

### Inhibition of Akt/protein kinase B activity sensitizes moderately- to un-differentiated gastric cancer cells to chemotherapy

Yao G. Wu, PhD, Xi M. Xu, PhD,  
Guang J. Yuan, PhD, Jun H. Li, PhD,  
Zhong Y. Zhou, MD, Wei Ge, MM.

Gastric cancer is the most common malignant tumor of the digestive tract in China. Development of resistance to chemotherapy also occurs frequently in tumor cells. Akt, also known as protein kinase B (PKB), is a 60kD serine/threonine kinase and functions downstream of phosphatidylinositol 3-kinase (PI-3K), controlling diverse cellular functions, including cell apoptosis, proliferation, differentiation, and glucose metabolism.<sup>1</sup> In the present study, we studied the activities of Akt/PKB in gastric cell lines with different differentiation degrees (MKN-28, well-differentiated; SGC-7901, moderately-differentiated; BGC-823, poorly-differentiated; HGC-27, undifferentiated), and explored the effects of Akt/PKB inhibition on cell survival rates and apoptosis rates with treatment of etoposide.

**Cell culture.** The current study was conducted between September 2005 and June 2006. The MKN-28 (well-differentiated), SGC-7901 (well-differentiated), BGC-823 (poorly-differentiated), and HGC-27 (undifferentiated) cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum, 100U/ml streptomycin, 100U/ml penicillin in a humidified 5% CO<sub>2</sub> atmosphere at 37°C. The cells were digested with 0.25% trypsin and 0.02% EDTA every 2-3 days, and passed at ratio of 1:3-1:5.

**Cell survival rate assay.** The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide colorimetric assay was used to determine the cell survival rate. Gastric cancer cells in the logarithmic phase were seeded in 96-well plates, and treated with 20μmol/L etoposide in the presence or absence of a 2 hour pretreatment of 40nmol/L wortmannin for 0 hours, 3 hours, 6 hours, 12 hours, and 24 hours. Cell survival rates were assayed at the above time points. When exposed to 20μmol/L etoposide within 24 hours, the 4 gastric cancer cell lines exhibited time-dependent inhibition of cell survival rates. Pretreatment with wortmannin potentiated the inhibitory effect of etoposide on cell survival rates in a time-dependent manner. Except for MKN-28, there was a significant decrease in cell survival rates in other cell lines with pretreatment of wortmannin compared to without ( $p < 0.01$ ).

**Measurement of cell apoptosis rate.** Cell apoptosis rate was determined with flow cytometry using double staining of annexin V and propidium iodide (PI). Gastric cancer cells were treated with 20μmol/L etoposide in the presence or absence of 2 hour pretreatment of 40nmol/L wortmannin for 0 hours, 3 hours, 6 hours, 12 hours, and 24 hours, followed by washing 2 times with 0.01mmol/L phosphate buffer solution. The cells were suspended with binding buffer and adjusted to a density of 1x10<sup>6</sup>/ml. Cell suspension (1 ml) was centrifuged at 200g for 5 minutes. The supernatant was then discarded. Cell pellets were resuspended in 80μl binding buffer, and incubated with 10μl annexin-V-fluorescein and 10μl PI solutions at room temperature in the dark for 10 minutes. After centrifugation and resuspension in binding buffer, the samples were analyzed by flow cytometry with excitation wavelength of 488nm. When treated with 20μmol/L etoposide within 24 hours, the 4 gastric cancer cell lines showed time-dependent elevation of cell apoptosis rates. Pretreatment with wortmannin enhanced the effect of etoposide on cell apoptosis rates in a time-dependent manner. The cell apoptosis rates were significantly increased in SGC-7901, BGC-823m and HGC-27 with pretreatment of wortmannin compared to without ( $p < 0.01$ ).

**Determination of Akt/PKB activity.** The Akt/PKB activity was determined using a non-radioactive protein kinase assay kit. Basal PKB activity was measured as follows. Gastric cancer cells in the logarithmic phase were digested, and total protein was extracted from the cell lysates. The total protein was then incubated with immobilized antibody specific for phospho-Akt/PKB to immunoprecipitate Akt/PKB. The latter was used to phosphorylate a specific substrate, GSK-3 fusion protein (GSK-3α/β). Phospho-GSK-3α/β was analyzed by western blot with its monoclonal antibody. Enhanced chemiluminescence (ECL) was used to detect phospho-GSK-3α/β, which indirectly reflects the activity of Akt/PKB. The Akt/PKB activities in gastric cancer cells treated with etoposide for 0 hours, 3 hours, 6 hours, 12 hours, and 24 hours were also measured with the above method. Non-radioactive protein kinase assay revealed that the basal activities of Akt/PKB in these cancer cell lines are in the contrary order of differentiation degrees, MKN-28 < SGC-7901 < BGC-823 < HGC-27. Treatment with 20μmol/L etoposide led to time-dependent induction in Akt/PKB activities within 24 hours in the 4 cancer cell lines. The Akt/PKB activity was undetectable in cell lines pretreated with wortmannin.

