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Academic Publishing & Translation

Biomedical Sciences

Comparative protection by desferrioxamine against hepato- and nephro-toxicity induced by azathioprine

Abdullah A. Alghasham, Muhammad Raza

Introduction Although the biochemical mechanism of toxicity to liver and kidney by azathioprine (AZA) have been fairly defined and several hypo-thesis have been proposed. The most documented hypothesis suggests an involvement of lipid peroxidation (MDA) preceded by glutathione (GSH) depletion. The involvement of peroxidative injury in hepato- and renal toxicity is based on several lines of evidence (1-6) and corroborate the postulated role of MDA in hepatic and renal manifestations. It is also well known that presence of transition metal plays a crucial role in the initiation and propagation of free radicals. A study of biochemical changes in liver and kidney following use of an iron chelator has not been reported.

Objectives To determine the effects of pretreatment of mice with DFO on the MDA and sulfhydryl contents in the kidney and liver tissues either with AZA or in combination with DFO and role of iron and other transition metals in the initiation and production of reactive moieties; and to assess the effects on the plasma levels of biochemical markers in AZA-treated mice and thus evaluate the possible participation of free radicals in its pathogenesis and possible protection by DFO.

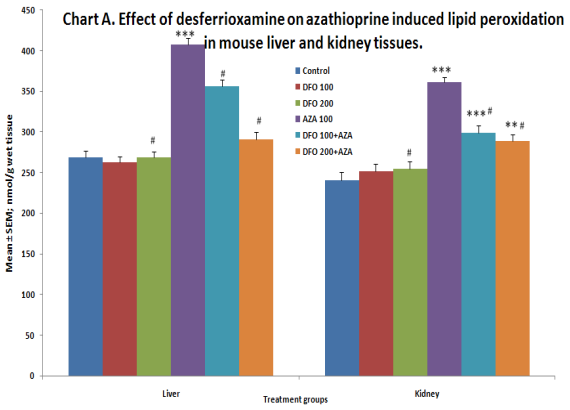
Methods Six groups of adult male Swiss albino mice were treated as follows. Group I: control group, saline for 5 days by intraperitoneal (i.p.) injection; group II and III: DFO 100 and 200 mg/kg body wt. i.p., for 5 consecutive days; group IV: AZA (100 mg/kg body wt.) as a single i.p. dose on the 5^{th} day; group V and VI (DFO 100 or 200 mg/kg for 5 days + AZA (100 mg/kg; single dose on 5^{th} day). A day after the last treatment, blood samples were obtained, and all the animals were sacrificed, the liver and kidneys were excised off the body, and biochemical analyses of tissues were performed. Hepatic and renal status was determined using plasma biochemical markers.

Results There was a significant increase ($P<0.001$) in MDA contents after treatment with AZA in both liver and kidney tissues when compared to control. Pretreatment with DFO prevented any increase in MDA contents in both the tissues significantly ($P<0.001$) but could not keep it to the base line when compared to AZA alone group (Chart A). Treatment of mice for consecutive five days had no significant effect on the levels of NPSH (Chart B) in liver and kidney tissues in comparison to control group. But, AZA at 100 mg/kg i.p. significantly reduced ($P<0.001$) NP-SH contents in both liver and kidney tissues of treated mice in comparison to control. Pretreatment of mice for five days with DFO afforded no protection against the declining levels of NPSH at both the dose levels, and this decline was significantly ($P<0.001$ and $P<0.01$) lower at 100- and 200 mg/kg doses, respectively, in the liver and kidney tissues. Treatment with AZA caused a significant ($P<0.001$) incline in all the parameters. The levels of plasma biochemical parameters (AST, ALT, LDH and creatinine) were significantly ($P<0.001$) lower in DFO treatment group from AZA group. Pretreatment with DFO significantly prevented a rise in all biochemical parameters when compared to AZA alone group, but remained significantly elevated than the control group.

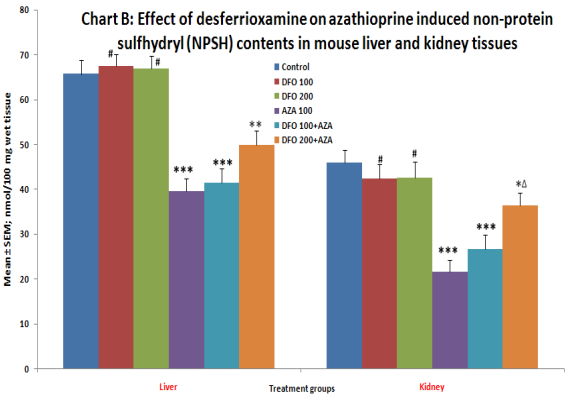
Discussion Our findings suggest that DFO is safe and useful for ameliorating azathioprine-induced hepato- and nephro-toxicity. The protection offered against azathioprine was comparable in preventing a rise in MDA.

However, a decline in NPSH contents in both liver and kidney tissues could not be recovered by either of the test doses of DFO. The plasma levels of aminotrans-ferases, LDH and creatinine that increased significantly by AZA also recovered significantly but could not recuperate to baseline; it

seems that DFO pretreatment is more effective in the recovery of LDH and creatinine levels to its baseline in this treatment regimen.



Six animals were used in each treatment group.
Treatments were given daily for consecutive five days for consecutive five days.
Statistical analysis was done by One Way ANOVA followed by Tukey-Kramer multiple comparisons test in each tissue independently.
All the groups were compared to control. **P<0.01 and ***P<0.001. #P<0.001, compared to azathioprine alone (group 4).



Six animals were used in each treatment group.
Treatments were given daily for consecutive five days for consecutive five days.
Statistical analysis was done by One Way ANOVA followed by Tukey-Kramer multiple comparisons test in each tissue type independently. All the groups were compared to control. *P<0.05; **P<0.01 and ***P<0.001. #P<0.001, and ΔP<0.05 compared to azathioprine alone (group 4).

References

1. Cates, L., and Li, V.-S. (1982) J. Pharm.Sci., 71: 308-311.
2. Hung, C.R., Wang, P.S., (2004). Eur. J. Pharmacol. 491: 61–68.
3. Rydkinaa, E., Sahni, S.K., Santucci, L.A., Turpina, L.C., Baggs, R.B., Silverman, D.J., (2004). Microb. Pathog. 36: 293–301.
4. Suntres, Z.E., Omri, A., Shek, P.N., (2002). Microb. Pathog. 32: 27–34.
5. Haque, R., Bin-Hafeez, B., Parvez, S., Pandey, S., Sayeed, I., Ali, M., Raisuddin, S., (2003). Hum. Exp. Toxicol. 22: 473–480.
6. Mansour MA. (2000). Life Sciences, 66(26): 2583-2591

Methylenetetrahydrofolate Reductase (MTHFR) and Angiotensin Converting Enzyme (ACE) Gene Polymorphisms among Saudi Population from Qassim Region

Abdullah AlGasham, Hisham Ismail, Moataz Dowaidar , Ahmad A Settin

Introduction Hypertension and cardiac diseases are multi-factorial disorders with genetic background determined by multiple gene polymorphisms.
MTHFR plays a central role in folate metabolism. Its involvement in the regulation of homocysteine concentration makes it a risk factor in cardiovascular disorders (CVD) [1]. ACE is a chloride and zinc dependent dipeptidyl carboxypeptidase. As a bioactive component of renin-angiotensin system (RAS) ACE plays a significant role in blood pressure regulation, fluid and electrolyte balancing, cardiovascular system development and vascular remodeling [2].

Objectives To assess the frequency of MTHFR and ACE gene polymorphisms as potential genetic risk factors for hypertension and cardiovascular disorders among Saudi subjects from Qassim region;

Methods This work included 273 adult healthy unrelated subjects from Qassim Region. Their DNA was analyzed for genetic polymorphisms of MTHFR (677C/T and 1298 A/C) as well as ACE (I/D) using real-time PCR.

Results Carriers of the mutant MTHFR; 677 T allele (CT+TT) and that of the 1298 C allele (CC+AC) constituted 33.7% and 48.9% of studied subjects respectively; while carriers of ACE gene mutant D allele (DD+ID) represented 93.3% of subjects. The allele frequencies of MTHFR 677T, 1298C and ACE D alleles were 18.7%, 29.45% and 72.5% respectively. Haplotype analysis of characterized chromosomes revealed that 2.5% were likely to carry the 3 mutant alleles together, 30.91% were likely to carry two of the three mutant alleles and 51.92% were likely to carry one mutant allele (Tables 1 and 2).

Discussion Saudis, particularly in Qassim region, are affected by hypertension and coronary artery diseases [3]. So far, very little information was available about the genetic background of Saudi subjects in terms of their susceptibility to hypertension and CAD. To our knowledge, this is the first report of analysis of three interactive gene polymorphisms that have a probable role in the susceptibility for EHand CAD among Saudi population. This study showed that the mutant allele MTHFR 677T frequency (18.7%) in subjects from Qassim was higher than that reported previously in Riyadh region (14.8%), Bahrain (12.6%) and Jordan (16%) [3].

Table 1 Genotype and allele frequencies of studied gene polymorphisms tested by Hardy-Weinberg Law of genetic equilibrium

Genotypes	Number	%	HWE χ^2 (P)
MTHFR 677C/T (n=270)			
CC	179	66.3	0.0016 (p>0.05)
CT	81	30	0.0148 (p>0.05)
TT	10	3.7	0.0311 (p>0.05)
C allele	219.5	81.3	
T allele	50.5	18.7	
MTHFR 1298A/C (n=269)			
AA	138	51.1	0.122 (p>0.05)
AC	105	38.9	0.438 (p>0.05)
CC	27	10	0.499 (p>0.05)
A allele	190.5	70.55	
C allele	79.5	29.45	
ACE D/I (n=269)			
DD	139	51.7	0.041 (p>0.05)
ID	112	41.6	0.200 (p>0.05)
II	18	6.7	0.305 (p>0.05)
D allele	195	72.5	
I allele	74	27.5	

This study also showed that the mutant allele MTHFR 1298C frequency in studied subjects from Qassim (29.45%), was lower than was reported in several other middle-eastern countries. Regarding the ACE gene polymorphism, this study showed that the ACE DD genotype frequency in Qassim region (51.7%) was higher than reported in other Mediterranean and Asian countries. Analysis of chromosomal haplotypes revealed that the 3 studied mutant alleles were likely to be carried by 2.5% of the characterized chromosomes while 2 mutant alleles were carried by 30.91%.

We conclude that Saudis in Qassim Region are carriers of relatively considerable amount of genetic alleles predisposing them to hypertension and cardiac diseases. This gives a warning to local health authorities for adoption of competent programs for prevention as well as early diagnosis and management.

Table 2 Haplotype frequencies of studied gene polymorphisms

Haplotypes	Number	Total
Total	524#	100%
Haplotypes with 3 mutant alleles		
MTHFR_ 677T/1298C/ACE_D	13 (2.48%)	2.48%
Haplotypes with 2 mutant alleles		
MTHFR_ 677C/ 1298C/ACE_D	96 (18.32%)	30.91%
MTHFR_ 677T/ 1298C/ACE_I	5 (0.95%)	
MTHFR_ 677T/ 1298A/ACE_D	61 (11.64%)	
Haplotypes with one mutant allele		
MTHFR_ 677T/ 1298A/ACE_I	20 (3.82%)	51.92%
MTHFR_677C/ 1298C/ACE_I	42 (8.02%)	
MTHFR_677C/ 1298A/ACE_D	210 (40.08%)	
Haplotypes with normal alleles		
MTHFR_677C/ 1298A/ACE_I	77 (14.69%)	14.69%

References

1. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ. 2002 Nov 23; 325(7374):1202.
2. Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). Thromb Haemost. 1997 Jul; 78(1):523-6.
3. Charles DH, Ness AR, Campbell D, Smith GD, Whitley E, Hall MH. Folic acid supplements in pregnancy and birth outcome: re-analysis of a large randomised controlled trial and update of Cochrane review. Paediatr Perinat Epidemiol. 2005 Mar; 19(2): 112-24.

Endogenous GD3 ganglioside induces apoptosis in U-1242 MG glioma cells

Ola M. Omran, Hany E. Saqr, Allan J. Yates*

Introduction GD3 ganglioside induces apoptosis in several cell types, but the molecular events through which this occurs are largely unknown. We investigated the apoptotic effects of GD3 expression using U-1242 MG glioblastoma cells, as these cells

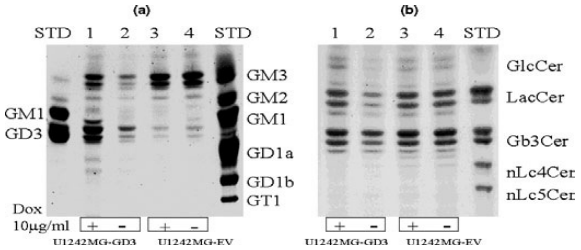
synthesize almost exclusively GM3 and GM2 but not GD3. Using flow cytometry, we selected glioma cells (U1242MGGD3 clone) that express high levels of GD3 in response to doxycycline.

Expression of GD3 was associated with apoptosis as verified by annexin-V binding, TdT-mediated dUTPnick end-labelling assay (TUNEL), and EGFP degradation. GD3- induced apoptosis occurred via caspase-8 activation.

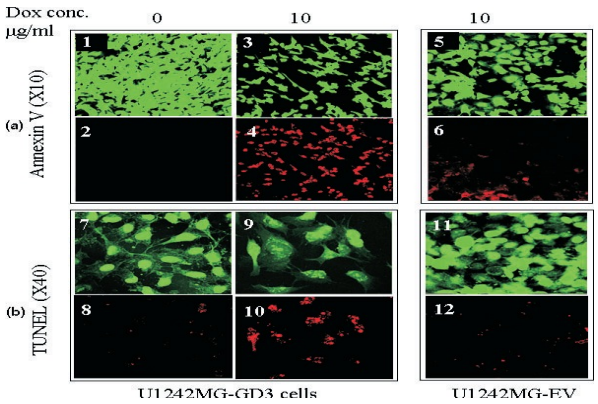
Objectives To investigate the biological effects of the expression of endogenous GD3; to investigate the molecular mechanisms of apoptosis in the U-1242MG human glioma cell line; and to investigate the molecular mechanisms through which GD3 induces apoptosis;

Methods *Cell culture:* Parental U-1242 MG cells were grown in minimal essential medium with 10% calf serum supplemented with penicillin/streptomycin (80 U/mL and 80 lg/mL) and Fungizone (0.22 lg/mL). Cells transfected with Tet-system vectors were grown in minimal essential medium supplemented with the same antibiotics in 10% Tet-system fetal bovine serum. *Plasmid construction,TetOn tri-cistronic construct, GD3 synthase construct and Generation of stably transfected cell lines:* We transfected U-1242MG with these two constructs. We selected U42-GRD2 cells on the basis of high GD3 expression under the effect of doxycycline. *Detection of GD3 expression using:* Flow cytometry; reverse transcription–polymerase chain reaction; immunocytochemistry and chemical analysis. *Effects of GD3 expression on cell growth:* Apoptosis assays; caspase-8 inhibition; Western immunoblotting; and determination of proliferative index.

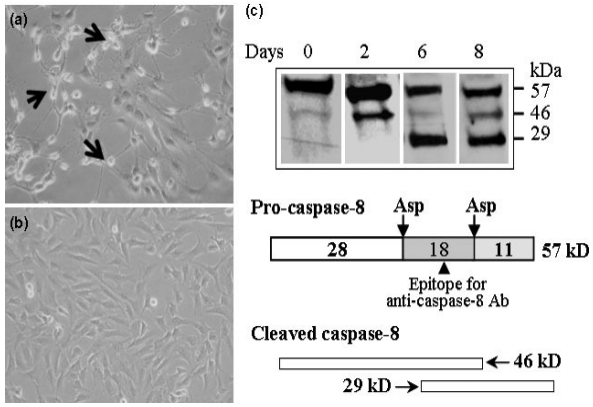
Results Modification of the TetOn system; detection of doxycycline-induced GD3 expression using the TetOn system:



Thin layer chromatographic separation of gangliosides and neutral glycolipids: (a) U1242MG-GD3 and U1242MG-EV cells were treated with 10 lg/mL doxycycline (a, lanes 1 and 3; b, lanes 1 and 3) and without Dox (a, lanes 2 and 4; b, lanes 2 and 4) for 6 days, after which total glycolipids were isolated and analyzed.

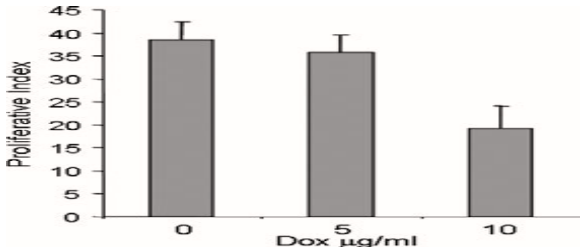


Endogenous GD3 induces apoptosis in U1242MG-GD3. U1242MG-GD3 and U1242MG-EV cells were exposed to 0 or 10 lg/mL doxycycline (Dox) for 6 days, then either (a) incubated with Annexin V conjugated to Alexa Fluor 647, or (b) a TUNEL assay was performed.



Flow cytometric analysis for enhanced green fluorescent protein (EGFP) can be used as an indicator of apoptosis. The arrow indicates U1242MG-GD3 cell population with low EGFP expression. B) GD3-induced apoptosis is mediated by caspase-8.

Doxycycline treatment of U1242-GD3 cells decreases the proliferative index. U1242-GD3 cells were grown with and without doxycycline for 6 days, at the end of which they were exposed first to MIB-1 antibody and then to Alexa 647- conjugated secondary antibody. Green fluorescent positive cells express the Tet-On gene, and provide an estimate of the total cell number.



Discussion The present study was designed to determine if endogenous GD3 induces apoptosis in U-1242 MG glioma cells. To investigate this we used a newly modified TetOn system, which enabled us to strictly regulate the expression of GD3. From the cells originally infected with these constructs, we selected a population of cells with the maximum controllable Dox regulated GD3 expression using flow cytometry. Fluorescence intensity from Alexa Fluor 647 in these cells indicates that U1242MG-GD3 cells express high levels of GD3. U1242MGGD3 cells exhibited morphological changes of apoptosis after 4–6 days exposure to Dox. GD3-induced apoptosis was also confirmed using the TUNEL assay. Here we report that GD3-induced apoptosis in U-1242 MG cells via activation of a caspase-8. Collectively, these findings are compelling evidence that increased levels of endogenously synthesized GD3 induce apoptosis in U1242MG-GD3 cells.

We conclude that GD3 synthesis in U-1242 MG human glioma cells can be controlled by our modified TetOn system. Increased expression of GD3 synthase caused U-1242 MG cells to undergo apoptosis determined by cytological appearance, increased annexin V binding, DNA fragmentation, and decreased expression of EGFP. Regulated GD3 expression using this system will be extremely useful in future studies on the mechanism of GD3-induced apoptosis in human glioma cells.

References

1. Bhunia A. K., et al. GD3 recruits reactive oxygen species to induce cell proliferation and apoptosis in human aortic smooth muscle cells. J. Biol. Chem. 277 (16): 396–402.
2. Castro-Palomino J. C., et al. Synthesis of ganglioside GD3 and its comparison with bovine GD3 with regard to oligodendrocyte apoptosis mitochondrial damage. Chemistry (7): 2178–84.
3. Saqr HE, et al. Endogenous GD3 ganglioside induces apoptosis in U-1242 MG glioma cells. J Neurochem 2006 Mar; 96(5): 1301-14.

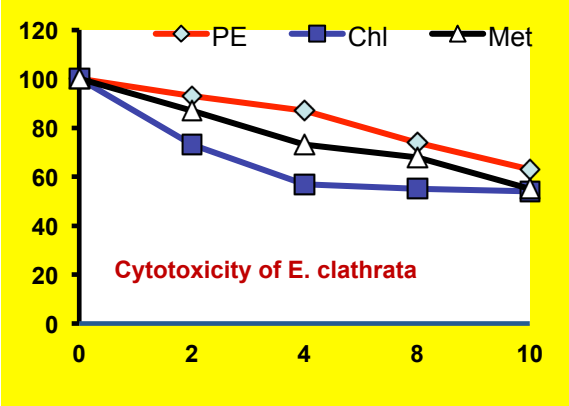
Anticancer activity of Egyptian marine alga *Ulva rigida*

Tarek A. Salem and Atef M. Ibrahim

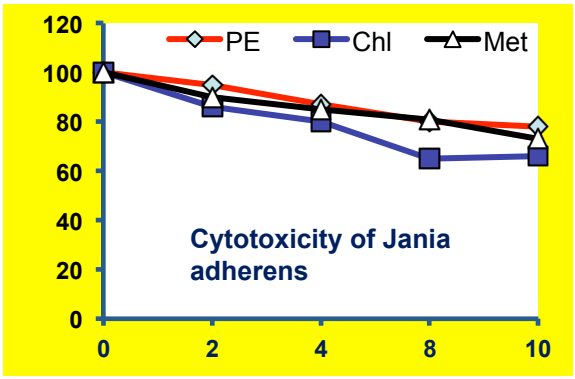
Introduction Marine organism–derived medicines have several features that make them particularly suitable for consideration as sources of anti-neoplastic agents. For example, the vast majority of marine invertebrates have only primitive immune systems, and thus, they produce toxic substances as a form of defense; these substances would be expected to have high potency and low solubility, given that they are immediately and tremendously diluted by water. Therefore, an increasing number of compounds derived from sponges, algae, mollusks, and other marine organisms are being tested for their therapeutic effects against cancer and other diseases in clinical and preclinical trials**.** The objectives are to investigate the cytotoxic activity of different extracts of four Egyptian marine algae on the Ehrlich ascites carcinoma (EAC) cell line in vitro. Further study was conducted in vivo to evaluate the antitumor activity of different extract of *Ulva rigida* against solid tumor induced in female mice by EAC cell line.

