to various studies [2,3,5]. The causes of increase lipids and lipid peroxides were due to alteration of functions of erythrocyte membrane. This inhibits the activity of antioxidant enzymes leading to accumulation of radicals which causes maximum lipid peroxidation and tissue damage in obesity. P-MDA values were directly related to socioeconomic status, the lower class had minimum oxidative stress (2.29±0.75 nmol/ml). The middle and upper class had high oxidative stress (2.36±0.75 nmol/ml). According to inhabitance rural girls had reasonably acceptable value of MDA (2.31±0.73 nmol/ml) while urban girls had higher level of the same (2.36±0.77 nmol/ml). MDA was also directly proportional to blood pressure. As the blood pressure increased MDA level also increased (1.81±0.54 nmol/ml v/s 2.82±0.58 nmol/ml). The study was also in accordance to report by Bjorntop et al [6], Reddy et al [2] who reported that significant increase in plasma lipid peroxides, free radical generation, MDA levels were obtained in urban inhabitants compared to rural. Indirectly, indication is that the changes of LDL oxidation were higher in obese state and could be one of the etiological factors in obesity. These presumptions are supported well by reports of other workers like Olusi et al [3]. Obesity induces systemic oxidative stress and increase oxidative stress in accumulated fat is one of the underlying causes of dysregulation of adipocytokines and development of metabolic syndrome and plays critical role in pathogenesis of various diseases [7,8]. Thus our study, in general, correlates with most of the available reports.

Summary and Conclusion

Oxidative stress marker-MDA was well within acceptable range as per ATP III guidelines in total, Gujarati and Non Gujarati girls.MDA increased with age. In total girls the average level of MDA for age<20 years was 2.31n mole/ml of serum while for age >20 years it was 2.39n mole/ml of serum. The same observation was seen in Gujarati girls (2.27v/s 2.37). Oxidative stress was maximum for middle and upper socio economic class when compared with lower class; Rural inhabitants showed less oxidative stress as compared to urban inhabitants (2.31nmol/ml of serum v/s 2.36nmol/ml of serum). Non vegetarian category showed high oxidative stress (2.39nmol/ml of serum v/s 2.33nmol/ml of serum;) Oxidative stress was maximum for girls with family history of CAD along with total cholesterol as compared to the respective values for the other family history of diseases. The increasing trend of MDA was FH CAD>FH HT = FH DM > without FH; FH CAD>FH HT > FH DM > without FH and that of TAA was FH CAD <FH HT < FH DM < without FH. Effect of maize diet was quite evident and almost equal (P<0.001) on all parameters in total, Gujarati and Non Gujarati girls. The oxidative stress showed improvement in normal, overweight and obese girls, most significantly on overweight and obese girls after taking the diet although the MDA levels were high but in acceptable range before the diet was taken. MDA levels were significantly reduced after the diet (P<0.001). When matched for age, MDA was significantly decreased for both the groups after the diet. When the two groups were compared with each other MDA was statistically significant (P<0.001). Effect of maize was seen for all socio economic classes. Highly significant changes were observed for almost all parameters in rural and urban categories for oxidative stress when matched for diet (P<0.001). For the vegetarian group of girls MDA level improved (P<0.001). MDA was significantly changed in the comparative analysis in family history of hypertension and coronary artery disease before as well as after the diet [10]. The girls of high normal category of blood pressure were more significantly affected by the diet which was evident by their improved MDA profile [11,12].

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