**Discussion.** Gastric cancer is the most common malignant tumor of the digestive tract in China,

and accounts for the second leading cause of death among malignant tumors. Surgery is the mainstay of treatment for gastric cancer. As an adjuvant therapy after surgical resection, chemotherapy has received considerable attention in the treatment of gastric cancer. However, development of insensitivity or resistance to chemotherapy occurs frequently in tumor cells.<sup>2</sup> Novel agents are, therefore, needed to sensitize the resistant cancer cells. Akt is a 60kD serine/threonine kinase and functions downstream of phosphatidylinositol 3-kinase (PI-3K). Activation of PI-3K generates phosphatidylinositol 3,4-bisphosphate, which may induce the membrane translocation of Akt coincident with its phosphorylation and activation. Akt is activated in response to insulin and growth factors, and upon activation, it phosphorylates several substrates including glycogen synthetase kinase-3 (GSK-3), Bax, Caspase-9. Activation of Akt kinase activity is inhibited by wortmannin or LY294002, inhibitors of PI-3K.<sup>3</sup> Over activation of Akt has been demonstrated in gastric cancer, and is correlated with clinicopathological parameters and poor outcome.<sup>4</sup> In our study, we determined the basal activities of Akt/PKB in 4 gastric cancer cell lines with different differentiation degrees, and found that Akt/PKB activity was inversely correlated with the degrees of differentiation of cancer cells, which may account for the reason why over activation of Akt is associated with poor outcome in gastric cancers.

Akt/PKB plays an important role in cell survival.<sup>5</sup> In our study, treatment with etoposide within 24 hours resulted in time-dependent inhibition of cell survival rates and induction of cell apoptosis rates in cancer cell lines. However, there was a marked induction of Akt/PKB activity in a time-dependent manner during the course of etoposide treatment. The induction of Akt/PKB activity may confer protection against etoposide-induced death because inhibition of Akt/PKB by wortmannin enhanced the effects of etoposide. Cell survival rates and apoptosis rates were significantly decreased and elevated respectively in SGC-7901, BGC-823, and HGC-27 cell lines after treatment with etoposide + wortmannin compared with etoposide alone. However, we found that the chemotherapy-sensitizing effect of wortmannin was related to the degree of differentiation of cancer cells. There was no significant difference in cell survival rates and apoptosis rates between treatments of etoposide + wortmannin and etoposide alone in MKN-28, a well-differentiated cell line. The MKN-28 had less activity of Akt/PKB than other cell lines, which may explain the lack of chemotherapy-sensitizing effect by wortmannin.

In conclusion, Akt/PKB activity is inversely correlated with the degree of differentiation of gastric cancer cells, and inhibition of Akt/PKB activity

may sensitize moderately- to undifferentiated cancer cells to chemotherapy, which maybe extrapolated to clinical practice in selecting the patients for combined chemotherapy with Akt/PKB inhibitor.

Received 26th December 2006. Accepted 24th March 2007.

From the Center of Oncology (Wu, Xu, Ge) and the Department of Gastroenterology (Yuan, Li, Zbou), Renmin Hospital of Wuhan University, Wuhan, Hubei Province, China. Address correspondence and reprint requests to: Dr. Wei Ge, Center of Oncology, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China. Tel. +86 (27) 88041911 Ext. 2281. Fax. +86 (27) 88077386. E-mail: Gewei514@126.com