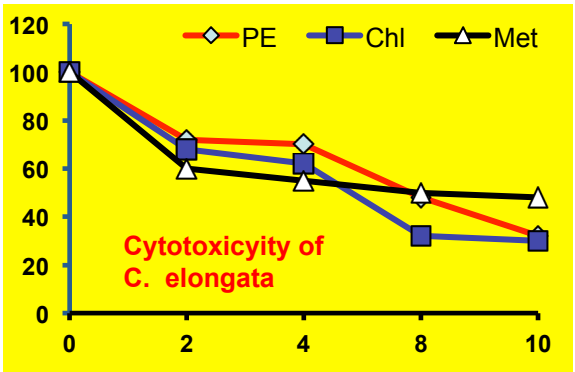
Methods Four marine algae were from Abu-Qir gulf, Alexandria, Egypt. These algae were identified as *Ulva rigida, Enteromorpha clathrata, Jania adherens and Corallina elongata* . Algae extracts were prepared and lyophilized. The cytotoxic activity of these algae extracts was determined in vitro against Ehrlich ascites carcinoma (EAC) cell line. The antitumor efficacy of *Ulva rigida* extracts was investigated in vivo against solid tumor-bearing mice induced by EAC cell line. Solid tumor was induced in Albino mice, except normal control group; by injecting $2x10^5$ EAC cells subcutaneously. Twenty-four hours post EAC inoculation, 0.1 ml of tested extracts was injected subcutaneously for 3 consecutive days. Lipid peroxidation, antioxidant level and VEGF were assayed.

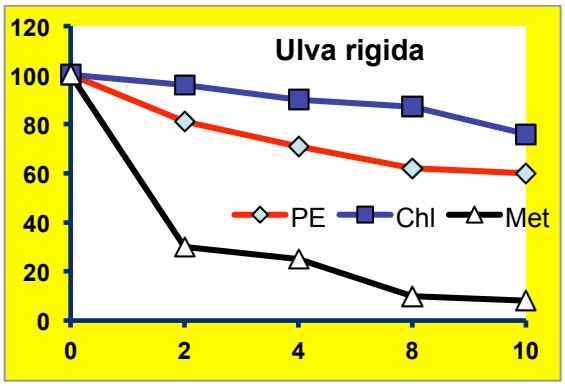
Results Varied inhibitory effects of algal extracts on the proliferation of EAC cell line in vitro were shown. Moreover, *Ulva rigida* exhibited the most potent antiproliferative effect on EAC cell line in a dose-dependent manner. In vivo results showed that methanol extract of *Ulva rigida* inhibits the growth of solid tumor induced in mice by EAC cell line. Treatment with methanol or chloroform extract of *Ulva rigida* resulted in significant reductions in the level of lipid peroxidation. The maximal protective effects against hepatic lipid peroxidation (64.8 ± 4.5 and 69.6 ± 13.3 nmol/g of tissue) were observed when solid tumor bearing mice treated with 80 and 100 µg/ml of methanol extracts of *Ulva rigida*, respectively. The maximal augmentation in antioxidant enzymes activity was observed at the same concentrations of *Ulva rigida* in SOD and CAT activities; these increments are associated with the reduction in tumor volume revealing the antitumor activity of *Ulva rigida*. In addition, vascular endothelial growth factor was maximally reduced in tumor-bearing mice treated with these concentrations of *Ulva rigida* alga. This finding revealed the antiangiogeneic role of *Ulva rigida*.



In conclusion, this study shed light on the antitumor activity of some marine algae grown in Egyptian coast. This study showed that *Ulva rigida* exerts its antitumor activity through augmentation of antioxidant status and antiangiogeneic efficacy.







References

4. Lukes T, Eric A, Kerry M, Patricia F. William H. Gerwick marine natural products as anticancer drugs. Mol Cancer Ther. 4(2): 333–42.
5. Om-Ali Y. Tarek A. S. Mohamed FE. Protective role of Egyptian propolis against tumor in mice. Clin Chim Acta 318; 11 – 16.
6. Paterson I. Anderson EA. The renaissance of natural products as drug candidates. Science, 310 (5747): 451-3.
7. Aleem AA. Monem MAS. Khalifa HE. BeiderM. El- Ghandour, IA. Galal YG. Marine algae. J. of Sustainable Agric; 19: 41-8.

Experimental simulation for accidental exposure to uranium pollutants in drinking water: a histological study on the possible impact on cerebral cortex of adult albino rats

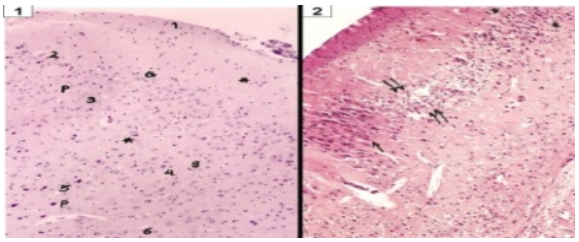
Amany Osman

Introduction Uranium (U; atomic number 92) is a naturally occurring radioactive element having three different natural radioactive isotopes (U-238, U-235 and U-234). The isotopes are separated to increase the concentration of one isotope relative to another; a process known as *enrichment***.** The resulting enriched fraction (U-235) is the best fuel for nuclear power reactors and for making nuclear weapons. The remaining part after removal of the enriched fraction is known as *depleted uranium* (DU) and contains mainly U-238 which is 60% less radioactive than the natural uranium and hence most preferred for civilian applications. For instance, it is used in counter weights on the wings of airplanes, in ceramic glazing and in X-ray shields. The last decades witnessed the military use of DU as a new weapon entering the international conflicts as in the Gulf and Balkan wars. U-238 is an alpha-radioactive emitter which is both chemically toxic and mutagenic. Moreover the microparticles of uranium U-238 are aerosolized. Apart from being respired, aerosolized radioactive uranium particles are brought back by wind and rain to soil and water. Hence, a great concern has been raised to face the problem of uranium contamination of water sources. A number of critics have asserted that exposure to DU has resulted in a variety of adverse health effects.

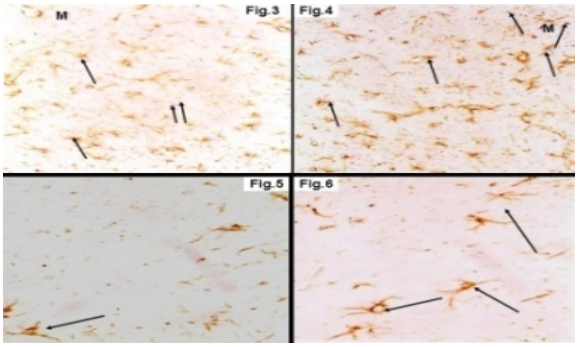
The objective of this study is to evaluate the histological alterations in the cerebral cortex of albino rats subjected to oral ingestion of a soluble uranium compound.

Methods Ten control adult male albino rats received daily 1 ml of ordinary tap water by orogastric intubation for 90 days. Another group of 15 experimental rats received 60 μg/kg body weight dissolved uranium in 14.21 μml uranyl acetate added to 1 ml tap water by the same route and for the same duration. Specimens from the left fronto-temporal area of the cerebral cortices of both groups of animals were processed for light microscopic examination by routine hematoxylin and eosin stain and immunohistochemical labeling for glial fibrillary acidic protein as well as for transmission electron microscopy.

Results The applied dose and duration of exposure to uranyl acetate in drinking water proved to induce focal degenerative changes in some neurons of the cerebral cortex, which was associated with moderate increase in the neuroglial reaction.



Light photomicrographs (Mic. Mag. X 100) of: (1) control rat cerebrum demonstrating normal arrangement of the layers of the cerebral cortex.; (2) cerebral cortex of rats ingesting uranyl acetate revealing a localized intensely stained eosinophilic area↑; note the increased cellularity and disarrangement of the cortical layers ↑↑. Adjacent area has a disorganized vacuolated appearance *.



Light photomicrographs (DAB chromogen; Mic. Mag. X 100) of: (Fig 3) control cerebral cortex with immunolabeling for GFAP demonstrating average distribution of positively stained neuroglia cells (↑). (Fig. 4) cerebral cortex of rats ingesting uranyl acetate with immunolabeling for GFAP showing moderate increased density of the positively stained glial cells (↑) in the molecular (M) layer; (Fig 5) deep cerebral cortex of control rats immunolabelled for GFAP showing few scattered positive glial cells (↑) per high power field; (Fig 6) deep cerebral cortex of the rats ingesting uranyl acetate immunolabelled for GFAP revealing more abundant positive glial cells (↑) per high power field.

Conclusions Regular, short-termed monitoring of uranium levels in all sources of drinking water is mandatory at the local as well as the national ranges. The population at risk for high rates of exposure should be subjected to periodic assessment of uranium level in urine. The efforts of the national and international health organizations together with the governments should be directed to limit the expanding utilization of uranium compounds in civilian and military applications.

References

1. Miller AC, Mc Clain D. A review of depleted uranium biological effects: in vitro and in vivo studies. Rev Environ Health 2007; 22 (1): 75-89.
2. Ghosh S, Kumar A, Pandey B N, Mishra K P. Acute exposure to uranyl nitrate causes lipid peroxidation and histopathological damage in brain and bone of Wistar rat. J Environ Pathol Toxicol Oncol 2007; 26 (4): 255-61.

Effects of hydroquinone on epidermal non-keratinocytes

Noaf K Al-Aqeel and Eltohami M Abdel-Mageed

Introduction Hydroquinone is crystalline substance that consists of a benzene ring and two hydroxyl groups. It is incorporated at various concentrations in over 200 types of hair and skin cosmetic preparations and medications. It is renowned for its skin lightening capabilities but it reportedly elicits inflammatory and allergic skin responses (Stoppler, 2006). Two types of non-keratinocytes (melanocytes and Langerhans cells) seem to be the affected cells (Fernandes et al., 2004; Kooyers and Westerhof, 2006). Information on the effect of hydroquinone on the volume densities and ultrastructure of non-keratinocytes is meager or altogether lacking.

The objective of this study is to identify the effects of topical application of 4% hydroquinone on the volume densities and electron microscopic features of melanocytes and Langerhan's cells.

Methods The guinea pig was used for this study because of structural similarity between its epidermis and that of humans; thirty adult male animals were divided into two groups. The first group comprised 10 animals and was used to study the normal volume densities and electron microscopic features of non-keratinocytes of the thin and thick skin. The second group of 20 animals was used to study the effect of hydroquinone on non-keratinocytes. Hydrquinone cream (4%) that was daily applied onto the skin of the ear auricle of 10 animals of this group at a dose of 1 g/cm^2 for 35 days. The auricle of the remaining 10 animals was treated similarly by lipobase (the vehicle). The

volume density (Vv) of melanocytes was determined by point counting on skin sections stained immunohistologically for HMB45 (Fig 1) whereas NSE (Fig 2) method was used for Merkel cells. Point counting on epidermal sheets stained by the ATPase histochemical method (Fig 3) was used to determine the volume density of Langerhan's cells. A light microscope fitted with an eyepiece graticule of 121 points was used (Fig 3) for point counting. For electron microscopy ultrathin sections of the epidermis, stained with uranyl acetate and lead citrate, were examined with a Joel transmission electron microscope. Point counting was performed on electron micrographs superimposed with a transparent sheet of 100 equidistant points. The volume densities of non-keratinocytes and their components, the relative standard error, and the minimum sample size were obtained using histomorphometric methods.

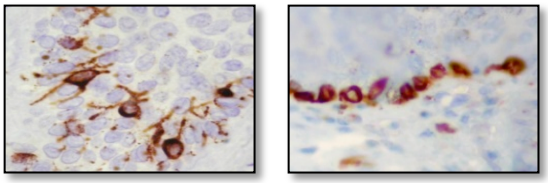


Figure 1 (left): melanocytes immunohistologically staining +ve (brown) to HMB45; Figure 2 (right): Merkel cells staining +ve (brown) to NSE (neuron specific enolase).

Results The volume density Vv of Melanocytes as revealed by this study was highest (0.06) in the epidermis of the perinasal region and lowest in the thick skin of the foot (0.03), whereas Langerhans cell Vv was highest (0.15) in the skin of the back and lowest (0.04) in the thick skin of the footpad. Merkel cells were lacking in epidermis of the ear auricle and very scarce in other locations. Application of hydroquinone induced a notable increase in the skin thickness (403.5um thick compared to a thickness of 91um in controls). There was a notable increase in Vv of the dermis (0.7 compared to 0.55 in controls). This was apparently the result of the vascular congestion and edema noted in this in the papillary dermis. Hydroquinone as seen in this study (Fig 4) resulted in an apparent decrease in melanocyte Vv (0.063 down to 0.013) and an increase in Langerhans cell Vv (0.12 up to 0.17). Moreover, it caused an apparent decrease in volume density of melanosomes within melanocytes (From 0.1 down to 0.063). These results apparently explain the skin lightening effect and the skin reactions that hydroquinone elicits. Electron microscopic examination of hydroquinone treated epidermis revealed normal keratinocytes with reduced melanin granules and normal Langerhans cells. Melanocytes, on the other hand, showed abnormal RER and melanosomes (Fig 5). These melanosomal changes augment the hypothesis that hydroquinone affect oxidation of tyrosine and hamper formation of dopaquinone and melanin (Nordlund, 2007).

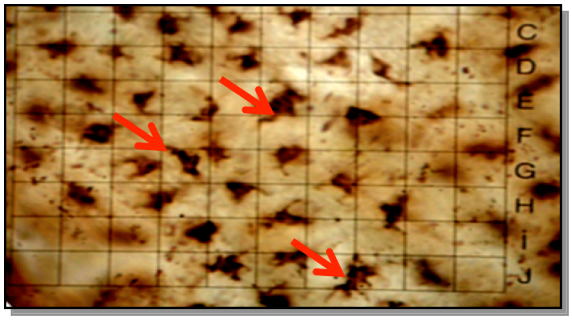


Figure 3: ATPase +ve Langerhans cells (arrows) in an epidermal sheet. Point counting graticule is superimposed.

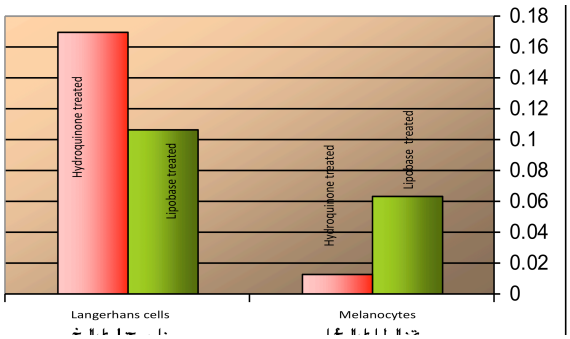


Figure 4: Comparing Vv of Langerhans cells and melanocytes in hydroquinone treated and control skin

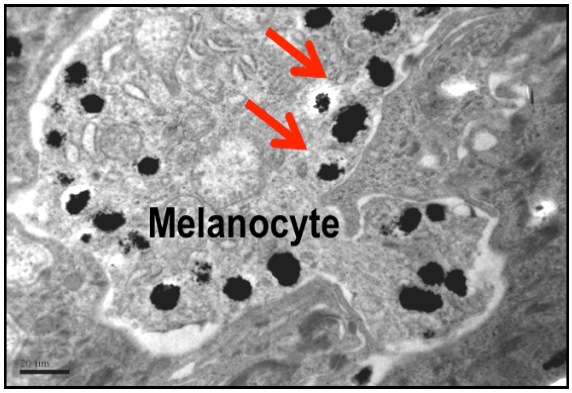


Figure 5: Electron micrograph of a melanocye of hydrquinone treated skin showing deformed melanosones (arrows)

References

1. Aherne, W and Dunnil, M (1998). Morphometry New York : Lippincott
2. Fernandes et al. (2004). Increase of melano-genesis…. Tissue Cell. 36, 95-105
3. Koyers T and Westerhof W (2006). Toxicology and health risks of hydroquinone. J Eur Acad Derma tol.20. 541-44
4. Stoppler M. (2006). FDA proposes hydroquinone ban. Federal Register 71, 51146-55.
5. Nordlund J (2007). Hydroquinone.. Exp Rev Dermatol. 2, 283-87

Effect of cyclosporine a on the pancreas in rabbits: A light and an electron microscopic study

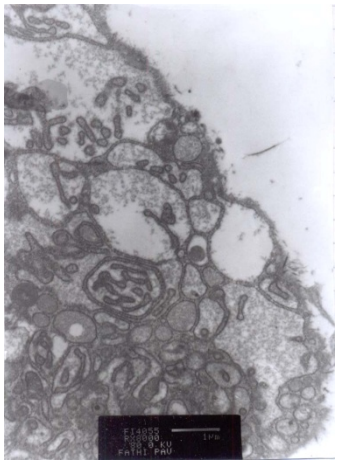
Fathy Ahmed Fetouh and Eman M.A. Abdelghany

Introduction Cyclosporine A (CsA), a neutral lipophilic cyclic undecapeptide isolated from the fungus *Tolypocladium inflatum gams*, was first identified in 1976 during screening for novel antibiotic agents. This molecule exerted a rather wide spectrum of biologic activities, including antiparasitic, fungicidal and anti-inflammatory effects. It was subsequently discovered to be a powerfull immunosuppressive agent [1]. The drug exerts its major therapeutic effects by inhibiting T-cell activation [2]. The use of cyclosporine A has improved quality of life and survival of transplant patients. CsA has largely contributed to the decrease in morbidity, rejection episodes and hospitalization days in these patients.

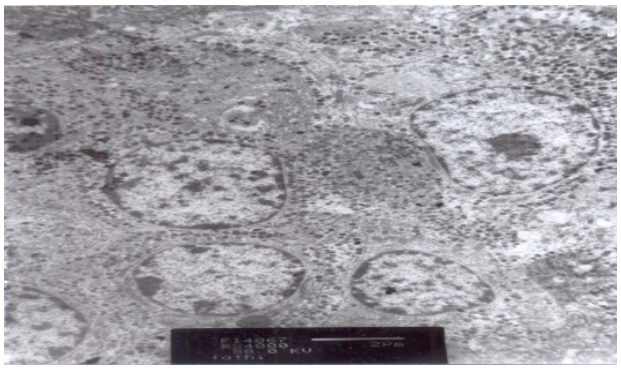
The present work aimed to study the histological and ultra-structural effects of cyclosporine A on the exocrine and endocrine parts of the pancreas.

Methods Two groups of Egyptian adult rabbits were used for this study (3-4 rabbits for each). One group was used as a control and the other group (experimental) was treated with cyclosporine A as oral solution in a dose of 15 mg/kg for 10 days. The animals were killed by cervical dislocation and the pancreatic specimens were taken from tail of the pancreas. The specimens were fixed in a buffered glutaraldehyde 2.5% solution. Semi-thin sections of 1 μm thick were obtained, stained with 1% toluidine blue and examined by light microscopy and ultrathin sections for electron microscopic examination.

Results The cyclosporine A had adverse effects on the pancreas. The acinar cells showed marked decrease in their zymogen granules and some showed degranulation at their apices. Abundant cytoplasmic vacuoles were observed. The rough endoplasmic reticulum was decreased swollen and vacuolated. The mitochondria were few and degenerated with no transverse cristae. The nucleus was deformed, irregular and dark. In islet cells, the beta cell showed marked decrease in its secretory granules, the mitochondria were observed as numerous and elongated. The endoplasmic reticulum appeared as few scattered stacks. The alpha cells were not affected structurally and appeared with their normal secretory granules and endoplasmic reticulum.

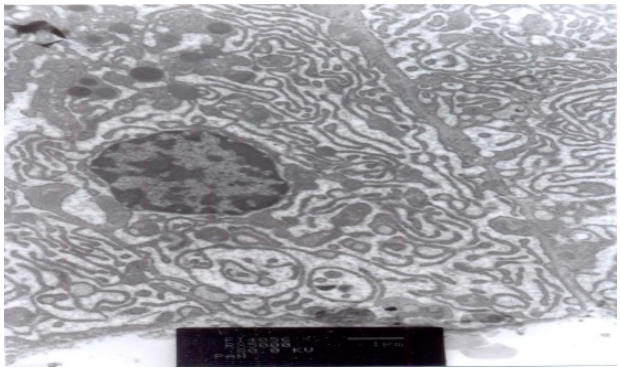


An electron photomicrograph of the pancreas of rabbit treated with CSA; the acinar cells are filled with abundant cytoplasmic vacuolization (V) .



An electron photomicrograph of ultrathin section in the pancreatic islet of rabbit treated with CsA. It shows that, the alpha

cells are numerous and characterized by electron-dense secretory granules (SG) and rough endoplasmic reticulum (rER).



An electron photomicrograph of ultrathin section in exocrine pancreas of rabbit treated with CsA showing two acinar cells. The nucleus (N) is dark, deformed, dilated cisternae of rough endoplasmic reticulum (rER)

Discussion In the present study, cyclosporine A (CsA) had adverse effect on the exocrine part of the pancreas where, the acinar cells showed cytoplasmic vacuolization and pyknotic nuclei. The capillaries appeared congested. By electron microscopic examination in the present study, the acinar cells showed marked decrease in zymogen granules and some acini showed degranulation at their apices. The granules appeared as low density. Abundant cytoplasmic vacuolization with no exocytosis was observed. Lopez-Miranda et al. [2] related the pancreatic injury to the dose and found that total pancreatic amylase and protein contents showed a dose- dependent decrease and suggested that, CsA produces a toxic effect on exocrine pancreatic function. Hirano et al. [3] explained the exocrine pancreatic injury in which CsA causes redistribution of cathepsin B from the lysosomal fraction to the zymogen fraction indicating colocalization of lysosomal enzymes with pancreatic digestive enzymes. So, the lysosomal enzymes play important roles in the pathogenesis of this injury.

References

1. Edwardson JM: Effects of immunosuppressant cyclosporine A and FK 506 on exocytosis in the rat exocrine pancreas in vitro. Brit J Pharmacol 1993; 108(4): 892- 900
2. Lopez- Miranda J, Blanco- Molina A, Lopez- Segura F, Nicolas- Puiggari M, Torre- Cisneros J and Perez- Jimenez F: The effect of cyclosporine on exocrine function of the rat pancreas: an in vitro study. Transplantation 1991; 51(3): 562- 5.
3. Hirano T, Mandabe T, Printz H, Tobe T. Cytotoxic effects of cyclosporin A on the exocrine pancreas in rats. Surg Gynecol Obstet 1992; 175 (6): 495-500.

Effect of green tea extract on lead toxicity in different organs of rats

Abdel-Raheim Meki, Abdullah Alghasham, EL-Sayed EL-Deeb

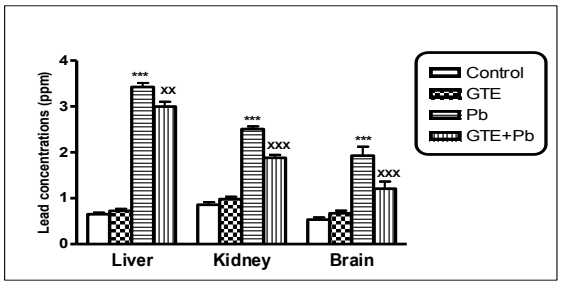
Introduction Health hazards from increased lead (Pb) exposure as a result of industrial and environmental pollution are recognized (Juberg et al., 1997). Lead poisoning is considered to be one of the most difficult environmental health problems, since it does not show any unique manifestation during its early stage (Patrick, 2006). Lead has been found to produce wide range of biochemical and physiological dysfunctions in humans and laboratory animals (Courtois et al., 2003). Several mechanisms have been proposed to explain the Pb-induced toxicity. Many investigators proposed that one possible mechanism of Pb toxicity is the disturbance of prooxidant and antioxidant balance by generation of reactive oxygen species (Wang et al. 2001). Adonaylo and Oteiz, (1999) reported that Pb-associated tissue injury in the vital organs is resulted from the oxidative stress. Some studies suggested potential role of antioxidants to ameliorate Pb toxicity (Hsu et al., 1998). The increasing interest in the health properties of tea extract and its main catechin polyphenols have led to a significant rise in scientific investigation for prevention and therapeutics in several diseases (Mandel et al., 2006). Green tea extract (GTE) due to its content of catechins reveals strong antioxidative activity, which is manifested by its ability to inhibit free radical generation, scavenge free radicals and chelate transition metal ions that catalyze free radical reactions (Ostrowska and Skrzydlewska, 2006).

The present study was conducted to investigate the biochemical and histo-pathological effects of Pb toxicity on liver, kidney and brain of rats. Moreover, the antioxidative activity of GTE against oxidative stress induced by Pb toxicity was evaluated. The

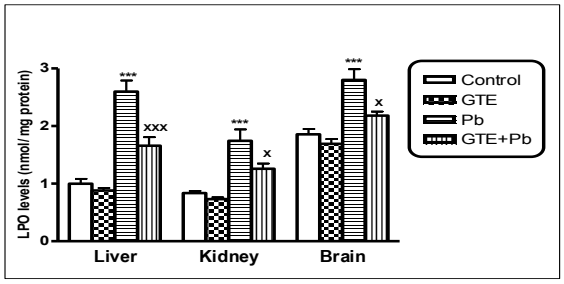
chelating property of GTE to reduce Pb burden in rat tissues was detected.

Methods Four groups of male rats (each 15 rats) were utilized as following: Controls, GTE – treated rats (1.5 % w/v), Pb –treated rats (0.4 % lead acetate in dist. H2O), Pb + GTE -treated rats. The rats received GTE and/or lead orally in drinking H2O for 6 weeks. The biochemical measurements were performed in plasma, erythrocytes and tissue of liver, kidney and brain. The histo-pathological examinations of the studied organs of different groups using light microscope were done. The levels of lipid peroxides (LPO), nitric oxides (NO), total antioxidant, glutathione (GSH), glutathione S-transferase (GST), superoxide dismutase (SOD) were detected using colorimetric methods. The concentrations of Pb in whole blood and tissues of liver, kidney and brains were determined using atomic absorption spectrometer.

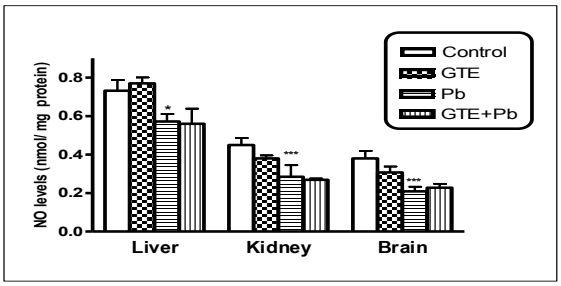
Results Pb concentrations in Pb-treated rats were significantly higher in whole blood and studied tissues than controls. Levels of LPO in Pb-treated rats were significantly higher in plasma, erythrocytes, liver, kidney and brain than controls. Plasma level of total antioxidant and levels of GSH and SOD activities in all studied tissues and activities of GST in only tissues organs of Pb-exposed rats were significantly reduced in comparison with controls. GTE co-administrated with Pb appeared more effective in reduction of Pb concentrations in tissues of the studied organs and whole blood as well as reductions of oxidative stress indices as detected by a significant decline of LPO in all studied tissues. The antioxidant status was improved in almost the studied tissues in Pb+GTE treated rats comparing to Pb-treated rats. The biochemical results were confirmed by histopathology.



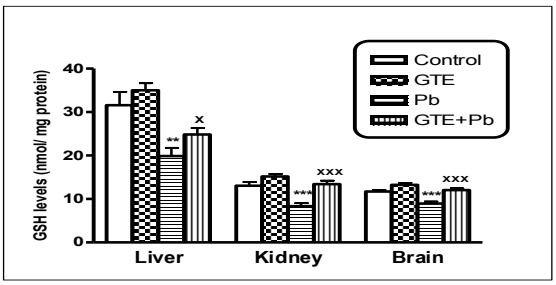
Lead concentrations in tissues of liver, kidney and brain of different treated groups. (mean ± SEM. ***P<0.001 for Pb vs controls; ^{xx}P<0.01 and ^{xxx}P<0.001 for GTE+Pb vs Pb)



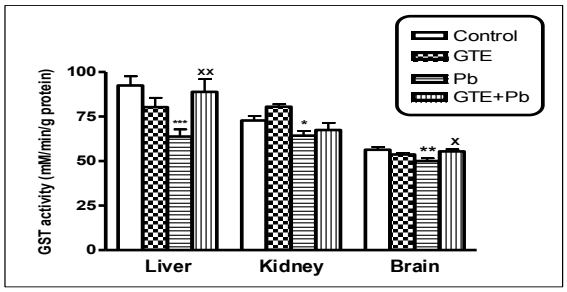
Lipid peroxide (LPO) levels in tissues of liver, kidney and brain of different treated groups (mean ± SEM. ***P<0.001 for Pb vs controls; ^{x}P<0.05 and ^{xxx}P<0.001 for GTE+Pb vs Pb)



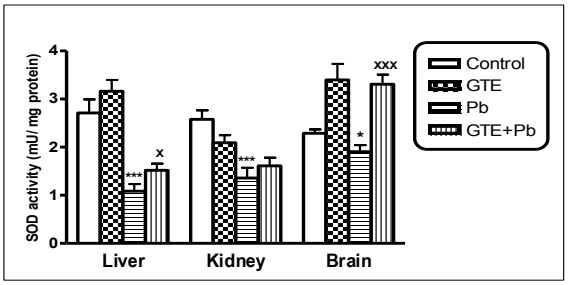
Nitric oxide (NO) levels in tissues of liver, kidney and brain of different treated groups. (mean ± SEM. *P<0.05, ***P<0.001 for Pb vs controls)



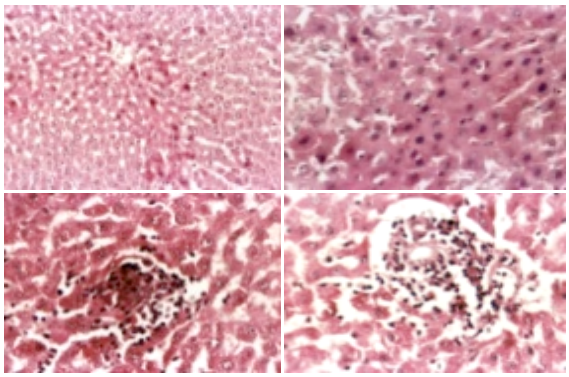
Glutathione (GSH) levels in tissues of liver, kidney and brain of different treated groups (mean ± SEM. **P<0.01, ***P<0.001 for Pb vs controls; ^{x}P<0.05 and ^{xxx}P<0.001 for GTE+Pb vs Pb)



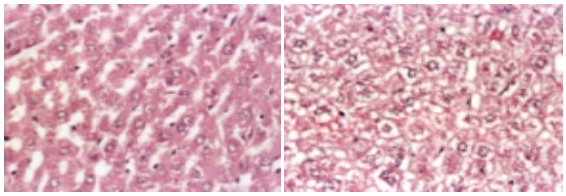
Glutathione S -transferase (GST) activities in tissues of liver, kidney and brain of different treated groups. (mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 for Pb vs controls; ^{x}P<0.05 and ^{xx}P<0.001 for GTE+Pb vs Pb)



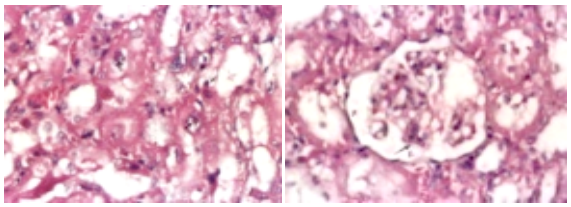
Superoxide dismutase (SOD) activities in tissues of liver, kidney and brain of different treated groups. (mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 for Pb vs controls; ^{x}P<0.05 and ^{xx}P<0.001 for GTE+Pb vs Pb)



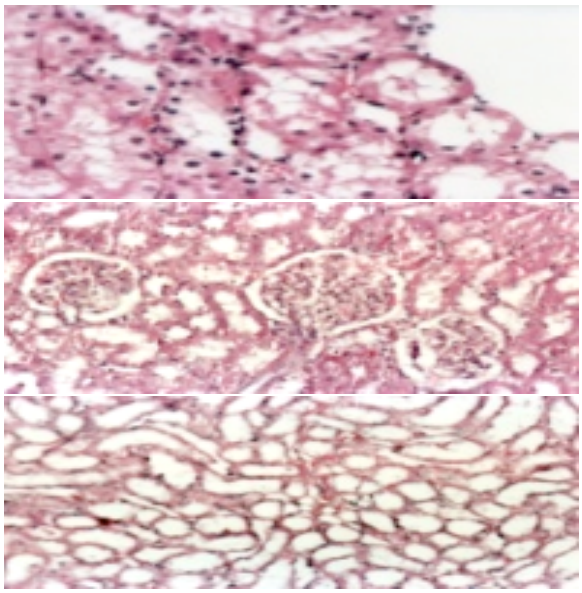
Liver of male rat intoxicated with lead acetate (clockwise): fatty change and hydropic degeneration of hepatocytes and multifocal hepatocytic coagulative necrosis (H&E .X 200); diffuse coagulative necrosis (H&E. X. 400); necrotic cells replaced by mononuclear leucocytes (H&E. X. 400); and interface Hepatitis (H&E.X.400).



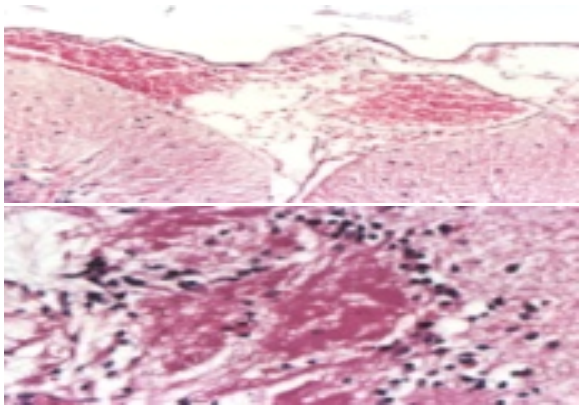
Liver of male rat treated with GTE+Pb showing Hydropic degeneration (L) and vacuolated nuclei (R)



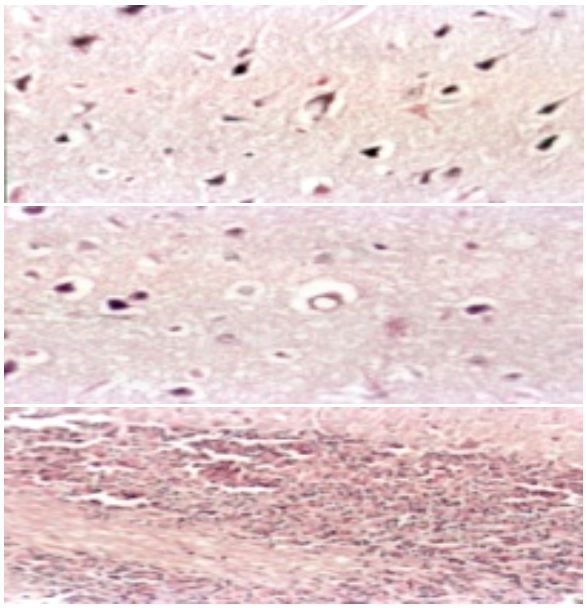
Kidney of male rat treated with Pb showing diffuse type of coagulative necrosis, intranuclear eosinophilic inclusion bodies (arrows), cast and cystic dilated tubules (H&E.X. 400).



Kidney of male rat treated with GTE+ Pb showing (from above): (I) Nearly normal kidney (just cloudy swelling, hydropic and fatty change besides apoptotic necrotic cells) (H&E.X. 400) and (II and III) Normal renal tubules and glomeruli with mild degree of necrosis (marked improvement in the histological structure) (H&E.X. 200).



Brain of male rat treated with Pb showing meningeal hemorrhage, edema and congestion (H&E. X. 200) (above), and cerebral infarction with mononuclear cells infiltration (H&E.X. 400) (below).



Brain of male rat treated with GTE+ Pb showing (from above): (I and II) Marked improvement " just vacuolated neurons (H&E.X. 400) and (III) Normal arrangement of the cerebellum layers and no spaces in between its granular and purkinje layers (H&E.X. 200).

Conclusions The accumulation of Pb in the studied tissues was associated with oxidative stress and tissue injury as detected by histopathology. The treatment of rats with GTE combined with Pb could enhance antioxidant/ detoxification system which consequently reduced the oxidative stress. The beneficial effect of GTE in the improving of antioxidant status was associated with reduction of Pb burden in the tissue organs, thus potentially reducing Pb toxicity and tissue damage.

Desferrioxamine protects against toxic damage to liver and kidney induced by cyclophosphamide

Muhammad Raza, Abdullah A. Alghasham

Cyclophosphamide (CP), a vital component of several chemotherapy and immunomodulation requiring protocols, have serious dose limiting side effects, including hepatotoxicity and nephrotoxicity. The most documented evidence in the literature suggests an involvement of lipid peroxidation (MDA) preceded by glutathione (GSH) depletion (1-4). In this study, desferrioxamine (DFO), an iron and other transition metals chelating agent that has already been documented as a scavenger (5) is used to ameliorate CP-induced hepato- and nephro-toxicity.

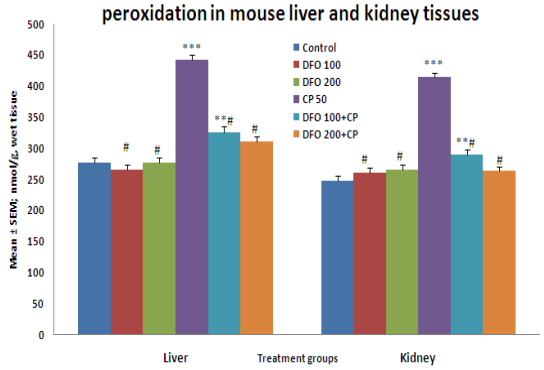
The objectives of this study were to know the role and presence of iron and other transition metals in the initiation and propagation of reactive oxygen moieties and overall free radical load with CP. It was also aimed to see the effects of pretreatment of experimental animals with DFO on the non-protein sulfhydryl (NP-SH) contents in both kidney and liver tissues either alone or in presence of CP. Furthermore, it was interesting to determine if DFO pretreatment have any effect on plasma activities of AST, ALT, LDH and creatinine in CP-treated mice.

Methods A total of thirty six adult male Swiss albino mice were divided into 6 equal groups. Group I: Control animals were administered normal saline for 5 days by intraperitoneal (i.p.) route. Group II and III, respectively: DFO (100 or 200 mg/kg body wt.) was administered i.p. for consecutive 5 days. Group IV: CP (50 mg/kg body wt.) was given as a single i.p. dose on the 5^{th} day of treatment. Group V (DFO 100 mg/kg + CP 50 mg/kg): animals were administered DFO i.p. 5 days before CP administration. Group VI (DFO 200 mg/kg + CP 50 mg/kg): animals were administered DFO (200 mg/kg/day) for 5 days along with a single i.p. injection of CP on 5^{th} day. One day after the last dosage animals from each group were sacrificed. Plasma was isolated and used for ALT, AST, LDH and creatinine assessments. Liver and kidney tissues were used for lipid peroxide and NP-SH determination.

Results In this study CP treatment significantly affected the levels of MDA in liver and kidney tissues as compared to control or DFO treated mice ($P<0.001$). The protection offered by DFO was comparable in both the tissues and there was no difference in level of protection at both the test doses of DFO in both tissue types. CP treatment caused a severe and significant decline in NP-SH contents when compared to control group ($P<0.001$). Pretreatment of mice significantly averted the decline in NP-SH by CP treatment as compared

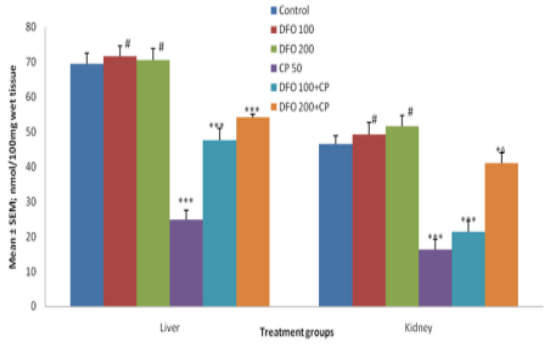
to CP alone (P<0.001). Despite the significant protection afforded by DFO the levels of NP-SH were significantly lower than the control group and did not recover to base line after CP treatment in combination treatment groups at both the test doses of DFO in liver and kidney tissues. CP treatment resulted in a significant elevation in levels of AST, ALT, LDH and plasma creatinine when compared to control group (P<0.001). Both dose levels of DFO for five days did not change plasma parameters. Pretreatment of mice with DFO followed by CP significantly protected against elevation in both the aminotransferases; but even then the levels of AST and ALT remained significantly high when compared to control group. However, DFO pretreatment prevented any significant rise in the levels of LDH and creatinine after CP that essentially remained closer to the control.

Chart 1: Effect of desferrioxamine on cyclophosphamide - induced lipid peroxidation in mouse liver and kidney tissues



Six animals were used in each treatment group.
Treatments were given daily for consecutive five days for consecutive five days.
Statistical analysis was done by One Way ANOVA followed by Tukey-Kramer multiple comparisons test in each column independently. All the groups were compared to control. **P<0.01 and ***P<0.001. #P<0.001 compared to cyclophosphamide alone (group 4).

Chart 2. Effect of desferrioxamine on cyclophosphamide induced non-protein sulfhydryl (NPSH) contents in liver and kidney tissue.



Six animals were used in each treatment group.
Treatments were given daily for consecutive five days for consecutive five days.
Statistical analysis was done by One Way ANOVA followed by Tukey-Kramer multiple comparisons test in each column independently.
All the groups were compared to control. ***P<0.001, #P<0.001, and $P<0.05 compared to cyclophosphamide alone (group 4).

Discussion Results of this study suggest that DFO administration may be useful in protecting CP-induced hepato- and nephro-toxicity as seen by a decline in free radical contents and a concomitant partial restoration of NP-SH. This corroborates the role of NP-SH (mainly glutathione) in the detoxification. This also suggests that lipid peroxidation may not become evident until glutathione activity is limited. However, both the test doses of DFO failed to avert a decline in NP-SH contents in both liver and kidney tissues after CP treatment to the control values. Iron and other transition metals are proposed to play a role in the production of free radicals. Being a chelator for transition metals, the role of DFO as a scavenger of metals becomes evident as indicated by the decreased levels of malondialdehyde (MDA). The plasma levels of ALT, AST, LDH (6) and creatinine (7) that increased significantly by CP also recovered significantly but essentially remained above the control values. However, DFO pretreatment is more effective in the recovery of LDH and creatinine levels to its base line in the CP treatment regimen.

References

1. Draeger, U., Peter, G., and Hohorst, H.-J. (1976). Cancer Treat Rep., 60: 355-359.
2. Topal, T., Oztas, Y., Korkmaz, A., Sadir, S., Oter, S., Coskun, O., Bilgic, H., (2005). J. Pineal Res. 38: 272–277.
3. Manesh, C., Kuttan, G., (2005). Phytomedicine 12: 487–493.
4. Abd-Allah, A.R., Gado, A.M., Al-Majed, A.A., Al-Yahya, A.A., Al-Shabanah, O.A., (2005). Clin. Exp. Pharmacol.Physiol. 32: 167–172.