## References

1. Martelli AM, Nyakern M, Tabellini G, Bortul R, Tazzari PL, Evangelisti C, et al. Phosphoinositide 3-kinase/Akt signaling pathway and its therapeutical implications for human acute myeloid leukemia. *Leukemia* 2006; 20: 911-928.
2. Lacueva J, Perez-Ramos M, Soto JL, Oliver I, Andrada E, Medrano J, et al. Multidrug resistance-associated protein (MRP1) gene is strongly expressed in gastric carcinomas. Analysis by immunohistochemistry and real-time quantitative RT-PCR. *Histopathology* 2005; 46: 389-395.
3. Kim JE, Tannenbaum SR. Insulin regulates cleavage of procaspase-9 via binding of X chromosome-linked inhibitor of apoptosis protein in HT-29 cells. *Cancer Res* 2004; 64: 9070-9075.
4. Kobayashi I, Semba S, Matsuda Y, Kuroda Y, Yokozaki H. Significance of Akt phosphorylation on tumor growth and vascular endothelial growth factor expression in human gastric carcinoma. *Pathobiology* 2006; 73: 8-17.
5. Tokar A, Yoeli-Lerner M. Akt signaling and cancer: surviving but not moving on. *Cancer Res* 2006; 66: 3963-3966.

---

## Random amplified polymorphic DNA typing of nosocomial *Candida albicans* isolates

Somansu Basu, MD,  
Harish C. Gugrani, PhD, FRCPath,  
Pooja Jain, PhD, Zafar K. Khan, PhD.

The incidence of candidiasis is dramatically on the rise due to the increase in the immuno-suppressed population, especially related to HIV infection, chemotherapy, and organ transplantation.<sup>1</sup> *Candida albicans* is the most frequent pathogenic species of candidiasis, and the mortality rate in candidiasis varies from 38-50%.<sup>2,3</sup> Because of its clinical importance, several typing schemes were developed to assess the strain identity. These typing methods are generally considered too variable to be of any practical value in epidemiological investigations.<sup>4</sup> With the advent of molecular genetics,

several DNA-based typing methods are being used for analyses, such as karyotyping, restriction enzyme analysis, pulse field gel electrophoresis, and multilocus enzyme electrophoresis; however, these methods are laborious and time consuming.<sup>5</sup> The analysis of *Candida albicans* by random amplified polymorphic DNA (RAPD) is rapid, convenient, as well as reliable, and helps better in understanding the epidemiological aspects of candidiasis.<sup>6</sup> In this study, isolates of *Candida albicans* from 2 hospitals, Sir Gangaram Hospital and St. Stephen's Hospital, in Delhi were analyzed by different random primers to establish the suitability of the RAPD typing for *Candida* strains.

Ten nosocomial isolates of *Candida albicans* were recovered from various clinical specimens, such as, bronchoalveolar lavage, blood, urine, pus, and plastic devices, and confirmed by Analytical Profile Index Candida. The genomic DNA was extracted according to the method described by Ausubel with minor modifications.<sup>7</sup> After ribonucleotidase treatment, 2 µl of extracted DNA template of 50ng/ml concentration was added into the mixture of 2.5 µl 10X buffer, 0.5 µl dNTPs mix, 0.2 µl Taq DNA polymerase, 1 µl RAPD primer, and 18.8 µl standard water to prepare the assay reaction mixture. Amplification parameters consisted of 45 cycles of denaturation at 94°C for 60 seconds, annealing at 35°C for 90 seconds, and extension at 72°C for 90 seconds. In the first cycle, the denaturation was carried out for 3 minutes, and 10 minutes for the final extension. Amplification reaction was carried out in an assay mixture of 25 µl using 6 different RAPD primers from Operon OPB-11 (5'-GT AG AC CC GT-3'), OPB 14 (5'-TC CG CT CT GG-3'), OPB 15 (5'-GG AG GG TG TT-3'), OPB 18 (5'-CC AC AG CA GT-3'), OPB 19 (5'-AC CC CC GA AG-3'), and OPB 20 (5'-GG AC CC TT AC-3') in Perkin Elmer Gene Amplifier 2400. Agarose gel electrophoresis of the polymerase chain reaction (PCR) product was carried out in 1.4% gel concentration, with 2 µl of DNA samples for 2 hours at 2 V/cm electric circuits. The molecular weight marker used in this study was 1 kb DNA ladder (Lambda DNA EcoRI/Hind III Digest, Sigma). Genomic DNA with A 260/A 280 ratio in between 1.8 to 2.1 was used, and the RAPD patterns generated was analyzed using Diversity Database software version 1.1 incorporated into gel documentation system from PDI, USA. Cluster analysis of the 10 *Candida albicans* was carried out using the unweighted-pair group method with arithmetic average, and the phylogenetic tree was constructed for all samples in each database.