5. Mansour MA. (2000). Life Sciences, 66(26): 2583-2591
6. Younes M, Siegers CP. (1985). Chem Biol Interact. 55(3):327-34.
7. Ding W, Shen H, Zhu H. (1998). Zhonghua Yu Fang Yi Xue Za Zhi.;32(5):278-80. Chinese

Prognostic value of P53, C-mycoprotein detection and DNA ploidy in some cases of hyperplastic and neoplastic colonic polyps

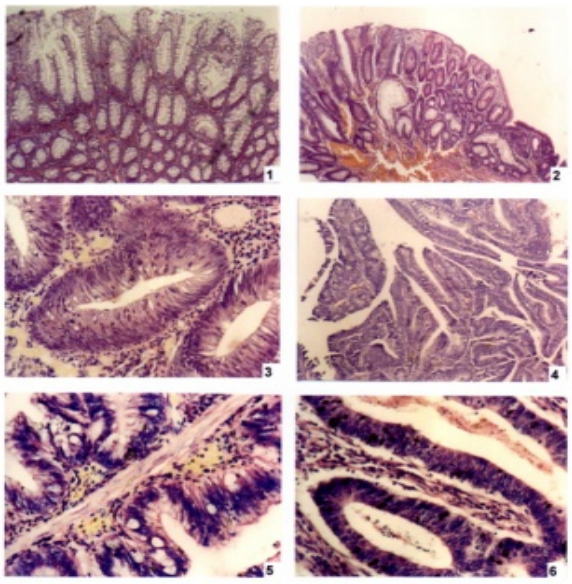
Susan A. Kato.

Introduction One of the most important mechanisms of tumor cell loss is apoptosis. This is commonly seen in adenomas and malignant large bowel tumors. Two particularly important mediators of apoptosis are the oncogenes c-myc and the tumor suppressor gene p53. DNA aneuploidy represents one of the earliest detectable cellular changes that may be considered as a specific marker for malignant transformation .The use of computerized image analysis (CIA) has proven a highly accurate quantitative and objective assessment of DNA content and has proven to be a reliable method for determination of ploidy in colorectal tumors.

This study aimed at correlating all types of colonic polyps, hyperplastic, adenomatous and malignant, using CIA measured ploidy pattern and DNA index (DI), as well as using immunohistochemical detection of c-myc and p53 antibodies.

The study included 30 cases of colorectal polyps and one control case of colitis. All were obtained as formalin-fixed specimens. The specimens were classified into three groups: *Group I:* 10 cases of hyperplastic colonic polyps, *Group II:* 10 cases of adenomatous colonic polyps (5 tubular, 2 tubulovillous and 3 villous), and *Group III:* 10 cases of malignant polyps (6 well differentiated, 2 moderately differentiated and 2 poorly differentiated). These were compared with one control case of colitis. All specimens were subjected to routine preparation and staining by H&E, Image analysis evaluation was done to determine DNA content by staining sections of 5µm thick of the polyps by Feulgen's stain, and Immunohistochemical studies were carried out on 5µm thick sections of formalin-fixed paraffin embedded specimens using anti- c- myc and p53 antibodies as described in their specification sheets.

Results All hyperplastic cases were diploid, negative for P53, and 80% of cases showed focal strong cytoplasmic c-myc immunostaining. Adenomatous polyps showed four diploid and six aneuploid cases. P53 nuclear immunostaining was seen in two cases and c- myc cytoplasmic immunostaining was positive in eight cases. All carcinomatous cases were aneuploid, P53 was positive in four cases, whereas pan cellular c-myc immunostaining was seen in seven cases.



H&E histopathologic figures

1: Hyperplastic polyp showing serrated appearance of surface columnar cells with abundant cytoplasm filled by mucous, regular basal nuclei. (X 100).

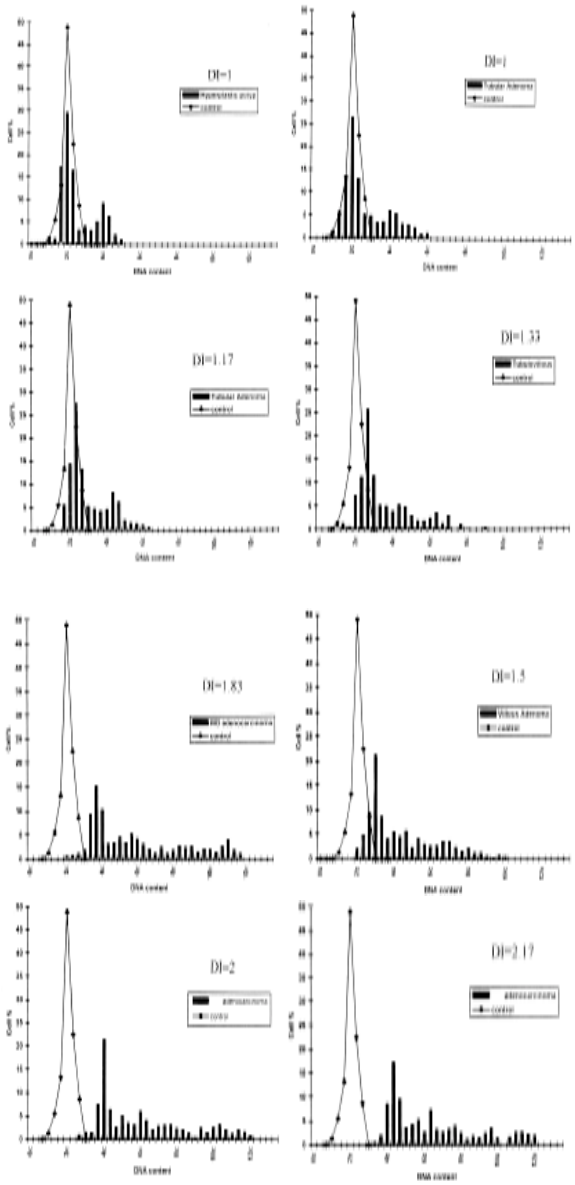
2: Tubular adenoma showing numerous glands, some of which show mild dysplastic nuclei, embedded in lamina propria. (X 100).

3: Tubular adenoma showing moderate dysplasia. Nuclei show pseudo stratification, mucin is decreased. (X 400).

4: Tubulovillous adenoma showing severe dysplasia. (X 100).

5: Villous adenoma showing severe dysplastic pseudostratified nuclei and marked reduction of mucin. (X 400).

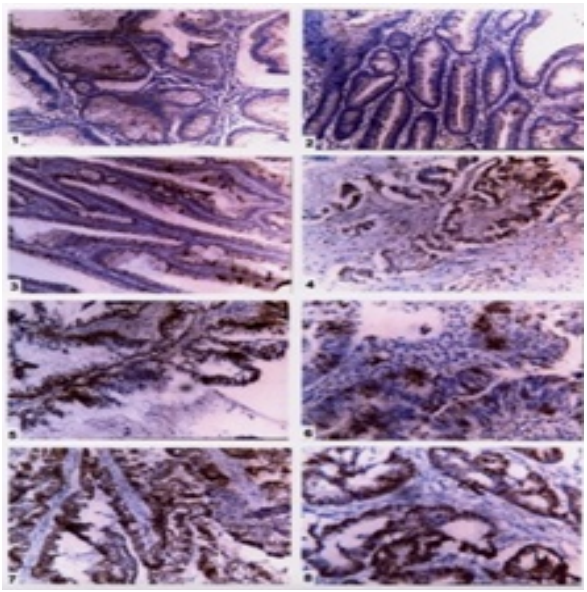
6: Well differentiated adenocarcinoma showing glandular spaces lined by malignant cells with pleomorphic hyper chromatic nuclei, and no cytoplasmic mucin. (X 400).



Histograms of different colonic polypi

1: Hyperplastic polyp, diploid pattern
2: Tubular polyp diploid pattern
3: Tubular polyp, aneuploid pattern
4: Tubulovillous adenoma aneuploid pattern
5: Villous adenoma, aneuploid pattern
6: Well differentiated adenocarcinoma, aneuploid pattern
7: Moderately differentiated adenocarcinoma, aneuploid pattern
8: Poorly differentiated adenocarcinoma, aneuploid pattern

Conclusions P53 was one of the most common genes important to colorectal carcinogenesis mutated late in the adenoma-carcinoma sequence. C-myc immunostaining was more noticed in dysplastic areas of adenomatous polyps than in normal or hyperplastic areas. The increase in aneuploidy incidence occurred in direction from hyperplastic polyps to tubular adenomas to tubulovillous to villous adenomas to carcinomas. Also the degree of aneuploidy of carcinoma cases was higher than that of adenomas and that of adenomas were higher than that of hyperplastic cases.



Immunostained sections by c- myc and p53

1: Hyperplastic polyp showing focal cytoplasmic brown staining by c- Myc antibody. (X 100).
2: Tubular adenoma showing heterogeneous cytoplasmic staining by C-myc antibody. (X 100)
3: Villous adenoma showing heterogeneous cytoplasmic staining by C-myc antibody. (X 100).
4: Moderately differentiated adenocarcinoma swing pan cellular staining by anti- c-myc immunostain. (X 100)
5: Tubulovillous adenoma showing strong nuclear staining by p53. (X 100)
6: Villous adenoma showing focal strong nuclear staining by p53. (X 200).
7: Villous adenoma showing focal strong nuclear staining by p53. (X 100).
8: Well-differentiated adenocarcinoma showing strong nuclear brown staining by p53 antibody. (X 200).

Evaluation of some biochemical markers as prognostic factors in malignant lymphoma

Hisham Ismail and Mohamed A. Soliman

Introduction Non-Hodgkin's lymphoma is a group of related disorders that possess a combination of clinical, histologic and immunopathologic features

[1]. NHL is characterized by abnormal proliferation or accumulation of B or T lymphocytes [2]. Patients with NHL may exhibit systemic symptoms such as fever, night sweats, cachexia; Which may be due to the release of cytokines by lymphoma cells [(1)]. Moreover, cytokines may play an important role in the pathogenesis of lymphoma [3]. Cytokines can either be produced by or exert effects on neoplastic or reactive cells [4].

This study was planned to check for the associations between levels of TNF-α, IL-2 and sCD44 in patients with different stages of NHL and the relation between these levels and tumor burden, presence of B symptoms and other prognostic criteria of the disease were evaluated.

Methods The study involved 52 patients (37 males and 15 females) suffering from NHL. They were selected from the Oncology Unit, Menoufiya University Hospital, Egypt. Ages ranged from 20-60 years; they were classified into 3 groups according to the Ann Arbor staging system and National Cancer Institute modified staging system. Group I involved 17 patients who were classified as stages I/II, group II involved 19 patients who were classified as stage III, while group III involved 16 patients who were classified as stage IV. The following laboratory investigations were done for the studied subjects: complete blood picture; estimation of serum albumin and LDH; estimation of C-reactive protein (CRP); assessment of TNF-α; Assessment of IL-2; and assessment of sCD44.

Results LDH and CRP levels were more significantly higher, while albumin level was significantly lower among patients with stage IV as compared to that of patients with stages I/II or III. The levels of TNF-α, IL-2 and sCD44 were significantly higher in NHL patients than in controls. The levels of both TNF-α and IL-2 were positively correlated with LDH and CRP and negatively correlated with albumin. The level of sCD44 was negatively correlated with both albumin and HB and positively correlated with CRP. There were significant positive correlation between the levels of TNF-α, IL-2 and sCD44. There was a significant association between the levels of both TNF-α and sCD44 and the presence of B symptoms. In conclusion, the occurrence of B symptoms in NHL may be attributed, at least in part, to high level of TNF-α. The increased levels of TNF-α IL-2 and sCD44 are associated with high tumor burden and poor prognostic criteria, and it is suggest that they can be used as prognostic markers in NHL.

Table 1

The studied parameter	Controls (n = 20)	Stage I/II (n= 17)	Stage III (n = 19)	Stage IV (n = 16)	Test of sig.	Sig
TNF- α (pg/ml)	4.70± 1.41	17.64± 9.07	154.01± 25.38**	345.93± 221.63**	K-W = 61.70	***
IL-2 (IU/ml)	0.99± 0.13	1.25± 0.64	11.52± 8.46**	11.68± 10.77**	K-W = 55.83	***
sCD44 (ng/ml)	7.51± 2.61	10.17± 3.47*	13.0± 5.03*	15.9± 4.6**	F = 7.03	**

Sig. * < 0.05; ** < 0.01; *** < 0.001;

Table 2

Parameters	TNF- α	IL-2	sCD44
LDH	0.85**	0.58**	0.26
Albumin	-0.79**	- 0.65**	- 0.38**
HB	-0.42**	-0.06	- 0.36**
CRP	0.58**	0.49*	0.34*
TNF-a	--	0.62**	0.45**
IL-2	0.62**	--	0.41**
sCD44	0.45**	0.41**	--

Sig. * < 0.05; ** < 0.01; *** < 0.001;

Conclusions The levels of TNF-α, IL-2 and sCD44 were all increased in NHL and were related to tumor burden. Furthermore, there was a significant association between high levels of TNF-α and sCD44 and occurrence of B symptoms. Association of TNF-α, IL-2 and sCD44 with the stage of the disease, and clinical and laboratory parameters of IPI may suggest a prognostic and may help to give an idea about the possible prognosis and to decide on appropriate therapeutic approaches for individual patients.

References

1. Akisik E; Bavbek S and Dalay N (2002): CD44 variant exons in leukemia and lymphoma. Pathol Oncol Res, 8 (1): 36-40.
2. El-Din HM; Attia MA; Hamza MR; Khaled HM; Thoraya MA; Eisa SA; (2004) hepatitis C virus and related changes in immunological parameters in NHL patients. Egypt J. Immunol. 11 (1): 55-64

3. Goto N; Tsurumi H; Takemura M; Hara T; Sawada M; Yamada T and Tomita Erythromycin (2006): Serum soluble tumor necrosis factor receptor 2 level determine clinical outcome in patients with aggressive NHL. Eur J Haematol, 77 (3): 217-25.
4. Niitsu N and Iijima K (2002): High serum soluble CD44 is correlated with a poor outcome of aggressive non-Hodgkin's lymphoma. Leuk Res, 26 (3): 241-8.
5. Liang X; Smoller BR and Golitz LE (2002b): Expression of CD44 and CD44v6 in primary cutaneous CD30 positive T-cell lymphoproliferative disorders. J Cutan Pathol, 29 (8): 459-64.

Proteomic response of healing corneal epithelial cells

Nikhat Ahmed Zulfiqar Naqvi, Shamim Mushtaq and A. A. Siddiqui

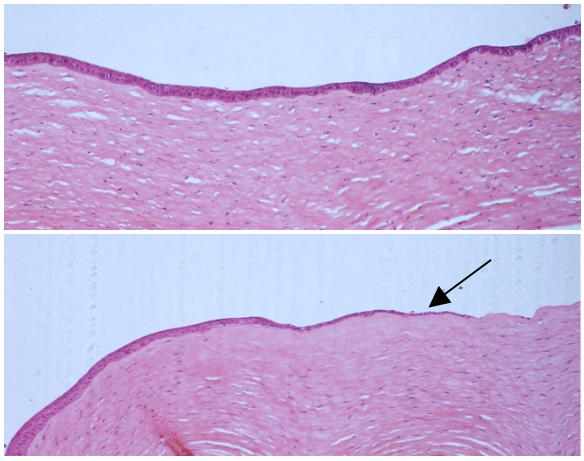
Introduction Corneal injury and delayed re-epithelialization lead to complications which often result in impaired vision. Elucidation of the underlying mechanism of corneal epithelial wound healing would provide useful information to effectively deal with this problem. We applied a) mRNA Differential Display, b) 2D-E ESI MSMS and c) GEarray to study the differential gene and protein expression during Corneal Epithelial Wound Healing

Methods The use of rabbit eyes (n=24) in this study was in conformity to the Declaration of Helsinki on the guiding principles in the Care and Use of Animals". All chemicals for organ and cell cultures were purchased from Sigma Ltd unless stated otherwise. Rabbit corneal epithelial organ cultures were prepared as described previously. Briefly the integrity of corneal epithelium was checked with fluorescein. After decapitation the eyes were processed on ice. Following washes with saline, the central corneas were demarcated and the epithelium within the trephined region was mechanically abraded and grouped as **migrating epithelium**, with controls without abrasion called **non-migrating epithelium**. The organ culture preparations were carried out in SHEM media. Progress of healing was monitored with Richardson stain every 12-24 hrs followed by histological studies. Proteins were extracted with CHAPS and estimated with BCA protein assay and subjected to 2-Dimensional Electrophoresis, ESI-MS/MS and Western blot analysis.

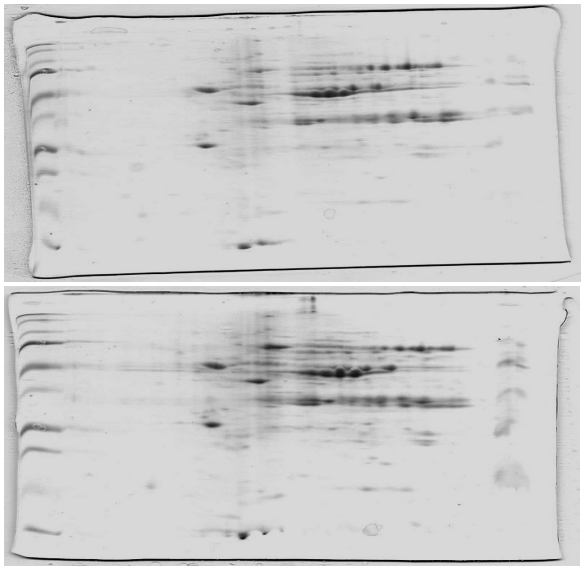
Differential Display Reverse Transcription Polymerase Chain Reaction (DDRT-PCR) was carried out on rabbit corneal (RC) epithelial migrating and nonmigrating organ cultures, to identify differentially expressed genes during corneal epithelial wound healing. Expression of DNA methyl Transferase1 (DNMT-1) was detected with western blot. Mechanical wounds were created in Human Corneal epithelial Cells (HCEC) and RC later incubated with or without 5-Aza cytidine (a demethylating agent) and Trichostatin A (a histone deacetylase inhibitor) and the rate of healing was monitored. Silencing of DNMT1 and DNMT3b genes was also carried and silencing efficiency was monitored using Real Time-PCR (Q-RT-PCR) and its effect on corneal epithelial wound healing was studied. GE array analysis of HCEC was carried out according to the instructions provided by the GE Health Care and the ECM proteins were analyzed.

Results

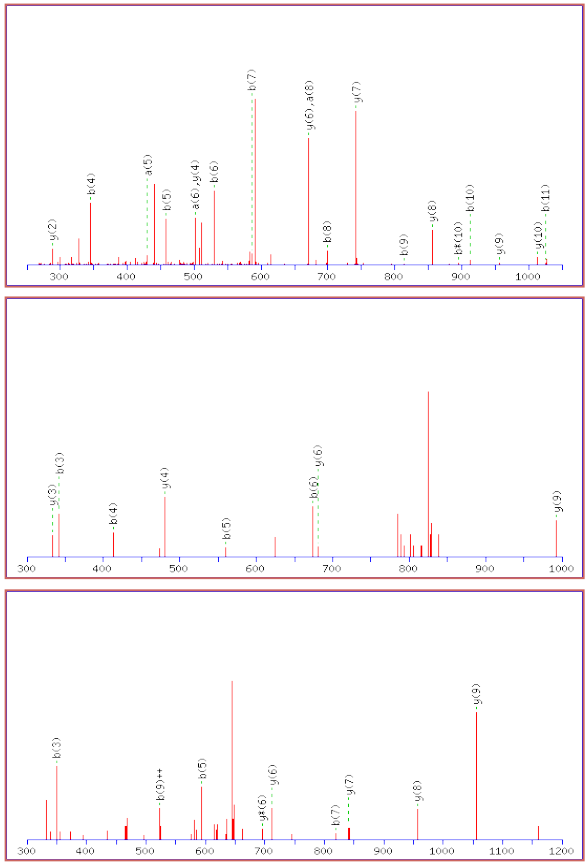
2DE followed by ESI MSMS, mRNA differential display and GE-array analysis revealed a number of genes/proteins to be differentially expressed in migrating and non-migrating corneal epithelia during wound healing; a number proteins regulated by DNA methylation are identified. DNMT1 and DNMT3b are differentially expressed in non-migrating and migrating corneal epithelia respectively.



Corneal tissue sections showing nonmigrating (above) and migrating (below) corneal epithelium, 48 hr post-wounding; arrow shows migration of single layer of epithelium to cover the wound.



2DE of Nonmigrating **(N)** and Migrating **(M)** corneal epithelia, at the active phase of migration (48 hr post wounding)



ESI MS/MS Spectra of proteins identified in Migrating (M) corneal epithelium, at the active phase of migration (48 hr post wounding) **(a)** HSP 70 **(b)** HSP8 **(c)** albumin. *Incomplete*

Expression of matrix metalloproteinases 2 and 9 in human trophoblasts of normal and preeclamptic placentas

Ola M. Omran, M. Shokry, Hisham Ismail, G. Omar, and M. Rezk

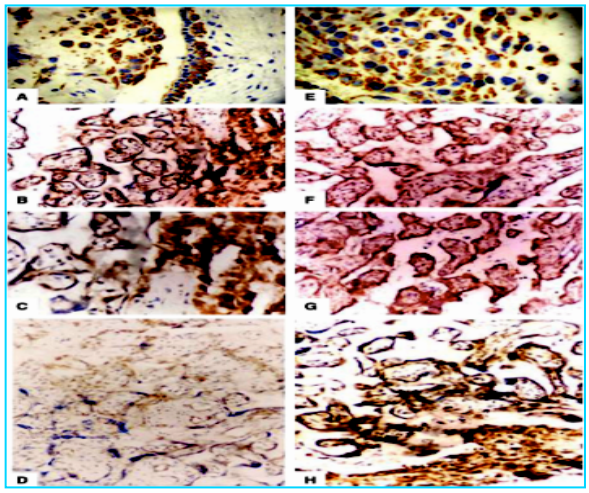
Introduction Preeclampsia is a common and serious complication affects 3% to 7% of all pregnancies. MMPS is a family of proteolytic enzymes, able to degrade extracellular matrix & basement membrane components. Several studies have examined the activity and localization of MMP2 and 9 *in vitro systems* (tissue culture and amniotic fluid). To date, studies that bear directly on the tissue localization and expression pattern of MMP2 & 9 (IHC) in human preeclampsia are limited.

This is a case–control study including preeclamptic placentas (N=40) and placentas of normal pregnancies (control group, N= 40). Through an immune-histo-chemical study, expression of MMP2 and 9 was examined using monoclonal antibodies against MMP 2 and 9. The negative control sections were treated in an identical manner with the omission of primary antibody. Positive control for MMP-2 was human placenta villous tissue (from first trimester). Positive control for MMP-9 was endothelial cells. Data were analyzed using SPSS for windows.

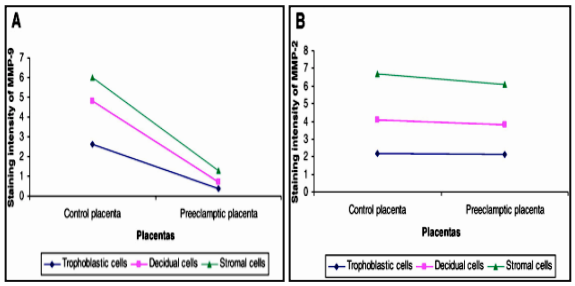
Results The incidence of intrauterine growth restriction was high and the mean birth weight was markedly low in patients with preeclampsia. Both MMP 2 & 9 proteins were frequently and strongly expressed in the majority of placentas of control group. MMP 9 expression was weak or absent in the majority of the preeclamptic placentas. In contrast, a strong MMP 2 protein expression was seen in the majority of the preeclamptic placentas.

	Control group N= 40	Preeclamptic group N=40	p+	Mild PE N=17	Severe PE N= 23	p++
Age (Mean (SE))	25.7 (0. 8)	24.1 (0.9)	NS	24.7 (1.1)	23.7 (1.2)	NS
Parity (Mean (SE))	1.5 (0.3)	1.2 (0.3)	NS	1.3 (0.4)	1.1 (0.4)	NS
Gestational age at delivery (Mean (SE))	37.4 (0.3)	35.8 (0.2)	<0.01	37.2 (0.5)	34.6 (0.4)	<0.01
Mode of delivery						
VD	34/40 (85%)	15/40(37.5%)	<0.001	15/17 (88.2%)	0/23 (0%)	<0.001
CS	6/40 (15%)	25/40 (62.5%)	<0.001	2/17 (11.8%)	23/23 (100%)	<0.001
Incidence of IUGR	1/40 (2.5%)	8/40 (20%)	<0.001	1/17 (5.9%)	7/23 (30.4%)	<0.001
Birth weight (kg) (Mean (SE))	3.214 (0.439)	2.127 (0.754)	<0.001	2.682 (0.374)	2.017 (0.409)	<0.009

Clinical characteristics of the study population and the neonates



Immunohistochemical staining of cytokeratin and metalloproteinases in control and preeclamptic placentas (A & E) Strong expression of cytokeratin in the extravillous trophoblast (A: ×200, E: ×400). (B–D) Strong MMP9 protein expression in the trophoblastic, decidual cells of the control placentas of uncomplicated placentas (B: ×200, C: ×400) versus weak expression in the chorionic villi of preeclamptic placentas (D: ×200). (F–H) Strong expression of MMP2 in the chorionic villi of the normal (F: ×200) and preeclamptic (G: ×200, H: ×200) placentas.



Staining intensity of MMP2 & 9 proteins in the normal and preeclamptic placentas; the variations in the staining intensities (all cell types) were statistically significant for metalloproteinase 9. No statistically significant differences were seen for MMP 2.

MMP-9 expression	Normal placenta (Control) N=40	Preeclamptic placenta N=40	*p value
Trophoblastic cells	35/40 (78.5%)	6/40 (15 %)	<0.001
Decidual cells	30/40 (75%)	5/40 (12.5%)	<0.001
Stromal cells	17/40 (17.5%)	8/40 (20%)	NS

Frequency of MMP-9 protein expression in the normal and preeclamptic groups

MMP-2 expression	Normal placenta (Control) N=40	Preeclamptic placenta N=40	*p value
Trophoblastic cells	30/40 (75%)	28/40 (70 %)	NS
Decidual cells	26/40 (65%)	23/40 (57.5%)	NS
Stromal cells	35/40 (78.5%)	31/40 (77.5%)	NS

Frequency of MMP-2 protein expression in the normal and preeclamptic groups

Discussion *Immunohistological localization of MMP2 and 9 in the control human placentas:* MMP 9 expression was prominent in trophoblastic and decidual cells, and weak in the mesenchymal cells of the of control placentas. In contrast, we found a prominent MMP2 protein expression in the trophoblastic and mesenchymal cells and moderate expression in the decidual cells. These preliminary findings are consistent with previous studies in animal and cell culture models (*in vitro* studies). Zymography with gelatin as substrate indicated that MMP 2 was present in increasing amounts in the amniotic fluid from day 70 of gestation to labour (days 140–145), and MMP 9 was detectable from day 125 to labour. Weak or absent MMP 9 protein expression in the majority of preeclamptic placentas concurs with previous observations. However, these findings need further confirmatory studies (Western blot and PCR). We demonstrated reduced/absent expression of MMP 9 in preeclamptic placentas compared to the control ones, which may be a factor that impairs trophoblast invasion and hence proper placentation. Our preliminary findings suggest that abnormalities of MMP expression may contribute to preeclampsia development.

References

1. Adly, M.A., et al., 2006a. Age-associated decrease of the nerve growth factor protein expression in the human skin: preliminary findings. J. Dermatol. Sci. 42, 268–271.
2. Adly, M.A., et al., 2006b. Expression of the heat shock protein-27 in the adult human scalp skin and hair follicle: hair cycle-dependent changes. J. Am. Acad. Dermatol. 54, 811–817.

3. Autio-Harmainen, H., et al.,1992. Simultaneous expression of 70 kilodalton type IV collagenase and type IV collagen alpha 1 (IV) chain genes by cells of early human placenta and gestational endometrium. Lab. Invest. 67, 191–200.
4. M. Shokry et al., 2009. Expression of matrix metalloproteinases 2 and 9 in human trophoblasts of normal & preeclamptic placentas: Preliminary findings. Experimental & Molecular Pathology 87, 219–225

Blood levels of apoptotic markers in diabetic patients in AL-Qassim region

Abdel-Raheim Meki, Abdullah A. Alghasham and Hisham Ismail

Introduction Diabetes mellitus is one of the major metabolic disorders worldwide. This metabolic disturbance is associated with high morbidity and mortality caused by micro-vascular complications [1]. Apoptosis plays an important role in normal tissue homeostasis. Dysregulated apoptotic modulators result in excessive or insufficient cell death fundamental to the initiation and progression of many human diseases including diabetes mellitus [2]. Oxidative stress as expressed by lipid peroxidation products, e.g. thiobarbituric–acid reactive substances (TBARS), is an apoptotic inducer [3]. It leads to dysregulated cell growth or apoptosis, which contributes to the development of inflammation and secondary complications of diabetes [4].

The objectives of this study are: to investigate the dysregulation of apoptotic modulators in type I and type 2 diabetes and to determine plasma levels of soluble Fas (sFas), sFas ligand (sFasL), tumor necrosis factor-alpha (TNF-α), TBARS and nitric oxide (NO); and to evaluate the relationships of these bio-indices with glycemic control, disease complications (diabetic neuropathy) and duration.

Methods Sixty diabetic patients (30 each of type 1 and type 2 diabetes mellitus) were sub-classified into non-complicated diabetic patients and patients with diabetic neuropathy. Patients were also categorized according to glycemic control (HbA1c ≤ 7% and HbA1c > 7%) and duration of diabetes (≤ 1 year and > 1 year). Each diabetic group was compared with its related age-matched control group. The plasma levels of sFas, sFasL, and TNF-α were determined using specific ELISA assays. The plasma levels of NO and TBARS were measured by colorimetric methods. Blood HbA1c was determined by chromatography.

Results In types 1 and 2 diabetes, plasma levels of sFas, TNF-α, TBARS and NO were significantly higher in non-complicated and complicated diabetes than controls. These indices were found significantly higher in complicated than non-complicated diabetic patients. Plasma sFasL level was insignificant in complicated in comparison with non-complicated type I and II diabetic patients. Significant increases in the investigated bio-indices were observed in type 1 diabetic patients with good and poor glycemic controls compared to controls. Poor glycemic control showed significantly higher levels of TNF-α in types 1 patients in comparison with good glycemic control. There were significant positive correlations among the investigated biochemical indices and indicators of glycemic control, oxidative stress and duration of the disease particularly in type 1 diabetes.

Discussion Types I and II diabetes are associated with dysregulated apoptotic modulators. Could the modulation of the observed dysregulation benefits diabetic patients? This is a point that needs further clarification. However, the increased level of apoptotic modulators detected in this study seems to be implicated in diabetic neuropathy and hence should be taken in the consideration as predictive marker for such complication and in strategies for therapeutic intervention.

References

1. Maejima K, et al., (2001): Increased basal levels of plasma nitric oxide in Type 2 diabetic subjects. Relationship to microvascular complications. J Diabetes Complicat. 15(3):135-43.
2. Grodzicky T and Elkon KB (2002): Apoptosis: a case where too much or too little can lead to autoimmunity. Mt Sinai J Med. 69(4):208-19.
3. Sata M, and Walsh K. (1998): Oxidized LDL activates Fas-mediated endothelial cell apoptosis. J Clin Invest, 102, 1682 – 1689.
4. Kostolanská J, Jakus V, Barák L.(2009): Glycation and lipid peroxidation in children and adolescents with type I diabetes mellitus with and without diabetic complication. J Pediatr Endocrinol Metab. Jul;22(7):635-43.

Methylene tetrahydrofolate reductase (MTHFR) and angiotensinogen converting enzyme (ACE) gene polymorphisms related to overweight and obesity among Saudi patients in Al Qassim

Ahmad Settin, Abdullah AlGasham, Moataz Dowaidar and Hisham Ismail

Introduction Obesity is an excessive accumulation of body fat and in its gross manifestation poses a real threat to health. It is the most prevalent, chronic medical condition in the developed, as well as in developing countries. Epidemiological studies have shown that low folate levels are associated with a high body mass index (BMI). This finding has potentially important health implications and warrant further investigation particularly for MTHFR gene polymorphisms to determine whether a causal relationship exists and the direction of this relationship. During the last three decades Saudi Arabia has seen a considerably rising rates for both overweight and obesity Qassim region is a tribal area in the middle zone of Saudi Arabia characteristically having high rate of consanguinity and familial diseases as obesity with diabetes and cardiovascular disorders.

The objectives of this study are to evaluate the association of polymorphisms with MTHFR and ACE genes with overweight and obesity among Saudi patients from Al Qassim region.

Methods This study includes 130 patients with overweight or obesity and 111 normal weight controls in the 18-40 years age group. The patients' DNA was analyzed for polymorphisms of MTHFR; 677C/T and 1298 A/C and ACE; I/D genes using real-time PCR.

Results Genotype and allele frequencies of studied polymorphisms in cases of overweight/obesity showed no significant statistical difference compared to that of controls (Table 1). However, on analysis of body mass index (BMI), cases showed higher mean ± SD values –although nonsignificant- among those carrying the mutant MTHFR 677 T allele (CT+TT vs. CC, 30.7±4.5 vs. 29.9±4.9), 1298 C allele (AC+CC vs. AA, 29.9±4.1 vs. 29.7± 5.5) and ACE D allele (ID+DD vs. II, 30.0±5.1 vs. 29.1 ± 2.8). In addition, controls having the DD and ID genotypes showed higher statistically significant values of BMI than those of the II genotype (22.0 ± 1.9, 21.7 ± 2.6 and 19.5 ± 2.3 respectively, $p<0.05$) (Table 2).

Table 1

Genotypes and Alleles	Overweight/ Obese n (%)	Control n (%)	P (χ2)	OR (95% CI)
MTHFR 677				
CC	89 (69.5)	69(62.7)	0.33	1.4 (0.8-2.3)
TC	34 (26.6)	36(32.7)	0.37	0.7 (0.43-1.3)
TT	5 (3.9)	5(4.5)	0.94	0.85 (0.24-3.03)
C	212 ()	174	0.36	1.3 (0.81-2.02)
T	44 ()	46	0.36	0.79(0.5-1.2)
MTHFR 1298				
AA	70(54.7)	51(46.4)	0.25	1.4(0.84 -2.3)
AC	45 (35.2)	47(42.7)	0.29	0.73(0.43-1.23)
CC	13 (10.2)	12 (10.9)	0.98	0.92 (0.42-2.12)
A	185 ()	149 ()	0.33	1.2 (0.84-1.84)
C	71 ()	71 ()	0.33	0.81 (0.54-1.19)
ACE I/D				
II	12 (9.5)	5(4.5)	0.21	1.23(0.76-6.55)
ID	54 (42.9)	44(39.6)	0.71	1.14 (0.68-1.92)
DD	60(47.6)	62(55.9)	0.26	0.72(0.43-1.2)
I	78 ()	54 ()	0.13	1.4 (0.93- 2.1)
D	174 ()	168 ()	0.13	0.72 (0.48-1.08)

Table 2

Genotypes	Overweight/obese Mean + SD	Controls Mean + SD
MTHFR 677		
CC	29.9 ± 4.9	21.7 ± 2.5
CT	31.1 ± 4.5	21.8 ± 1.8
TT	28.4 ± 4.6	23.5 ± 0.66
CT+TT	30.7 ± 4.5	21.97 ± 1.8
MTHFR 1298		
AA	29.7 ± 5.5	21.7 ± 1.9
AC	30.3 ± 4.1	21.8 ± 2.5
CC	28 ± 3.9	22.2 ± 2.6
AC+CC	29.9 ± 4.1	21.8 ± 2.5
ACE		
II	29.1 ± 2.8	19.5 ± 2.3
ID	30.2 ± 4.2	21.7 ± 2.6*
DD	29.5 ± 5.9	22.0 ± 1.9*
ID+DD	30.0 ± 5.1	21.9 ± 2.2*

Discussion In order to assess this association we selected cases with overweight/obesity without complications and compared them to unrelated matched controls of same ethnic background. Although no significant associations were found

comparing the genotype and allele frequencies of studied genes in overweight/obese cases and controls, we observed a trend for slightly higher BMI values among subjects carrying the mutant forms of these polymorphisms particularly that of the ACE DD and ID genotypes. This was markedly manifested in male subjects. Similar finding suggesting that ACE gene polymorphisms may influence the development of weight gain with a sex difference in males and females was previously reported. On the other hand, recent reports indicate a trend towards association of ACE I/D polymorphism with hypertension but not with obesity. We conclude that there is no solid association of polymorphisms related to MTHFR 677 and 1298 and ACE I/D genes with non-complicated overweight or obesity among Saudi subjects from Qassim Region. However, we recommend a further longitudinal study for follow up and genotyping of cases that might develop obesity complications.

Clinical Medicine

P Wave Dispersion (PWD) as a predictor of Atrial Fibrillation (AF)

Alaa Abd Elmoniem, Noor El-Hefny and Walid Wadi

Introduction AF is the most important risk factor for stroke; it has a deleterious effect on longevity with a doubling of all cause mortality [1]. The estimation of the probability of recurrent AF by using a simple parameter might guide the clinician in the management of these patients. P wave dispersion constitutes a recent contribution to non-invasive electro cardiology. This electrocardiographic measurement reflects a disparity in atrial conduction [2]. The objective of this study is to assess the value of P wave dispersion as a predictor of AF recurrence in patients with and without structural heart disease to detect and try to trace which group of patients is susceptible to recurrence of AF (the high risk group), in an attempt to follow and try to prevent the occurrence of serious complications among them.

Methods Sixty two patients who had AF and successfully converted to sinus rhythm were included. They were followed up for recurrence of AF for six months. Any patient with failed cardioversion, received antiarrhythmic agents 4 weeks before and after cardio-version, severe hypertension, ACS, heart failure, severe pulmonary disease, pulmonary embolism, sick sinus syndrome or patients with open heart surgery within 3 months were excluded from the study. All patients were subjected to the following: thorough history, standard 12 lead ECG for PWD calculation, Echo-doppler to measure LA diameter, Left ventricular systolic (EF%) and diastolic (E, A, E/A ratio) functions.

Results According to AF recurrence, the patients were classified into: Group I included 36 patients with recurred AF and Group II included 26 patients with preserved sinus rhythm (PSR). Maximum P wave duration was statistically significant longer in group I than that in group II ($P<0.04$) and at a cutoff point of >110ms sensitivity was 88.9% and specificity of 73.1% for patients with AF recurrence. PWD was of highly statistically significant values in group I (71±21ms) than those in group II(40±15ms) ($P<0.000$) with sensitivity of 75% and specificity of 88.5% at cutoff point >80.5ms. Statistically significant left ventricular diastolic dysfunction (in the form of impaired and pseudonormal relaxation) and increased left atrial diameter were more obvious in group I than those in group II($P<0.000$&$P<0.007$ respectively). On the other hand, there was insig-nificant decrease in EF% in group I than in group II. Logistic regression analysis for P max, PWD, LAD and EF% revealed that PWD is independent predictor for AF recurrence ($r=0.585$, $P<0.000$). Receiver operator curve for AF recurrence group showed that PWD had the largest area under the curve (AUC=0.975) than those of P max (AUC=0.885) or LAD (AUC=0.955).

Table (1): Patients' characteristics in both groups

Item no. (%)		AF recurrence		P value
		Recurred N=36	PSR N=26	
1-Age (mean± SD)		55 ±13	50 ±13	NS
2-Sex	Male	21 (58.3%)	14 (53.8%)	NS
	Female	15 (41.7%)	12 (46.2%)	
3-Hypertension	Hypertensive	21 (58.3%)	13 (50.0%)	NS
	Non-hypertensive	15 (41.7%)	13 (50.0%)	
4-DM	Diabetic	15 (41.7%)	10 (38.5%)	NS
	Non-diabetic	21 (58.3%)	16 (61.5%)	
5-Heart diseases	CAD	30 (61.6%)	22 (84.6%)	NS
	VHD	3 (19.2%)	1 (3.8%)	
	Lone AF	3 (19.2%)	3 (11.5%)	

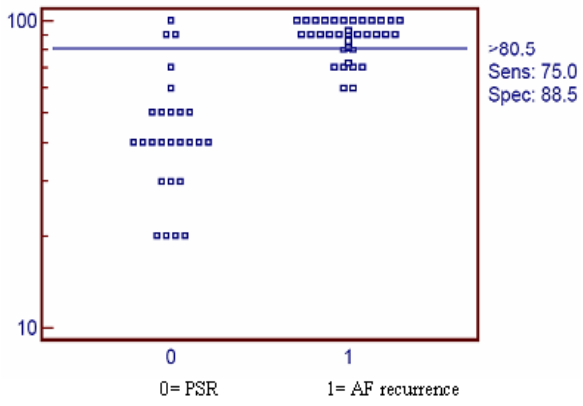
PSR =Preserved sinus rhythm, CAD =Coronary artery disease, VHD = Valvular heart diseases, NS = Non- significant

Table (2) The ECG and echocardiographic findings in both groups

Item Mean ± SD Or no. (%)	AF recurrence		P value
	Recurred N=36	PSR N=26	
1-Max P duration (ms)	116.33±10.00	105.46±8.45	0.04*
2-PWD (ms)	71±21	40±15	0.000***
3-LAD (cm)	4.40±.91	4.00±.87	0.007**
4-EF (%)	44.83±10.89	46.58±8.64	NS
5-Diastolic function			
NF	5 (13.9%)	16 (61.5%)	0.000***
IR	15 (41.7%)	3 (11.5%)	0.000***
PNR	16 (44.4%)	7 (26.9%)	0.000***

*PSR= Preserves sinus rhythm, PWD= P wave dispersion, LAD= Left atrial diameter, NF = Normal function, IR= Impaired relaxation, PNR= Pseudonorm relaxation * = significant, ** = highly significant, *** = Very highly signi*

Figure (1): Interactive plot after ROC curve for The PWD as regarding AF recurrence



Discussion Recurrence of atrial fibrillation after electrical cardioversion of chronic AF is not uncommon, where up to 57% of AF recurrence after cardioversion take place during the first 30 days after direct current shock therapy. However, it remains unclear which parameter (s) predict clinical recurrence [3].There is no universally accepted value that defines a prolonged PWD as calculated by 12-lead electrocardiography [4]; however, our results would point toward employing a higher cutoff to more accurately identify patients at risk for recurrent AF**.** We conclude that PWD, which is a non-invasive and simple parameter, could be applicable to predict the high risk group for AF recurrence after cardioversion, but a larger study is needed to consolidate these results.

Figure (2): Comparison ROC curves for PWD, LAD and P max

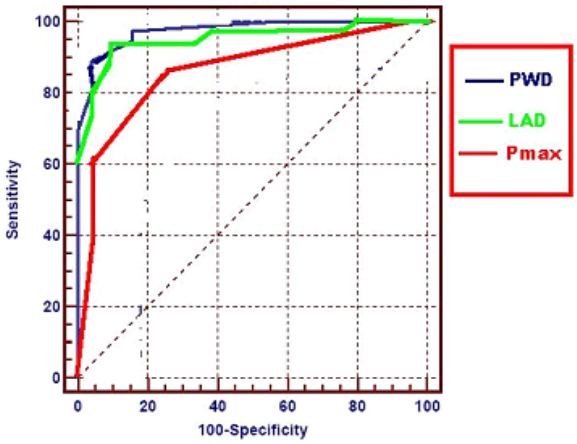


Table (3): The logistic regression analysis for predictors of AF recurrence.