The application of RAPD technology for strain delineation of *Candida albicans* has proven to be a valuable tool for clinico-epidemiological studies.<sup>6,8,9</sup>

Gyanchandani et al,<sup>10</sup> reported that RAPD of 19 *Candida albicans* showed non-identical profile when tested with 21 primers. The present study with oligonucleotide primers OPB-18, OPB-19, and OPB-20 did not produce any RAPD profile with the strains. However, the RAPD patterns of *Candida albicans* exhibiting intraspecific polymorphisms were obtained with OPB-11, OPB-14, and OPB-15 primers. However, unlike other primers, OPB-14 exhibited RAPD profile with all the 10 isolates. The OPB-15 showed profiles against 7 isolates only, while OPB-11 showed profiles to 6 isolates only. The range of molecular weights of DNA obtained by using OPB-11 was between 1.91 kb and 0.85 kb, while with OPB-14 it was 728 kb to 278 kb, and with OPB-15 it was between 1800 bp and 250 bp.

In the last few decades, there has been increasing reports of *Candida* infections in India.<sup>11</sup> In our study we found that 3 primers OPB-18, OPB-19, and OPB-20 were ineffective in producing profiles, but OPB-11 primer when used to generate RAPD profile, 3 clusters were clearly represented. One band of 1.4 kb was shared by all the strains when OPB-11 was used. Non-randomization was found in isolates from blood, urine, and pus of 3 unrelated patients; and between isolates of plastic device and skin of 2 unrelated patients in the same hospital. However, when OPB-15 was used to amplify the same isolates, it exhibited randomness in these strains. The OPB-15 primer therefore has better discriminatory power, with higher detection of strain variability, and is more efficient in reflecting intra-specific variation.

Received 16th December 2006. Accepted 17th March 2007.

From the Department of Medical Mycology (Basu, Guagnani), VP Chest Institute, University of Delhi, and the Department of Mycology (Jain, Khan), CDRI, Lucknow, India. Current address for correspondence and reprint request to: Dr. Somansu Basu, Department of Microbiology, Sur Hospital, Ministry of Health, Oman, PO Box 346, Postal Code 411, Sur, Oman. Tel. +968 25542619. Fax. +968 25561558. E-mail: somansu@rediffmail.com

## References

1. Pinto PM, Resende MA, Koga-Ho CY, Tendler M. Genetic variability analysis among clinical *Candida* spp. isolates using Random Amplified polymorphic DNA. *Mem Inst Oswaldo Cruz* 2004; 99: 147-152.
2. Pfaller MA. Nosocomial candidiasis: emerging species, reservoirs and modes of transmission. *Clin Infect Dis* 1996; 22: S89-S94.
3. Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A one enzyme PCR-RFLP assay for identification of six medically important species. *Nippon Ishinkin Gakkai Zasshi* 2006; 47: 225-229.
4. Pfaller MA. Strain variation among *Candida* species: application of various typing methods to study the epidemiology and pathogenesis of candidiasis in hospitalized patients. *Infect Control* 1987; 8: 273-276.

5. Xu J, Vilgalys R, Mitchell TG. Lack of genetic differentiation between two geographic samples of *Candida albicans* isolated from patients infected with human immunodeficiency virus. *J Bacteriol* 1999; 181: 1369-1373.
6. Bello MD, Gonzalez A, Barnabe C, Larrouy G. First Characterization of *Candida albicans* by Random amplified polymorphic DNA method in Nicaragua and comparison of the diagnosis methods for vaginal candidiasis in Nicaraguan women. *Mem Inst Oswaldo Cruz* 2002; 97: 985-989.
7. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, editors, et al. Short protocols in Molecular Biology. New York: John Wiley and Sons, Inc; 1999.
8. Vrioni G, Matsiota-Bernard P. Molecular typing of *Candida* isolates from patients hospitalized in an intensive care unit. *J Infect* 2001; 42: 50-56.
9. Chong PP, Lee YL, Chong Tan B, Peng Ng K. Genetic relatedness of *Candida* strains isolated from women with vaginal candidiasis in Malaysia. *J Med Microbiol* 2003; 52: 657-666.
10. Gyanchandani A, Khan ZK, Farooqui N, Goswami M, Ranade SA. RAPD analysis of *Candida albicans* strains recovered from different immunocompromised patients (ICP) reveals an apparently nonrandom infectivity of the strains. *Biochem Mol Biol Int* 1998; 44: 19-27.
11. Sengupta P, Ohri VC. Study of yeast species isolated from clinical specimens. *Med J Armed Forces Ind* 1999; 55: 319-321.