Item	R- square	P - value
PWD	.585	.000
PMAX	.056	.755
LAD	.570	.487
EF	.046	.479

References

1. Poli D. Age, CHADS2 define bleeding risk in anticoagulated AF patients. Thromb Res 2007;121:347-52.
2. TurhanH,KoseH,CelikT et al. Atrial fibrillation recurrence after cardioversion : is there is a simple electrocardiographic parameter to predict it ? Int J of cardiology 2006; 113(3):435-436.
3. Boriani G, Diemberger I, Biffi M et al., P wave dispersion and short-term vs. late atrial fibrillation recurrences after cardioversion, Int J Cardiol 2005; 101: 355–361
4. Boriani G,Diemberger I,BiffiM etal., PWD&short-term vs. late AFrecurrences after DC, Int J Cardiol 2005; 101: 355–361

Neonatal birth-weights and reference intervals in sonographically monitored normal fetuses

Khalid Shehzad

Introduction An important dilemma of pediatric care is the prediction of fetal birth as it is not only an indicator of the prenatal maternal nutrition, her socio-economic status and a part of normal growth milestones of the fetus, but is also concerned with the subsequent care of the newborn. Pakistani population is heterogeneous and very little data exists in the country with regard to birth weights at term in normal infants. This study evaluates the birth-weight normograms constructed by utilizing the subjects having a normal fetal ultrasound. The objective of this study is to determine the normal neonatal birth-weights and to establish the normal reference intervals (normal range) in sonographically monitored normal fetuses in a tertiary care hospital.

Methods This study enrolled 387 pregnant women with normal singleton pregnancy having fetal biometry by ultrasound. The ultrasound parameters used were bi-parietal diameter, head circumference, abdominal circumference and femur length. All women had delivered between 39 to 41 completed weeks of gestation. The birth-weight of the newborn was recorded within 15-30 minutes of delivery on an infant beam balance (Tanita- Japan, Capacity 20 Kg). The criteria for inclusion were normal singleton gestation having spontaneous onset of labor at term and vaginal or abdominal delivery.

Results Mean age of the mother was 26.3 ± 4.6 years, while the mean height of the mother was 158.9 ± 6.2 cm. The mean height of the father was 171.1 ± 8.8 cm. About 92% of women were housewives; 97% were literate (having 10+ years of education). The mean birth-weight was 3.06 ± 0.34 Kg, with the 5th percentile at 2.50 Kg and the 95th percentile at 3.61 Kg. The range was 2.5 to 4.1 Kg. The mean birth-weight for male newborns was 3.13 ± 0.33 Kg, while that for the female newborns was 2.96 ± 0.32 Kg (P < 0.001).

Table

Birth-weight (Kilogram)	Number (n)	Percentage (%)
2.5 – 3.0	197	50.9%
3.1 – 3.5	153	39.5%
3.6 – 4.0	33	8.5%
4.1 – 4.5	4	1.0%
Total	387	100%

Conclusions In this sample of newborns, the minimum birth-weight was 2.5 Kg, and no babies were low-birth-weight (< 2.5 Kg) according to the WHO criteria. However, the vast majority (51%) of babies weighed between 2.5 Kg and 3.0 Kg at birth. This sample belongs to a population that is relatively better-off in socioeconomic terms. Nonetheless, the birth-weight among female newborns is significantly lower than the mean birth-weight among male newborns.

References

1. Taha SA, Abdullah MA, Jowda MS, Akbar JU. Sizes at birth of live-born Saudi infants. Br J Obstet Gynaecol, 1984; 91 (12): 1197-202.
2. Assessment of growth. In: Behrman, Khiegman, Jenson, (edi).Nelson Textbook of Pediatrics. 17th ed. Philadelphia: Saunders, 2004: 58-62.

N-terminal pro-BNP in acute coronary syndrome patients with ST elevation (STE-ACS) versus non ST elevation (NSTE-ACS)

Ragaa Salama, Alaa El-Moniem, Nour El-Hefney and Tarek Samor

Introduction Pro-BNP was synthesized as a pro-hormone by cardiac myocytes then cleaved by enzyme to N-teminal proBNP (NT-proBNP) and BNP (brain naturetic peptide). Conventional cardiac markers, such as troponin-T (Tn -T), and creatine kinase (CK)-MB isozyme, detect the development of minor myocardial necrosis [1]. A direct release of BNP from ischemic cardiomyocytes and ischemia induced by increase in ventricular wall stress was postulated. Natriuretic peptides have cyto-

protective effect in myocardium related to cGMP accumulation and opening of ATP-sensitive K^+ channels which is potentially exploitable therapeutically [2].

The objective of this study is to investigate the differences in the secretion of NT-proBNP and conventional cardiac markers in patients with STE-ACS vs. NSTE-ACS as a trial to solve the dilemma of the early detection of myocardial ischemia in NSTE-ACS.

Methods Sixty two patients with acute coronary syndrome (ACS) were divided into 2 groups according to ECG: group1 with elevated ST segment (STE-ACS) and group 2 with non elevated ST segment (NSTE-ACS). Twenty healthy subjects with matched age and sex were enrolled as control group. In the sera of all subjects, levels of NT –proBNP, CK-MB and Tn- T were measured by different commercial kits. Serum CK-MB levels measured kinetically by UV method whereas Tn-T determined by chemiluminescent immunoassay using available kit. NT-proBNP measured by ELISA. SPSS version 16 was used for data analysis. Mann-Whitney U test, ANOVA test and ROC curve were used for comparing the data.

Results CK-MB and Tn-T were both significantly higher in STE-ACS patients compared with NSTE-ACS patients. Conversely, NT-proBNP was significantly higher in NSTE-ACS patients than STE-ACS especially within 4 hours from onset of chest pain (Figures 1, 2 and 3). This suggested a larger ischemic insult despite the smaller extent of myocardial necrosis compared with STE-ACS patients. Comparison between NT-proBNP, Tn-Tand CK-MB levels by ROC curve (Figure 4) revealed a marked difference of area under the curves with higher sensitivity and specificity of NT-proBNP in NSTE-ACS patients.

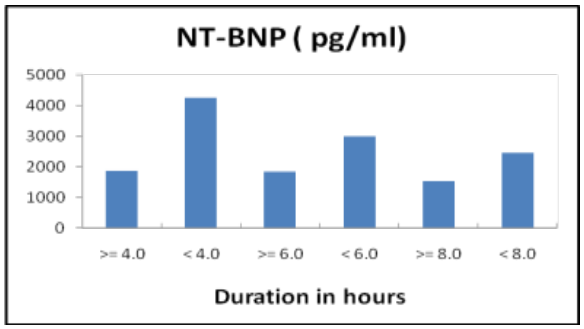


Figure 1: NT-proBNP in patients with ACS.

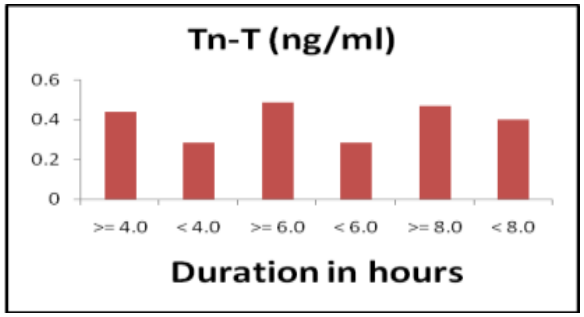


Figure 2: Tn-T in patients with ACS.

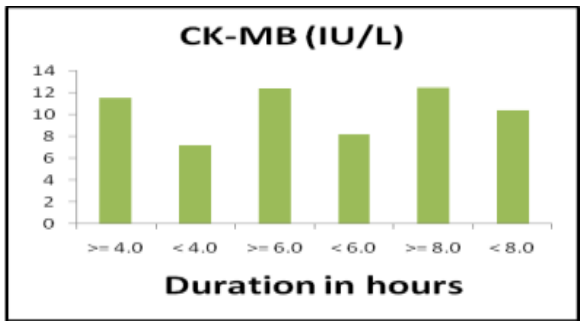


Figure 3: CK-MB in patients with ACS.

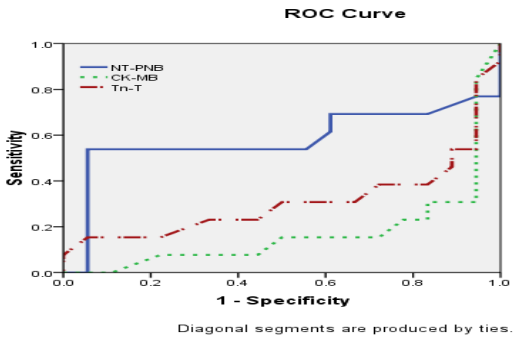


Figure 4: ROC curve in NSTE-ACS: The area under the curve was 0.58 (NT-pro BNP); 0.31 (Tn-T); 0.17 (CK-MB)

Discussion The increment of NT-proBNP in NSTE-ACS patients was inversely proportional to the duration of chest pain. It was more significant

increased when the duration of chest pain was ≤ 4 hours, compared to when the duration was between 6 and 8 hours. It increased during the hyper acute phase in NSTE-ACS patients, and wasn't raised by the process of myocardial necrosis but the ischemic insult per se. The ischemic area or area at risk showed different spectrum in these 2 groups. STEMI caused by acute total coronary occlusion, whereas NSTEMI associated with vulnerable plaque and sub-occlusive thrombosis [3]. NTpro-BNP may be a powerful indicator of long-term mortality in patients with ACS and Provide prognostic information above and beyond conventional risk markers [4]. We conclude that NT-proBNP may be a sensitive and specific marker in the early diagnosis of NSTE-ACS than other traditional cardiac markers. NT-proBNP might be superior to clinical judgment in the diagnostic evaluation of NSTE-ACS patients.

References

1. Haastrup B, et al.,Biochemical markers of ischemia for the early identification of acute myocardial infarction without St segment elevation. Cardiology 2000; 94: 254 – 261.
2. Rautureau Yet al., Acute actions of natriuretic peptides in coronary vasculature and I schaemic myocardium. Curr Pharm Des. 2004;10(20):2477-82.
3. Lorgis L et al., Prognostic value of N-terminal pro-brain natriuretic peptide in elderly people with acute myocardial infarction: prospective observational study. BMJ. 2009; 6 : 1605-1609
4. Omland T et al., Prognostic value of N-terminal pro-atrial and pro-brain natriuretic peptide in patients with acute coronary syndromes. Am J Cardiol 2002; 89: 463-5.

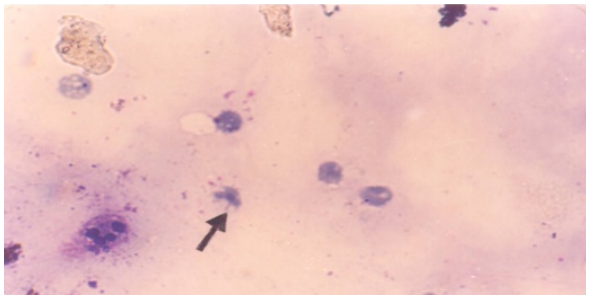
Acanthamoeba Eye Infection: A Prospective Study

Abdelmageed Imam

Introduction *Acanthameba* is a free living protozoan found in diverse environments including soil , water , and air . The first note on *Acanthamoeba* was made in 1930, and the first human eye infection was reported in 1974. *Acanthamoeba* has been isolated from the throat & nasal passages of healthy humans. It can infect the cornea of the human eye in immunocompetent individuals & cause keratitis (AK). *Acanthamoeba* may lead to blindness in 15% of cases if diagnosis & prompt treatment are delayed. The risk factors associated with AK include contact lens wearing, soil work and swimming in pools. AK is typically unilateral and patients usually suffer from eye pain, hyperaemia, and photophobia. The objective of this study is to explore acanthamoebic etiology among patients suffering from chronic keratitis, where the infectious agent remains unknown.

Methods This is a prospective analytic & descriptive study conducted at two referral eye hospitals in Khartoum. The study population included all patients who presented with chronic keratitis and who did not respond to standard therapy for bacterial, viral, or fungal infections during July 2003 to August 2005. Specimens were corneal swabs and corneal scrapes. We used light microscopy and culture techniques. A wet mount preparation from both the corneal swab & scrape was made. The specimen was suspended in 10 ml of 0.9% saline and centrifuged. Most of the supernatant was aspirated and the sediment suspended in the remaining fluid. A preparation was examined by microscopy for *Acanthamoeba*. Another similar specimen was cultured in a non-nutrient agar seeded with *Escerichia coli* bacteria and plates incubated at room temperature. Plates were examined daily for two weeks.

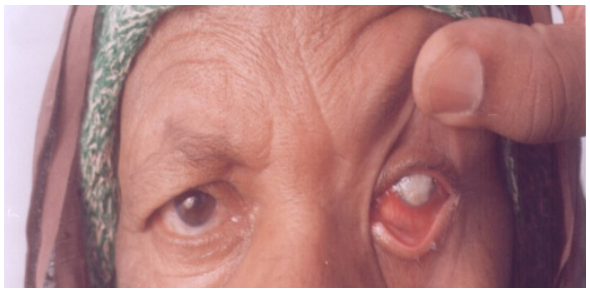
Results Only six (4 males and 2 females) of the 138 patients were positive for *Acanthamoeba*. The age range was 30-65 yrs (mean 47.5 yrs).



Acanthamoeba trophozoite (arrow). Giemsa stain (x 400**)**

This is the first study on Acanthamoeba eye disease from Sudan using culture and microscopy methods. Molecular methods are recognized laboratory methods for diagnosis of AK, but the high degree of

heterogeneity limited their use in our study.Isoenzyme analysis for diagnosis and species differentiation of Acanthamoeba can be used, however they are not always reproducible. For example, while distinct differences in the acid phosphatase and esterase isoenzyme profiles in Acanthamoeba pustulosa and Acanthamoeba palestensis were reported by one investigator; identical isoenzyme patterns for these two species were found by another investigator. Contact lens wearing is a common risk factor for AK, mainly from western countries. However, minor eye trauma was the main risk factor in our case series and these findings are in agreement with reports from India.



Thickly scarred cornea indicating a blind eye

References

1. Imam A. Pathogenic free living amoebae. Postgraduate Doctor, Middle East. 1989, 12 (9): 426-30.
2. Imam A, Mahgoub E. Blindness due to Acanthamoeba: first case report from Sudan. Int J Health Sci 2008; 2(2): 247-9.
3. Khan NA. Acanthamoeba: Bology and clinical significance. FEMS Microbiol Review 2006; 30: 564-95.

Intravitreal Bevacizumab for posterior capsule neovascularization

Mansour Al-Mohaimeed

Introduction Posterior capsule opacification and neovascularization has been reported as a rare condition that can happen in diabetic patients after extracapsular cataract extraction [1, 2] after pars plana vitrectomy in diabetic patients, or it can be idiopathic [2]. This condition is characterized by peripheral extraretinal vascular proliferation that extends along the anterior hyaloid to the posterior surface of the lens. Vascular endothelial growth factor (VEGF) has been determined to be a major regulator of pathologic ocular angiogenesis. The treatment of ocular neovascular diseases can be represented by inhibition of VEGF.

Case Report A 67-year-old male patient was presented to King Khaled Eye Specialist Hospital with a long standing history of diabetes and systemic hypertension. He underwent uncomplicated combined trabeculectomy and phacoemulsification with posterior chamber intraocular lens (IOL) implantation in right eye, and then he received panretinal photocoagulation (PRP) in both eyes for the proliferative diabetic retinopathy, which resulted in regression of new vessels. He was presented one year after the cataract surgery complaining of decrease vision for the last 2 months in the right eye. Examination demonstrated best corrected visual acuity (VA) of 20/400 in the right eye with controlled intraocular pressure (IOP). Gonioscopy showed a normal anterior chamber angle with no angle neovascularization. Anterior segment examination showed no iris neovascularization, a posterior capsule opacity (PCO) with massive neovascularization on the capsule (Figure1). Fundus examination revealed neovascularization on the disc with no other retinal neovascularization. PCO precluded the application of panretinal laser photocoagulation. The patient was offered an off-label intravitreal bevacizumab injection; a written informed consent was taken. The area was cleaned using topical 5% Povidone-iodine, a sterile lid speculum was applied. An intravitreal injection of 1.25 mg Bevacizumab was administered in the right eye 3.0 mm posterior to the limbus using a 30-gauge needle. After 9 days, neovascularization of the posterior capsule had resolved completely (Figure 2), and the visual acuity was 20/125, with controlled IOP. Nd:YAG capsulotomy was performed followed by an augmentation of PRP in the right eye. Two weeks later, his visual acuity was 20/60 with no recurrence of vascularization was documented.

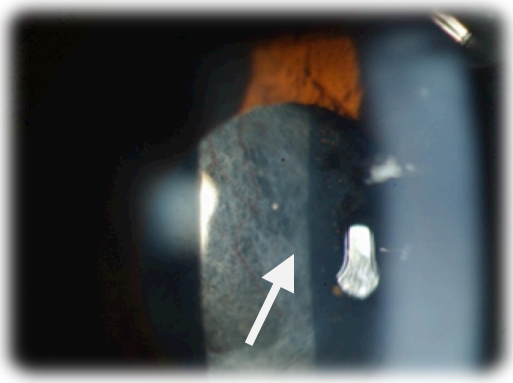


Figure 1: Slit lamp photomicrograph of the involved eye showing posterior capsule opacity with neovascularization (arrow).

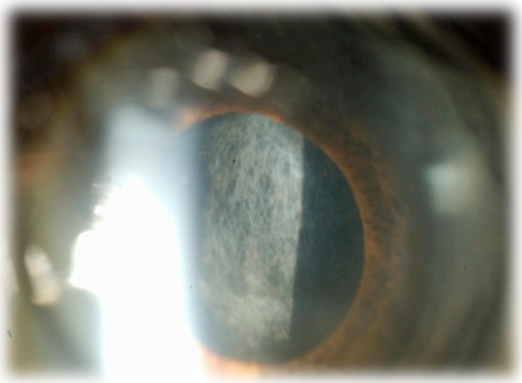


Figure 2: Neovascularization of the posterior capsule had resolved completely – 9 days after intravitreal injection of bevacizumab.

Discussion Posterior capsule neovascularization is a rare complication which can be caused by surgical intervention and systemic or ocular diseases. The anterior extraretinal fibrovascular tissue uses the anterior vitreous as a support, extends toward the lens, contracts, and causes peripheral retinal and ciliary body traction detachement [1]. With intravitreal anti-VEGF therapy, we have obtained a valuable, and most promising, new treatment of ocular neovascularization. Bevacizumab (Avastin) is a humanized, full-length monoclonal antibody that inhibits all isoforms of VEGF. It is approved as an intravenous treatment for metastatic colorectal cancer in February 2004. Several case series have shown promising results for using off-label intravitreal bevacizumab for the treatment of exudative age-related macular degeneration, proliferative diabetic retinopathy, neovascular glaucoma (NVG), oedema from retinal vein occlusion, and one single case of posterior capsule neovascularization [2]. In the previous reports about neovascularization of the capsule, other modalities of treatment were applied including argon laser photocoagulation of the vessels in the capsular bag, pars plana vitrectomy with removal of posterior capsule, cryoablation, endophotocoagulation of peripheral retina, membrane dissection with prophylactic placement of encircling band, and photodynamic therapy [1]. The current case demonstrates that a single intravitreal bevacizumab injection may be associated with rapid regression of posterior capsule neovascularization. Recently, one case report of posterior capsule neovascularization regressed by intravitreal bevacizumab injection was reported [3] and the published results are in full accordance with ours. In conclusion, intravitreal injection of bevacizumab followed by Nd:YAG capsulotomy seemed to be a beneficial treatment modality in our particular patient in whom Nd:YAG capsulotomy alone cannot be done due to the risk of bleeding.

References

1. Ayata A, Unal M, Ersanli D, Gulecek O, Sonmez M. Photodynamic therapy for posterior capsule neovascularization. J Cataract Refract Surg 2007; 33: 1131-2.
2. Avery RL, Pieramici DJ, Rabena MD, et al. Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Ophthalmology 2006; 113: 363-72.
3. Eren E, Kucukerdonmez O, Yilmaz G, Akova YA. Regression of neovascular posterior capsule vessels by intravitreal bevacizumab. J Cataract Refract Surg 2007; 33: 1113-5.

Prevalence of Social Phobia in Psychiatric Hospital Outpatient Clinics

Yasser Raya, Abdul Hameed Al- Yahya and Ashraf El-Tantawy

Introduction Social phobia is an anxiety disorder (also known as social anxiety disorder), characterized by the fear and/or avoidance of situations where an individual is subjected to the scrutiny of others. Social phobia is a prevalent disorder among

psychiatric outpatient clinic patients and their first degree relatives. Prior to the last decade, little attention has been specifically paid to social phobia in clinical, therapeutic and epidemiological studies.

This study aimed to: a) determine the prevalence of social phobia in psychiatric hospital outpatient clinics, and among first degree relatives of patients diagnosed with social phobia; b) examine the range of disability and the reduced quality of life associated with social phobia; and c) examine functioning across the subtypes of social phobia (the generalized and specific subtypes).

Methods This is a cross-sectional study of social phobia probands, their first degree relatives and control group (age ≥ 18 years). All participants gave informed consent before they were enrolled in the study. Forty three adult social phobia patients (Probands Group) out of 764 attendees of outpatient clinic of a major mental health hospital in Al-Qassim, and 114 persons of their first-degree relatives (Relatives Group) were enrolled in the study. Thirty comparison subjects (Control Group), who did not have social phobia, were also selected. Socio-demographic variables and additional information were obtained from medical records or through an additional interview. Probands, their first degree relatives and control group diagnosed as having psychosis, mental retardation or dementia were excluded. Semi Structured Psychiatric Interview was introduced to all patients. International Classification of Mental and Behavioral Disorders (ICD-10) was used for assigning a diagnosis. Physical disorders were assessed with a self report (yes or no checklist). Social Phobia Inventory (SPIN) was used to evaluate fear, avoidance and physiological discomfort. The Eysneck Personality Questionnaire Revised (EPQ-R) and the Beck Depression Inventory II (BDI-II) were also applied. Measurement of quality of life was carried out using the SF-36 questionnaire and the measurement of disability was carried out using the World Health Organization Disability Assessment Schedule (version 2.0) (WHO/ DAS II). Data analysis was conducted in SPSS version 13.0 (SPSS Inc., 2002).

All patients attended the outpatient clinic of Buraydah Mental Health Hospital, Al-Qassim, during May, 2008 and their 1st degree relatives were consented to participate in the study and screened by Social Phobia Inventory to determine the positive patients. Then were assessed for socio-demographic variables, inclusion and exclusion criteria. Patients with social phobia, their 1st degree relatives and the control group were screened for social phobia, depression and avoidant personality disorders using the tools described above.

Results Out of 764 patients attending the outpatient clinic, 43 (5.6%) were diagnosed to have Social Phobia by using SPIN and confirmed by ICD-10 criteria. Among their 1st degree relatives (n= 114) who completed the study, we found 15 (13.2%) had SP. The total patients who had Social Phobia among the studied groups were 58 (6.6%).

Among the Propands, 60.5%, among the Social Phobia patients 69.0% were female, compared to 36.7% in the control group. Unemployment was significantly higher among Social Phobia patients than the control group (41.4% vs. 20.0%).

The most common symptoms regarding the performance situation were speaking in public (22.4%), followed by taking part in a meeting or class (20.7%) and eating or drinking in the presence of others (5.2%). The commonest symptoms regarding interactional situation were talking to people because might sound foolish (20.7%); dealing with authority figures (17.2%); and returning items to a store (5.2%). The generalized subtype of Social Phobia was more common than specific subtype (96.5% vs. 3.4%). The avoidant personality disorder was significantly higher among Probands group (9.3%) and Relatives group (16.7%) than the control group (3.3%). The depressive disorders were a common co-morbidity with Social Phobia; their prevalence was significantly higher among Social Phobia patients (17.2%) than control group (6.7%). Moderate and severe depression, were also significantly higher among Social Phobia patients, compared to the Control group (6.9% vs. 3.45%). The quality of life was significantly lower among

Social Phobia patients than in the Control group, as follows: mental component summary (17.1 % vs. 8.7%); physical component summary (37.2 %vs. 12.4%); and total score (54.3 %vs. 21.1 %). It was also found that 44.8% of Social Phobia patients had a degree of disability (compared with 3.3% in the Control group). Most of the functionally disable patients had mild (50.0%) to moderate (42.3%) degree disability, while 7.7% of those identified with functional disability were classified as having severe disability. Finally, we found that high scores in SPIN and BDI-II were correlated with high score of WHO/DAS II and low score of SF-36 with higher correlation with BDI-II score than SPIN score.

Discussion This study confirms previous reports of the high prevalence of Social Phobia and also demonstrates disorder interferes substantially with meaningful aspects of patients' lives. We found that most patients were of generalized subtype (96.5%), a result that is in agreement with many previous studies, particularly among Saudi patients. We found that avoidant personality disorder was confined to the relatives of probands with social phobia. In all cases (both in probands and in relatives), avoidant personality disorder occurred. This observation, rather than reflecting true co-morbidity per se, most likely is due to the substantially overlapping criteria sets for the two disorders. In recent years we have come to recognize that social phobia is highly co-morbid with other conditions such as depression. Social phobia carried with it a large, independent burden of illness. This finding is consistent with the observation from clinical studies that social phobia is a serious illness. We conclude that social phobia is a common mental health problem in its generalized subtype. Depression and avoidant personality disorders may complicate treatment and increase the patients' functional disability and decrease their quality of life. High neuroticism and low extroversion are common in patients and relatives of individuals with SP. Early detection, prevention of consequences and early intervention may decrease the burden of illness.

Disability due to mental disorders and its relationship to severity of illness and quality of life

Abdul Hameed Al-Yahya, Yasser Raya and Ashraf El-Tantawy

Introduction Disability associated with mental illness is a major contributor to the global burden of disease. The present study looks at some aspects of disability and quality of life attributable to various six common mental disorders: dementia, schizophrenia, major depression, bipolar affective disorder, generalized anxiety disorder and obsessive-compulsive disorder.

The objectives of this study were to evaluate disability in dementia, schizophrenia, major depressive disorder, bipolar affective disorder, generalized anxiety disorder and obsessive compulsive disorder, and to compare the degree of disability with the severity of illness and quality of life among various disorders.

Methods Adult patients (≥ 18 years) using services of a major mental health hospital in Al Qassim, between January-June 2008, who were diagnosed as per ICD-10 criteria of mental and behavioral disorders, were included in the study. Patients with mental retardation, chronic debilitating illness, substance abuse disorder, psychiatric co-morbidity and organic brain disease other than dementia were excluded.

The tools used in this study included Mini Mental State Examination (MMSE) for dementia, Positive and Negative Symptoms Scale (PANSS) for schizophrenia, Hamilton's Rating Scale (HAM) for depression and anxiety, Young Mania Rating Scale (YMRS) and HAM for bipolar affective disorders (BAD) and Yale Brown Obsessive Scale (Y-BOCS) for obsessive compulsive disorder. The measurement of quality of life was carried out using SF-36 questionnaire. Disability was measured using the World Health Organization Disability Assessment Schedule (version 2.0) (WHO/ DAS II). Data were analyzed using the Statistical Package for the Social

Sciences (SPSS, 2002). Patients were counseled to be a part of the study and written consent was obtained from them or their responsible persons.

Results Out of a total of 196 patients, 109 were male and 87 female. These included 24 patients (12.2%) with diagnosis of dementia; 52 (26.5%) with schizophrenia; 32 (16.3%) with major depressive disorder (MDD); 18 (9.2%) with bipolar affective disorder; 43 (21.9%) with generalized anxiety disorder (GAD); and 27 (13.8%) with obsessive compulsive disorder (OCD). A majority of patients in all diagnostic groups except GAD were male. Patients suffering from dementia or schizophrenia had extreme disability as per the WHO/DAS II (70.8% and 51.9%), followed by BAD (16.3%) and MDD (9.2%). GAD and OCD patients had no proportion of patients with extreme disability. Comparison of disability in various disorders indicates that dementia is the most disabling of the mental disorders, followed by schizophrenia. MDD and BAD disorders are the next two disability causing disorders in order of their degree of disability caused. GAD and OCD disorders cause the least amount of disability. Dementia, schizophrenia, MDD and BAD showed low quality of life especially in the mental component summary of the SF-36 but GAD and OCD showed better quality of life. Correlation tests showed a correlation between disorder disability as measured by WHO/DAS II and the quality of life as measured by SF-36 for each disorder. Correlation between illness severity, disability and quality of life are as follows: a) dementia: relation of MMSE scores to cognitive deterioration is negative. The same is reflected in the correlation tests between MMSE scores and WHO/DAS II as well as between MMSE scores and SF-36. The correlation appeared strong on the WHO/DAS II and with The SF-36; b) schizophrenia: strong positive correlation with WHO/DAS II on all three components of the PANSS scale was shown, and similar results were obtained on SF-36; c) major depressive disorder (MDD): highly significant positive correlation with WHO/DAS II and between WHO/DAS II and the SF-36; d) bipolar disorder (BAD): severity of BAD and disability measurement tools revealed a positive correlation on the WHO/DAS II as well as the SF-36; e) generalized anxiety disorder (GAD): correlation tests showed a positive correlation between the severity of anxiety as measured on the Hamilton anxiety rating scale and both WHO/DAS II and the SF-36. This correlation was more significant in WHO/DAS II than in SF-36; f) obsessive-compulsive disorder (OCD): severity of illness measured with Y-BOCS has a significant positive correlation with disability but compulsions tend to have a weak negative correlation with disability scores; the correlation between disability and total Y-BOCS score, though positive, was not statistically significant.

Discussion Our findings indicate that significant amount of disability occur in a varying proportion of patients in all the disorders under study. The degree of disability tends to correlate with the severity of the disorder as reflected in the rating scales and with the quality of life. This is a replication of studies done earlier. Dementia and schizophrenia were the most causes of disability. This finding is in agreement with Chaudhury, *et al*., 2006, who found schizophrenia and dementia are the two major disability causing disorders among their seven studied psychiatric disorders. Both BAD and MDD have been implicated in causing disability in several studies. Recent population study found that MDD and BAD to be the two major disability-related disorders. Disability by the WHO/DAS II scale in patients with GAD and OCD; were 51.2% and 59.3% respectively, with only 4.7% of GAD has severe disability. Both disorders are affecting all areas of dysfunction except self care and life activities and they do not need to be admitted in the hospital. Previous studies, were similarly able to show the disabling propensity of OCD. Unlike Olfson *et al*., (1997) study which failed to correlate GAD with disability, Kessler *et al*., (1999), had demonstrated that GAD is independently associated with greater dysfunction. The findings from other studies of a strong independent association between mental disorders and disability and decreased quality of life was replicated in our study.

Obstetrical and fetal outcomes of a new management strategy in patients with intra-hepatic cholestasis of pregnancy

Hossam O. Hamed, Hani A. Al Shobaili, Ahmad Al Robaee, Abdullateef A. Alzolibani, Ahmad F. Aminand Salah Roshdy

Introduction The adverse maternal, obstetrical, and fetal effects encountered in ICP are varied. A policy of early induction of labor at 36-37 weeks gestation has been adopted by many investigators to avoid risk of IUFD. However, there has been a marked debate regarding the gestational week at which IUFD occurs. On the other hand, there are some concerns whether this clinical practice itself could be the cause of increased perinatal mortality as elective delivery at that age carries the risks of prematurity including RDS. We hypothesize that allowing pregnancy to continue in ICP patients until expected date of delivery (EDD) or spontaneous onset of labor could be associated with lower risks of iatrogenic preterm labor and respiratory distress syndrome without an additional increase in risk of IUFD.

The objective of this study was to determine the incidence, obstetrical and fetal complication rates of intra-hepatic cholestasis of pregnancy (ICP) in patients managed expectantly to 40 weeks gestation.

Methods This was a prospective cohort study conducted between February 2008 and January 2010 in Qassim Region of Saudi Arabia and including a total of 21,960 pregnant women, who were screened for ICP using specific criteria for diagnosis. The course of pregnancy was monitored to 40 weeks gestation or spontaneous onset of labor, whichever came first. The measured outcomes were compared with a cross-matched group of healthy pregnant women. Continuous variables were analyzed using the t-test, while χ^2-test was used for comparing percentages of categorical variables.

Results The incidence of ICP was 0.35% among screened patients (76 out of 21,960). There was no significant difference between groups in gestational age at delivery, preterm labor, intrauterine fetal death, cesarean section, or respiratory distress syndrome. There were higher intrapartum non-reassuring fetal heart rate patterns and meconium stained amniotic fluid in the ICP group ($P < 0.01$, and $P < 0.0001$, respectively).

Table 1 Obstetrical and fetal outcomes in both groups

	ICP-group (n=76)	*Controls (n=200)*	*P-value*
Gestational age at delivery (mean ± SD)	36.63±2.57	37.24±1.9	0.21
Preterm birth:	6 (7.8)	13 (6.5)	0.68
Spontaneous preterm labor	3 (4.0)	5 (2.5)	0.29
Iatrogenic preterm labor	9 (11.8)	18 (9.0)	0.42
Intrauterine fetal death	1 (1.3)	2 (1.0)	0.82
Meconium stained amniotic fluid	18 (23.6)	5 (2.5)	0.0001
Non reassuring intrapartum CTG.	8 (10.5)	6 (3.0)	0.01
Cesarean Section:	6 (7.8)	12 (6.0)	0.65
Elective	9 (11.8)	17 (8.5)	0.31
Emergency	15 (19.7)	29 (14.5)	0.15
Postpartum hemorrhage	4 (5.2)	9 (4.5)	0.41
Neonatal outcome			
5 min-Apgar score median, range	9 (6-10)	10 (8-10)	0.37
Pediatric care unit admission	12 (15.7)	10 (4.9)	0.001
Respiratory distress syndrome	2 (2.6)	3 (1.5)	0.28

Table 2 Maternal biochemical assays and umbilical artery Doppler sonogram in both groups (mean, SD and range)

	ICP- group (N=76)	*Controls (N=200)*	*P-value*
Total bile acid (μmol/L)	28.95±3.93 [19.2- 36.3]	6.51± 0.8 [1.2- 9.6]	0.001
ALT (IU/L)	182.21±22.49 [134 -229]	49.35± 18.36 [18 – 76]	0.001
Total serum bilirubin (μmol/L)	10.5±2.19 [7.4 -14.8]	4.77±1.22 [2.4 -7.8]	0.05
Doppler indices			
S/D ratio	2.43±0.41 [1.88–3.72]	2.40±0.43 [1.58–3.78]	0.43
Pulsatility index	0.92±0.16 [0.66–1.27]	0.91±0.19 [0.51–1.32]	0.79

Discussion The incidence of ICP in Qassim region of Saudi Arabia is low compared to worldwide figures. The expectant management of ICP to 40 weeks is associated with comparable pregnancy course to normal women provided good fetal monitoring, and moderate rise of maternal serum bile acids. Careful intrapartum fetal monitoring is necessary to avoid more likely birth asphyxia. Large randomized clinical trials to compare both early delivery and expectant management lines are highly recommended to establish an effective management strategy to

reduce rates of adverse obstetrical and fetal outcomes.

Comparison between Mannitol 20% and hypertonic saline 7.5% for cerebral resuscitation in severely head injured patients with intra-cranial hypertension

MH Alsharkasy, AH Altouny, HA Elshatoury and HA Ewila

Introduction Elevated intracranial pressure (ICP) represents the most important cause of morbidity and mortality in patients suffering from severe traumatic head injury. Early management of the elevated intracranial pressure is advantageous in preventing secondary brain insults and improving outcome. Infusion of hyperosmolar solutes is one of the modalities currently used for management of intracranial hypertension after severe head injury. Mannitol 20% is considered as the reference solute, but it has limitations and may fail to decrease critically elevated intracranial pressure. Hypertonic saline solutions have received renewed attention in clinical practice as osmotic agent for cerebral resuscitation. It may be more beneficial than other osmotic diuretics because they augment intravascular volume and cardiovascular performance in addition to reducing intracranial tension and improving cardiovascular elastance.

This study compares the effect of 20% isovolume of mannitol with 7.5% hypertonic saline on intracranial pressure (ICP), hemodynamics and cardiac performance in patients with severe head injury and high intracranial pressure.

Methods Fifty-six patients admitted to intensive care unit fulfilling the inclusion criteria were allocated randomly into two groups. Group (A), 28 patients, received a bolus dose infusion of 2 ml/kg mannitol 20% within 10 minutes and group (B), 28 patients, received a bolus dose infusion of 2ml/kg hypertonic saline 7.5% within 10 minutes. Extradural catheter tip pressure transducer was inserted in all patients. Central venous catheter and urinary catheter were also inserted. Continuous monitoring of ICP was done for all patients and data of first 12 hours was recorded. Measurement of hemodynamic variables (HR, BP, and CVP) and cardiac output variables (CI, SVI, and EF) were assessed through transthoracic electrical bioimpedance using BOMED NNCCOM3 cardiodynamic apparatus at different time intervals after infusion of the tested solution. Urine output and blood electrolytes were also evaluated. Number of additional doses of the tested solution was recorded.

Results Both groups were similar regarding sex, age, and GCS at admission. There was significant decrease in ICP after 30 minutes and one hour after infusion in both groups (from 21±2.5 to 9±2.25 and 8±1 mmHg in HTS group and from 21±1.8 to 8±1.75 and 9±1.05 mmHg in mannitol group). Furthermore, in HTS group, there was significant increase in cardiac index (CI), stroke volume index (SVI), and ejection fraction (EF). There was also mild, but insignificant, increase in mean arterial pressure and central venous pressure (CVP). Heart rate showed non-significant decrease. On the other hand, in mannitol group, there was a significant decrease in CVP, while all other cardiac and hemodynamic variables showed no significant changes.

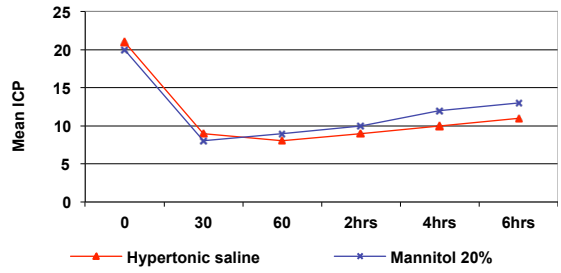


Figure 1 Changes in Intracranial pressure (ICP) at different time intervals among study groups.

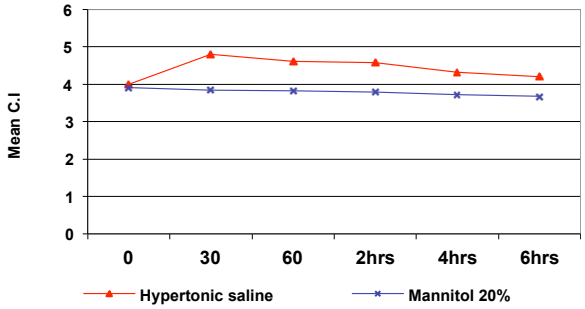


Figure 2 Changes in cardiac index (CI) at different time intervals among study groups.

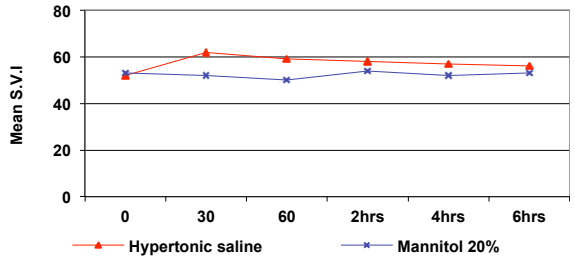


Figure 3 Changes in Stroke volume index (SVI) at different time intervals among study groups.

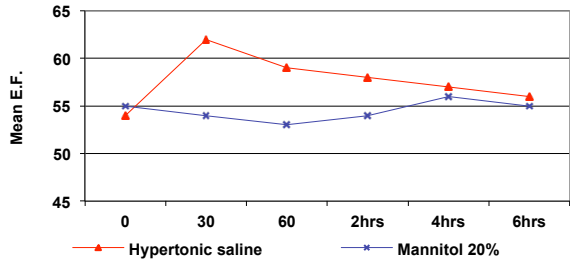


Figure 4 Changes in Ejection Fraction (EF %) at different time intervals among study groups.

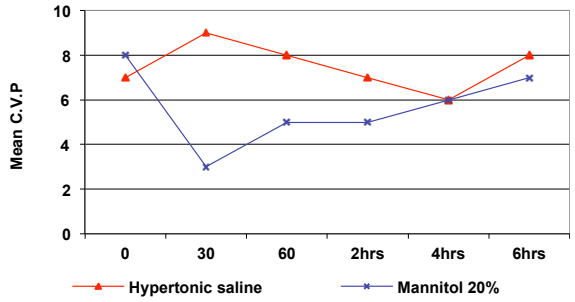


Figure 5 Changes in central venous pressure (CVP) at different time intervals among study groups.

Discussion HTS 7.5% is effective in reducing ICP to the same extent when compared to mannitol but for longer duration of action. Furthermore, HTS 7.5% has the advantages of maintaining hemodynamics and improving cardiac performance. So we recommend that HTS 7.5% may take place of mannitol in early management of severe head trauma patients with increased intracranial pressure especially when hemodynamic instability is present.

References

1. Hung Sheng J, Rozet, I, Tontisirin Nuj, Muangman S, Vavilala M: Effect of Equiosmolar Solutions of Mannitol versus Hypertonic Saline on Intraoperative Brain Relaxation and Electrolyte Balance. Anesthesiology November 2006. 107(5):697-704
2. Hayden W, David C, Bala V: The Use of Hypertonic Saline for Treating Intracranial Hypertension after Traumatic Brain Injury. Anesth Analg 2006; 102:1836 –46.
3. Klosen P JA, Nielson JO, Tonnesen E.: Acid base and electrolyte changes after hypertonic saline (7.5%) infusion. Scand J Clin Lab Invest. 2005; 65(1):13-22.
4. Claire Battison, BA Hons; Peter J. D. Andrews MD: Randomized, controlled trial on the effect of a 20% mannitol solution and a 7.5% saline / 6% dextran solution on increased intracranial pressure after brain injury Crit Care Med 2005; 33:196 –202.

Complete rectal prolapse in adults: clinical and functional results of Delrome procedure combined with postanal repair

Nabil Hamrah, Ayman AlGadaa and Yahyia AlAshry

Introduction Increased awareness of the functional abnormalities associated with rectal prolapse has resulted in the realization that appropriate surgery should not be directed only at a reduction of the prolapse. Faecal incontinence occurs in about 70% of cases, difficulty with evacuation of the rectum in 50% and constipation in up to 28%**.** The ideal surgical technique should therefore be based not only on the elements of simplicity, recurrence and complications but should also take into account the treatment or at least the alleviation of the functional abnormalities so commonly associated with rectal prolapse**.**

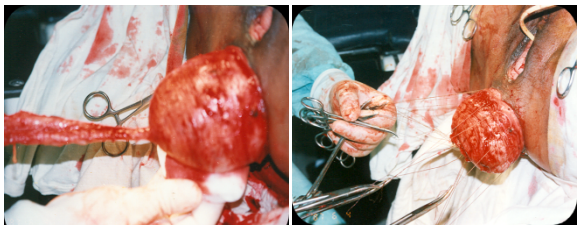
The objective of this study is to develop techniques to correct anatomical abnormalities and improve the functional outcome of Delorme's procedure by addition of postanal repair.