---

### Assessment of nutritional status and lifestyle pattern among Saudi Arabian school children

*Fayssal M. Farahat, MSc, MD,  
Kamlesh P. Joshi, MD, DNB,  
Falah F. Al-Mazrou, BSc.*

Though the prevalence of obesity varies greatly in different populations of the world, various studies have shown a significant increase in the prevalence of overweight and obesity among children.<sup>1</sup> The epidemiologic transition in developing countries as a result of urbanization, migration, sedentary life styles, and changing dietary habits are recognized as some of the factors in the development of overweight and obesity in childhood.<sup>2</sup> As health-related behaviors and beliefs established during childhood have been linked to patterns of behavior in adulthood, therefore, the schools can play an important role in the health promotion of children and represent the best opportunity to acquire a healthy lifestyle not only through knowledge, but also attitude and behavior.<sup>3</sup> The purpose of the study was to describe the nutritional status among school children in healthy cities in the Kingdom of Saudi Arabia (KSA), using the 85th and 95th percentiles of body mass index (BMI) for age and gender as cut-off points.

This was a cross-sectional study carried out in the provinces of KSA from December 2004 to January 2006. Using a stratified random sampling technique, the sample subjects were chosen from 3 selected provinces, namely, Western, Northern, and Eastern of the 5 provinces. The ongoing 'healthy cities' program in these provinces made it convenient to carry out a study in these regions and give recommendation for its expansion. From each selected province, 3 cities each from Western and Northern provinces, and 2 cities from the Eastern province were selected randomly from a list of cities. Four schools (2 boys and 2 girls intermediate schools) from each city were randomly selected, except for one city in the Eastern province, where the study was conducted in 2 girl schools only due to certain administrative reasons. Two classes from each school were randomly selected and thereafter, each class was considered as a cluster in which all students (average 27 students per class) were enrolled in the study. Thus, a total of 30 schools were surveyed, wherein a total of 60 classes, namely, 28 boys and 32 girls (grades 1-3) were included in the sample. Approval was taken from the Ministry of Education and the Principal of the school to carry out the survey in the chosen schools and classes. The health survey questionnaire used in this study was modified and adapted from the Global School Health Survey (GSHS), which basically assesses the overall health of school students. This instrument included questions on food consumption, daytime physical inactivity, and smoking habits and, was subsequently translated to Arabic vernacular by the investigators. Furthermore, the questionnaire was pilot tested separately on boys and girls and, accordingly adapted for implementation in KSA. In each randomly chosen class, all students were briefed on the survey health questionnaire, and subsequently administered and supervised while filling the questionnaire by the investigators. Their basic demographic details were initially recorded. Subsequently, anthropometric measurement, namely, one trained male and female nurse carried out height and weight of participating school children for boys and girls. For each child, BMI was estimated by age and sex, and compared to the BMI latest World Health Organization/National Center for Health Statistics (WHO/NCHS) 2000 reference.<sup>3</sup> The cutoff percentiles used to classify the nutritional status of the children were underweight, BMI  $p < 10$ ; normal, BMI  $p \geq 10$  to  $p < 85$ ; overweight,  $p \geq 85$  to  $p < 95$ ; and obese, BMI  $p \geq 95$ .

The study group included a total of 1454 children in the 3 provinces, in the age range from 12-19 years, with the mean and median age as 15 years. Out of all participating children, 45.2% were boys, and 54.8%

**Table 1.** Region-wise prevalence of overweight and obesity among school children.

Regions	Boys n (%)		Girls n (%)		Statistical significance	
	Overweight	Obesity	Overweight	Obesity	Overweight	Obesity
Eastern	8 (6.5)	20 (16.3)	39 (17.2)	35 (15.4)	$\chi^2=7.8, p=0.005$	$\chi^2=0.04, p=0.81$
Northern	24 (10.1)	43 (18.1)	46 (18.3)	24 (9.5)	$\chi^2=6.5, p=0.01$	$\chi^2=7.50, p=0.005$
Western	26 (9.2)	36 (12.7)	47 (15.7)	37 (12.4)	$\chi^2=5.6, p=0.017$	$\chi^2=0.02, p=0.90$

girls. Among boys, significant differences in nutritional status were noted according to age, with a shift to overweight and obesity among the younger age groups ( $\chi^2=16.16, p=0.008$ ). Among girls, no significant differences were observed according to age. Significant difference in overweight was observed between boys and girls with a higher prevalence among girls. Although the prevalence of obesity was higher among boys, however, no significant difference was observed between boys and girls. The nutritional status in both boys and girls was assessed by regions. Significant differences in nutritional status were observed in different regions both in boys and girls. However, a higher prevalence of overweight and obesity was reported among boys in the Northern Province ( $p=0.025$ ) and among girls in the Northern and Eastern Province ( $p=0.034$ ), compared to other provinces. Girls below 15 years were significantly more physically inactive for more than 5 hours in the daytime compared to boys. Initiation of smoking at a younger age and for a longer duration was observed among boys with an increasing proportion of children in higher age groups. Furthermore, a relationship of BMI with lifestyle pattern showed that the difference in the dietary habits, particularly vegetables ( $\chi^2=10.2, p=0.003$ ) and fruits ( $\chi^2=6.2, p=0.044$ ), were significantly related to difference in the BMI in different regions. However, physical activity and smoking did not significantly relate to BMI.

The present study shows that overweight and obesity in Saudi Arabian school children is high and reaffirms what other authors have observed in similar population groups.<sup>1,2</sup> With regard to inter-provincial differences, our study observed that overweight is highest both in boys and girls in the Northern region and also, boys had significantly higher obesity compared to girls, and in the Western region girls were significantly more overweight than boys (**Table 1**). Differences in overweight and obesity in another study,<sup>1</sup> could be explained by the difference in the reference standard used in assessment of nutritional status. In this regard, it has been noted that cutoffs to define obesity based on WHO/NCHS reference represents similar absolute BMI values between different national reference data sets, and so this provides a degree of consistency in

definitions among nations.<sup>3</sup> Also, a systematic review/critical evidence appraisal concluded that the evidence base for use of national BMI reference data is sufficiently strong for a recommendation for its adoption in clinical practice and epidemiology.<sup>4</sup> Wang<sup>5</sup> in his research on cross-national comparison of childhood obesity, based on the data sources from 1992-1994, showed that childhood obesity is related to socio-economic status with family income as a primary indicator. However, these differences in various studies could also be partly due to a secular trend over the past 10 years, and other attributing factors such as eating habits, lifestyle pattern, income differences, and genetics. It has been noted that economic development in KSA during the last 30 years has changed nutritional, and lifestyle habits.<sup>1</sup> These changes have influenced the quality and quantity of food intake and predispose people to a sedentary life.<sup>1</sup> To substantiate, our study has also observed differences in dietary habits in different regions with more boys and girls in the Western region, than Northern and Eastern region, preferring vegetables and fruits in their diet. In our study, the overall prevalence of obesity among younger children exhibited higher obesity prevalence in both genders, however only in boys was the differences in age groups significant. In a cross-national comparison of childhood obesity, the findings show that in both Russia and China, but not the USA, the prevalence of obesity and overweight was higher among children than among adolescents.<sup>5</sup> The study points towards the need for further research to investigate whether the gap is due to the differences in children's and adolescents' social and behavioral factors, such as diet and physical activity, or if it is because of the WHO/NCHS standard, which is based on data from the USA.<sup>5</sup> It is suspected that by using the WHO/NCHS standard we might have underestimated the obesity problem among adolescents in China, Russia,<sup>5</sup> and similarly also in KSA.

Physical activity is noted as one of the most important contributing factors to the increase in childhood obesity.<sup>1,2</sup> Boys and girls at 15 years and above are shown to be equally physically inactive, except girls below 15 years were more physically inactive. In a study on adult Saudis, it was shown that overweight and obesity prevalence is significantly high among the Saudis, where Saudi males have a significantly higher

prevalence of overweight, while the Saudi females have more obesity.<sup>2</sup> This predicts the future trend of overweight and obesity among boys and girls in the present study, with the current lifestyle pattern, putting Saudi school children at high risk of becoming obese in adulthood. This study therefore presents the urgent need to implement comprehensive lifestyle modification intervention programs in schools that addresses nutrition and physical education, and other healthy lifestyle habits, targeting school children, and parents, to reduce the risk of overweight and obesity and its health consequences in adulthood.

Received 26th November 2006. Accepted 24th March 2007.

From the General Directorate of Preventive Health, Ministry of Health, Riyadh, Kingdom of Saudi Arabia. Address correspondence and reprint requests to: Dr. Fayssal M. Farabat, General Directorate of Preventive Health, Ministry of Health, PO Box 2903, Riyadh 11461, Kingdom of Saudi Arabia. Tel. +966 (1) 4791073. Fax. +966 (1) 4785751. Email: [fmfayssal@yahoo.com](mailto:fmfayssal@yahoo.com)

## References

1. El-Hazmi MAF, Warsy AS. A comparative study of prevalence of overweight and obesity in children in different provinces of Saudi Arabia. *J Trop Pediatr* 2002; 48: 172-177.
2. Abalkhail B. Overweight and obesity among Saudi Arabian children and adolescents between 1994 and 2000. *East Mediterr Health J* 2002; 8: 470-479.
3. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 2000; 320: 1240-1245.
4. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. *Adv Data* 2000; 314: 1-27.
5. Wang Y. Cross-national comparison of Childhood Obesity: the epidemic and the relationship between obesity and socioeconomic status. *Int J Epidemiol* 2001; 30: 1129-1136.

---

## The study of diabetes mellitus in Gorgan, Iran

*Gholamreza Veghari, MSc,  
Abdoljalal Marjani, PhD,  
Hamidreza Joshaghani, PhD.*

Diabetes Mellitus (DM) is a serious disease and it causes a number of chronic diseases the same as cardiovascular disease, renal failure, and neuropathy. Globally, it is expected that the prevalence of DM will double from 2000 to 2030, while public awareness remains less.<sup>1</sup> Several studies have demonstrated that DM has a strong negative impact on the health-related quality of life, especially in the presence of

complications.<sup>2</sup> It is commonly linked with ecological factors such as food habit, physical activity, and obesity. These factors can increase, decrease or prevent the side effects of DM. Therefore this study was carried out to determine anthropometrical status, some biochemical indexes, and nursing supervision on 334 diabetic patients attending the Gorgan Diabetic Clinic.

The cases (109 men and 225 women) were chosen by random sampling over 3 months. The questionnaires, weight, and height were recorded for all. Serum triglycerides (TG), cholesterol, and fast blood sugar (FBS) were determined using laboratory kits (enzymatic methods), and the spectrophotometry technique (model JENWAY 6105 UV/VIS). Hemoglobin A1c (HbA1C) was measured via electrophoresis. All of the cases agreed to participate in this study. The data was saved on computer, and analyzed by Statistical Package for Social Sciences, version 11.5 software;  $\chi^2$  and correlation tests were used to compare the groups. The  $p < 0.05$  was considered to be significant.

In this study, 24.9% (83) of patients suffered from insulin dependent DM (IDDM), while 75.1% (251) suffered from non insulin dependent DM (NIDDM). With regard to dwelling place, 15.6% lived rurally, and 84.4% lived in an urban area. Seventy-seven percent were illiterate or had elementary education, 30.4% had been patients for 15 years. Body mass index (BMI) over 25 kg/m<sup>2</sup> was observed in 49.9% of IDDM patients, and 85.2% of NIDDM patients. The HbA1C in 85.3% of patients was over 8%, which is not suitable for blood glucose control. Fasting blood sugar of  $\geq 110$  mg/dl was observed in 73.2% IDDM, and 86.4% NIDDM (Table 1). The level of serum cholesterol in 40.6% of IDDM and 9.1% of NIDDM patients and serum TG in 1.73% and 86.4% of these patients were higher than normal range (Normal range: Cholesterol; 140-250 mg/dl and TG; 50-170 mg/dl). There were statistically significant differences between the 2 diabetes types on basis of serum FBS ( $p < 0.005$ ) and serum cholesterol ( $p < 0.0004$ ). There is a reverse significant correlation between mean serum TG ( $p < 0.01$ ,  $r = 0.098$ ), serum cholesterol ( $p < 0.01$ ,  $r = 0.193$ ) and BMI ( $p < 0.01$ ,  $r = 0.172$ ) with literacy, but it has a positive correlation with HbA1C.

Some studies show that food pattern, low physical activities, and genetic factors are original agents for obesity, DM, and cardiovascular disease. In this study, the prevalence of overweight was observed in more than 80% of NIDDM, but it was less in IDDM. It is known that high body fat is common in NIDDM. High body fat causes insulin resistance and decreases glucose metabolic rate. Therefore, these patients should achieve an optimal weight for better control of blood glucose. The HbA1C was measured for control of blood glucose

**Table 1** - Comparison of biochemical indexes between 2 types of diabetes mellitus (DM).

Types of DM	N	FBS (mg/dl)*			HbA1c (%)		TG (mg/dl)			Cholesterol (mg/dl)*		
		<60	60-110	>110	<8	>8	n (%)	>50	50-170	>170	>140	140-250
IDDM	82	2 (2.4)	20 (24.4)	60 (73.2)	15 (18.3)	67 (81.7)	2 (2.4)	46 (56.0)	33 (40.2)	9 (10.9)	65 (79.2)	7 (8.5)
NIDDM	250	0 (0.0)	34 (13.6)	216 (86.4)	35 (14.0)	214 (85.6)	2 (0.8)	118 (47.2)	131 (52.4)	6 (2.4)	176 (70.4)	69 (27.6)
<b>Total</b>	<b>332</b>	<b>2 (0.6)</b>	<b>54 (16.3)</b>	<b>276 (83.1)</b>	<b>50 (15.0)</b>	<b>281 (84.6)</b>	<b>4 (1.2)</b>	<b>164 (49.4)</b>	<b>164 (49.4)</b>	<b>15 (4.5)</b>	<b>241 (72.6)</b>	<b>76 (22.9)</b>

IDDM - insulin dependent diabetes mellitus, NIDDM - non insulin dependent diabetes melitus, FBS - fasting blood sugar, TG - triglyceride, \* $\chi^2$  test is statistically significant between the 2 groups.

during the previous 3-4 months. In this study, 85.3% of patients did not have good blood glucose control. In this case, IDDM and NIDDM are similar. The study by Matini et al<sup>3</sup> on patients at Kashan Hospital, Iran reported a lower ratio in comparison with this study. There is no significant correlation between literacy and HbA1C. Other studies are necessary to detect the factors effecting low blood glucose. Serum TG in NIDDM is higher than IDDM in this study. Navaii et al<sup>4</sup> in Islamshahr observed that the mean TG serum in women was higher than men, and reported obesity and hyperlipidemia among diabetic patients. In this study, the level of serum cholesterol in IDDM was higher than NIDDM diabetic patients. The study of Amini et al<sup>5</sup> among <40 year old people in Isfahan, showed similar results in diabetic patients. Serum TG and blood pressure among diabetic patients is higher than healthy people.

We conclude that most patients in this study have 2 main problems; they have no knowledge about their diet and blood glucose control. The high serum TG and cholesterol, high blood pressure and obesity, are also problems. Therefore, educational planning is necessary to decrease the rate of diabetic complications. More studies are necessary to fully investigate the status of DM in rural and urban area of Gorgan, Iran.

Received 27th December 2006. Accepted 24th March 2007.

From the Department of Biochemistry and Nutrition (Veghari), School of Medicine, and the Department of Biochemistry (Marjani, Joshaghani), Golestan University of Medical Sciences, Gorgan, Iran. Address correspondence and reprint requests to: Dr. Gholam R. Veghari, Department of Biochemistry and Nutrition, School of Medicine, Gorgan, Iran. Tel. +98 (171) 4421651. Fax. +98 (171) 4421289. Email: veghary@yahoo.com

## References

1. Wild S, Roglic G, Green A, Sincere R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; 27: 1047-1053.
2. Redkop WK, Koopmanschap MA, Stalk RP, Rotten GE, Wolfenbuttel BH, Nielsen LW. Health-related quality of life and treatment satisfaction in Dutch patients with type 2 diabetes. *Diabetes Care* 2002; 25: 458-463.
3. Matini M, Moiré R, Vali GR. Diabetes mellitus and its control at Shahid Beheshti Hospital in 1997 FEYZ. *Journal of the Kashan University of Medical Sciences and Health Services* 2001; 9: 26-21.
4. Navaii L, Mehrabi Y, Kimagar M, Azizi F. Epidemiologic survey on hypertension and its relation with obesity, diabetes and hyperlipidemia in Islamshahr, Iran. *Journal of the Shabed Beheshti University of Medical Sciences and Health Services* 1990; 2: 52-40.
5. Amini M, Mehr MN, Aminorroaya A. The incidence of type 2 diabetes in 25-70 year old symptomatic women referring to Isfahan Endocrine & Metabolism Research Center from 1996 to 2002. *Journal of the Zanjan University of Medical Sciences and Health Services* 2004; 46: 40-33.