Methods This study was performed at the Department of General Surgery, Zagazig University Hospital (Egypt) and King Saud Hospital, Onayzah (Saudi Arabia) on 20 adult patients (6 males and 14 females with a mean age of 60 years) with complete rectal prolapse for which double perineal surgical attack, in the form of Delorme's procedure and postanal repair, was done. All patients presented with complete rectal prolapse at least 5 cm in length. The associated disorders included constipation (4 patients, 20%), variable degrees of incontinence (15 patients, 75%). Only one patient had no associated

functional problems. The median follow up period was 65 months.

Results Postoperative pain was easily controlled in 12 patients by injection of xylocaine 2% in the epidural catheter (2 cc diluted in 10 cc saline given 8 hourly for 3 doses). In the other 8 patients, pain was controlled by intramuscular injections of diclofenac 75 mg 8 hourly. The median follow up period for patients was 65 months (range: 10-120 months). Only two patient developed recurrence of rectal prolapse. Four patients developed complications during immediate postoperative period: three of them developed postoperative bleeding and were treated successfully by an anal pack; the fourth patient developed rapid elevation of blood pressure (240/140 mmHg) and was controlled medically in the ICU. Perineal wound infection and disruption of skin only occurred in 2 patients, one male and one female and healed by granulation in three weeks. As regards the bowel habit, the 4 patients (3 males and 1 female) who were complaining of constipation showed postoperative improvement. The degree of anal incontinence improved postoperatively in 11 patients (11/15) (73.3%). One patient remained incontinent to solid stool, one to liquid and flatus and two patients to flatus.

Discussion The complication rate found in this study (20%) (4/20) was higher than that of Senapati et al. (6%) and Tobinand Scott (8%). The addition of postanal repair may be the cause of this higher rate of complication. Although, Oliver et al. reported a complication rate of 25% after performing Delorme's operation alone on 40 patients with complete rectal prolapse. Lieberth et al., in their study of 76 patients, reported a recurrence rate of 14.5%, which is near to our results, while Fang et al. in their retrospective study reported a very low recurrence rate (6.6%). In this study, 2 patients developed recurrence of rectal prolapse (10%). This relatively low percentage of recurrence in our study may be due to the lower number of patients and the short-term follow up period. Plusa et al. reported 69.2% (9/13) improvement in incontinence after Delorme's operation alone. Other studies working on Delorme's operation alone reported a higher postoperative incontinence (Senapati et al.; Lechaux et al.). The addition of postanal repair to Delorme's operation in our study appears to result in a better improvement in incontinence. Pudendal neuropathy may be the cause of failure of postoperative improvement in the degree of faecal incontinence in 4 of patients. In this study, no patient described serious problems with evacuation after surgery, a result exactly similar to that of Plusa et al. who had 3 patients complaining of constipation before Delorme's operation and all had been improved after surgery. On the other hand, Senapati et al. reported postoperative constipation in 15% of their patients who underwent Delorme's operation alone.



The complete mucosal tube (Left). The initial plicating sutures being inserted (Right).

We conclude that addition of postanal repair to Delorme's procedure at the same time for repair of complete rectal prolapse in adults corrects anatomical defects and improves functional results. We recommend this method in the treatment of complete rectal prolapse especially in the elderly who are complaining of fecal incontinence.

References

1. Plusa SM; Charig JA; Balaji V; WattsA and Thompson MR (1995): "Physiological changes after Delorme's procedure for full-thickness rectal prolapse." Br. J. Surg., 82:1475 – 1478.
2. Senapati A; Nicholls RJ; Chir M et al. (1994): "Results of Delorme's procedure for rectal prolapse." Dis Colon Rectum, 37: 456.
3. Tobin SA and Scott IHK (1994): "Delorme's operation for rectal prolapse." Br. J. Surg., 81: 1681.
4. Oliver GC; Vachon D; EisenstatTE et al. (1994): "Delorme's procedure for complete rectal prolapse in severely debilitated patients: an analysis of 41 cases." Dis. Colon Rectum, 37: 461.
5. Lieberth M, Kondylis LA, Reilly JC and Kondylis PD (2009): "The Delorme repair for full-thickness rectal prolapse: a

retrospective review". American Journal of Surgery,197 (3): 418-423.
6. Fang CB, CandeláriaPde A, Klug WA, Capelhuchnik P. (2008): "Surgical treatment of rectal prolapse by the Delorme technique and rectopexy". Rev Assoc Med Bras., 54(2):142-5.
7. Liberman H, Hughes C, Dippolito A. (2000): Evaluation and outcome of the delorme procedure in the treatment of rectal outlet obstruction. Dis Colon Rectum., 43(2):188-92.
8. Lechaux JP; Lechaux D and Perez M (1995): "Results of Delorme's procedure for rectal prolapse: advantages of a modified technique." Dis. Colon Rectum, 38: 301.

Damage control orthopedic surgery (DOC): Is there an Influence on outcome?

Osama A. Amin, Kojok Mustafa and Hatem Hussein

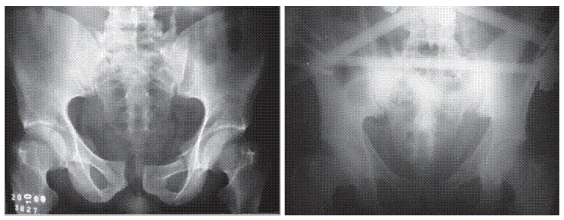
Introduction It has been convincingly demonstrated that the damage control strategy minimizes the second trauma of orthopedic surgery. This concept was developed by general surgeons about a decade ago for the management of abdominal injuries after blunt trauma. Since then, many authors have advocated limiting the extent of the initial surgery for blunt trauma and performing intensive care treatment after hemorrhage control has been achieved. Likewise, clinical studies have demonstrated an increased incidence of multiple organ failure (MOF) after an initial surgery of long duration. Meanwhile, the damage control strategy has been adopted by traumatologists to treat extremity fractures.

The aim of the study is to evaluate the concept of damage control by immediate external fracture fixation (DCO) and consecutive conversion osteosynthesis. Is it time savings and safe?

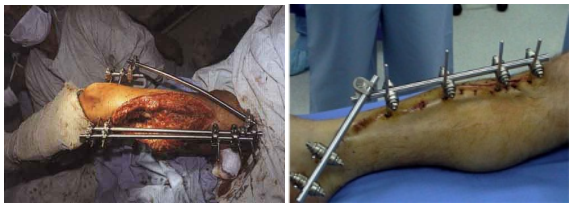
Methods Thirty-nine patients (30 males and 9 females), aged 3-82 years, (with a mean of 38.1±19.9 years) with orthopaedic-type severe poly-trauma were managed by damage control orthopedics from January 2008 to October 2009 at KFSK and BCH. Thirty one out of the 39 patients were sent to the intensive care unit (ICU). The ISS7 ranged from 17 to 57, with an average of 31.9. The control group consisted of 54 patients (47 males and 7 females) with multiple injuries with a mean age of 42.9 ± 16.9 years and an ISS of 30.2 ± 6.3). Damage control orthopedic surgery included: a) Immediate life-saving surgery,, which was required in 17 cases; b) Surgery for controlling heavy bleeding, which was required in 11 cases; c) Wound bleeding control, infection control, washing, dressing, and temporally closing the wound; and d) Provisional minimally-invasive external fixation, which was performed in 39 patients.

Results The treatment group from patients with multiple injuries with additional orthopedic injuries consisted of 39 patients with a mean age of 38.1±19.9 years and an ISS of 31.9 ±9.2). All these latter patients were exclusively treated according to DCO. None of the patients underwent ETC. Major accompanying injuries (Abbreviated Injury Scale Score [3]) were thoracic trauma, brain injury, and abdominal trauma in 79.5%, 51.3%, and 25.6%, respectively. The control group consisted of 54 patients with multiple injuries with a mean age of 42.9 ± 16.9 years and an ISS of 30.2 ± 6.3. The overall (ISS) showed a comparable level between both groups if one considers the Abbreviated Injury Scale score for extremity trauma to be responsible for the difference between 31.9 in DCO and 30.2 in control group patients. DCO patients had an even higher incidence of severe trauma to the trunk. Immediate fracture fixation was required in 60 fractures. Fractures concerned the femur in 25 and the tibia in 20 cases. Unstable pelvic fractures were found in 13 and complex fractures of the upper extremities in 2 cases. Of all fractures, 72.9% were closed and 27.1% were open. DCO required a mean of 38.7±20.5 minutes (SEM, 3.3) per patient including the time needed for soft-tissue management in the 27.1% that were open fractures. Operation time differed for closed fractures (average, 29.2 minutes; range, 15–55 minutes) and open fractures (average, 48.7 minutes; range, 25–125 minutes) because of soft-tissue management. Accordingly, blood loss averaged 232.1 mL (SEM, 16.7) for external fixation with soft-tissue management, whereas virtually no blood loss was observed for external fixation alone. Transfixation of neighboring joints was necessary in

40% of all fractures, comprising 13 unstable pelvic and 20 metaphyseal fractures. Three patients developed five complications caused by external fixation. Two of the patients developed pin track infection requiring local debridement. Five patients died before definitive treatment as a result of severe traumatic brain injury (n =2), multiple organ failure (n =1), pulmonary embolism (n =1), and myocardial infarction (n =1). The mortality rate was 12.8%.



Male patient 33 yrs old presenting Type B1 fracture (Left); and postop radiograph showing anatomic reduction (Right).



Open fracture femur Type IIIB treated by external fixator (Left); Open fracture tibia Type IIIA treated with external fixation and after fracture union (Right).

Discussion Damage control aims to eliminate three death-threats (metabolic acidosis, low body temperature and coagulation disorder) resulting from hemorrhagic shock, in which the traditional complicated resuscitation procedures are simplified and, thus are possibly performed timely before the patient falls into irreversible shock. It recommends that patients should stay in the ICU till their physiological states are able to withstand the definitive surgery. Most common complications associated with external fracture fixation are pin-track infections, deep infections, and non-unions. A low mortality rate of 12.8% indirectly hints an improved survival of the DCO patients versus controls. The study clearly demonstrates the enormous time savings and reduction of blood loss during initial treatment if patients with multiple injuries are treated according to DCO. We feel that DCO is a safe strategy in severely injured patients with multiple injuries who are too critically ill for ETC and for those patients who cannot be safely assigned to the clinical pathway of early total care.

Impact of Obesity on Fetomaternal Outcome in Pregnant Saudi Females

Meher-un-Nisa

Introduction Worldwide obesity is the most prevalent, chronic medical condition [1]. The rate of obesity in pregnant women is rising, increasing the significance of its impact on obesity-related pregnancy complications. Pregnancy complications associated with maternal obesity can be broadly grouped into those primarily affecting the mother and those primarily affecting the fetus, neonate or older child. Obesity influences not only the chance of conception but also reduces the response to fertility treatment, and increases the risk of miscarriage, congenital anomalies as well as pregnancy complications like gestational diabetes, pregnancy induced hypertension, cesarean delivery, macrosomia and infections in addition to potential adverse effects on long term health of both mother and infant.

The aim of this study is to determine frequency of obesity and its adverse effects on fetomaternal outcome in pregnant Saudi females.

Methods This is a prospective cohort study conducted over eight months (November 2008 to June 2009) in the Maternity and Children Hospital (MCH) Buraydah, Saudi Arabia and including 1000 pregnant Saudi patients admitted through OPD and emergency. The patients' height was recorded once and the weight was recorded twice, once at the beginning (roughly corresponding to the pre-pregnancy weight) and once at the end of pregnancy. The difference between the two weights was taken as net weight gain during pregnancy. Pre-pregnancy weight was used to calculate the body mass index (BMI) using Weight (Kg) / Height^2 (m). The sample was divided into 5 groups based upon the BMI: < 18.5, 18.5-24.9, 25-29.9, 30-39.9 and $\geq$40,

classified as underweight (lean), normal weight, overweight, obese and morbidly obese, respectively. The normal weight group was used as control group. Data were also collected regarding complications of obesity in pregnancy and labor. All data were analyzed using SPSS version 13.0 (SPSS Inc., 2006).

Results The frequency of weight distribution in the subjects was as follows: underweight 2%; normal weight 31%; overweight 33%; obese 30%; and morbidly obese 4%. Compared with normal weight women, both overweight and obese women had a significantly increased risk of gestational diabetes, preeclampsia, Cesarean delivery, and delivery of a macrocosmic infant ($P < 0.05$).

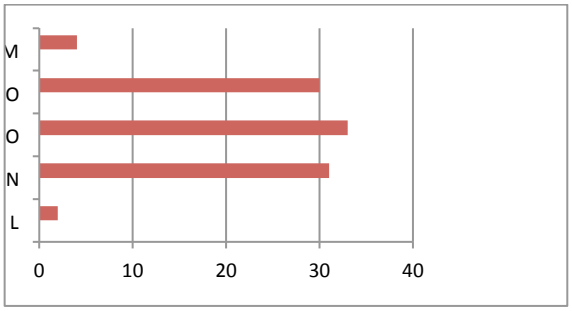


Figure 1 Percentage distribution of subjects by BMI category

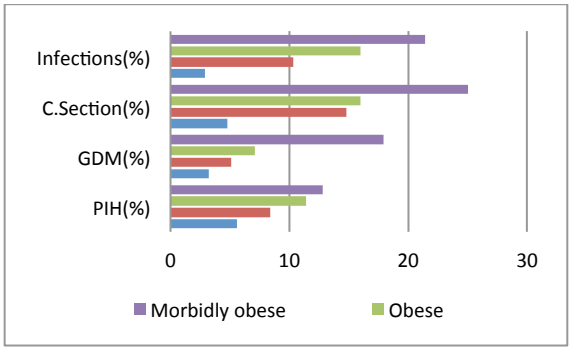


Figure 2 Incidence (%) of complications by BMI category

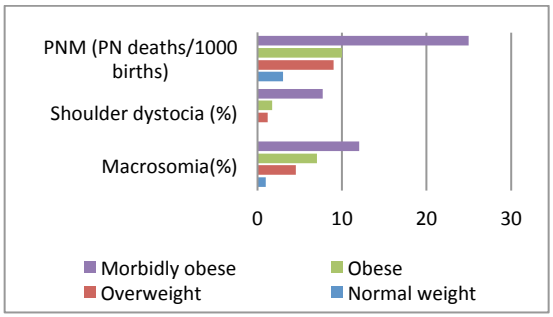


Figure 3 Perinatal mortalit (PNM) and incidence (%) of shoulder distocia and macrosomia by BMI category

Discussion Our study results are comparable to similar national and international studies. With regard to distribution of our subjects by BMI category, previous studies in Saudi Arabia have found similar results, e.g. Al Nuaim *et al,* in two separate studies [4, 5] reported 29.4% and 27.0% of Saudi females to be overweight, while Rasheed *et al* [6] in 1994 reported an obesity prevalence of 26.1% and morbid obesity in 4.5% of the Saudi females. We found that adverse pregnancy outcomes are associated with obesity. We conclude that obesity is a growing problem among pregnant Saudi women and that it is associated with an increased risk of fetomaternal complications like preeclampsia, gestational diabetes, cesarean delivery, and delivery of a macrocosmic infant.

References

1. Legato MJ. Gender-specific aspects of obesity. Int J Fertile Womens Med. 1997; 42:184–197.
2. Norman RJ, Clark AM. Obesity and reproductive disorders. Reprod Fertil Dev. 1998; 10:55–63.
3. World Health Organization. Obesity: preventing and managing a global epidemic. World Health Organ Tech Rep Ser 2000; 894:1–4.
4. Xx
5. Xx
6. Xx

Medical Education

Blended Learning in Orthopedics Course: An Evaluation Study

Osama A Amin and Mohammed Saqr

Introduction In Medical education the adoption of E-learning has not matched the pace with education technologies developed and there has been a growing debate whether e-learning is suitable for medicine or not. The problem is worse when it comes to clinical phase in colleges of medicine. Qassim College of Medicine has adopted e-learning

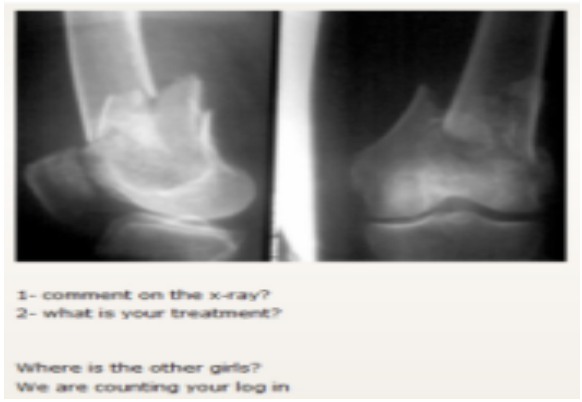
as an educational method two years ago where it is utilized in most curses taught in the college. The question was whether this has added to our education or not, and how our students received it.

This study aims at evaluation of different e-learning techniques in teaching a clinical course (Orthopedics) with traditional face-to-face lectures and bedside teaching using the blended approach.

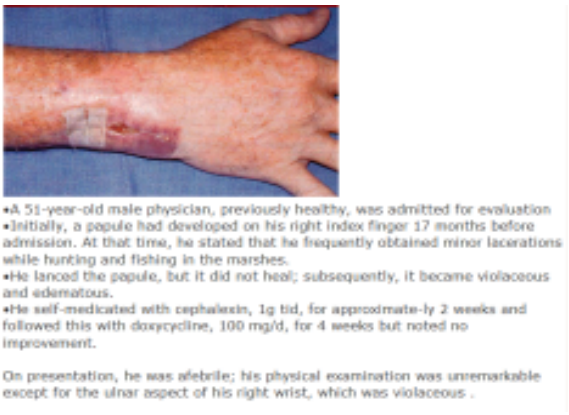
Methods The study was conducted among 4th year medical students (n=46), of whom 37 participated in the feedback. The course design included: course objectives, assessment methods and references; multimedia (X-rays, videos, patient images), enhanced case discussions with simulated patient scenarios (mediated by the supervisor and extended training beyond the limited classroom time); questions and answers discussions usually initiated by the instructor covering daily issues; downloadable lectures and materials; online multiple choice formative exams, which offered the students an opportunity to know their strengths and weaknesses; videos for selected orthopedic procedures; and E-portfolios containing evidence of student performance in clinical training, bedside learning and case presentations. The course served as a common place for all course updates, news and announcements. It included an electronic attendance sheet and a course evaluation survey.

Results Students reported that they had more contacts with their tutors and colleagues (75.7%), better possibility of self-assessment and feedback (83.8%), flexible learning (75.7%) and faster and easier information retrieval (83.8%). They reported that they could access information at any time (91.9%). They found the learning easier compared to conventional teaching (70.3%) and that E-learning helped them understand the surgery better (54.1%). Moreover, 18.9% of students rated the experience as excellent and 64.9% as good; 72.9% of them recommended that e-learning be used in every course in their program.

E-learning website: clinical discussions, enhanced by images of real patients, or X-ray questions



Course materials and students seminars available for download



Patient scenario based online discussions moderated by the instructor on the E-learning website

A clinical examination video available for students online.

Discussion The results from our survey indicate that the students' performance - although not reported due to problem of multiplicity of variables - and the tutor's experience markedly enhanced due to E-learning experience. E-learning is helpful to students in terms of better communication between peers and tutors, easier access to information and better chance for self-assessment throughout the course. Findings from our study are consistent with similar studies in other settings. It should be emphasized that the nature of course, the available resources, and the technical skills of tutors and students (in terms of use of computer and internet) play a significant role in the process. We conclude that implementation of blended learning in orthopedics helped students get easy access to information, better interact with tutors and improve their understanding of the subject.

Evaluation of mini-essay questions (MEQ) and multiple choice questions (MCQ) as a tool for assessing the cognitive skills of undergraduate students at the Department of Medicine

Moeen-uz-Zafar and Badr-Aljarallah

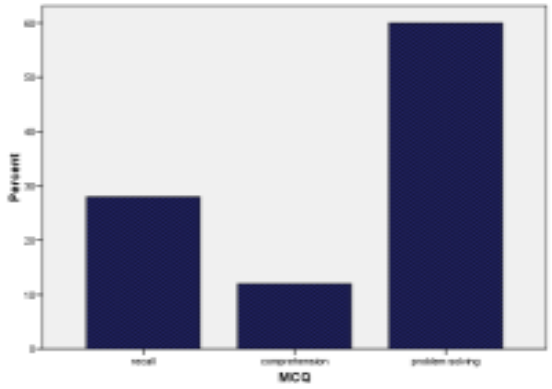
Introduction The evaluation of skills of undergraduate medical students is a very critical task in medical education [1]. There are three domains of skills that need to be evaluated (cognitive, affective and psychomotor). There are three levels of cognitive domain, Level I is recall, Level II is understanding and Level II is problem solving [2, 3], whereby the major emphasis is on developing and evaluating the problem solving skills [1-3]. MEQ and MCQ may test the problem solving skills. We evaluated both types of questions in testing the problem-solving skills of the students. The objectives of this study are to compare MCQ and MEQ in their ability to test different levels of the cognitive domain, and to detect item writing flaws in the construction of a question.

Methods This was a cross sectional study, whereby 50 MCQ and 50 MEQ were randomly chosen for evaluation. These questions were extracted from the final and mid-term examinations conducted during the period 2005-2009. Each question was analyzed separately by two assessors to give it a score according to modified Bloom's taxonomy as per set criteria. Each question was evaluated for any item writing flaw like: error in formatting, spelling etc.

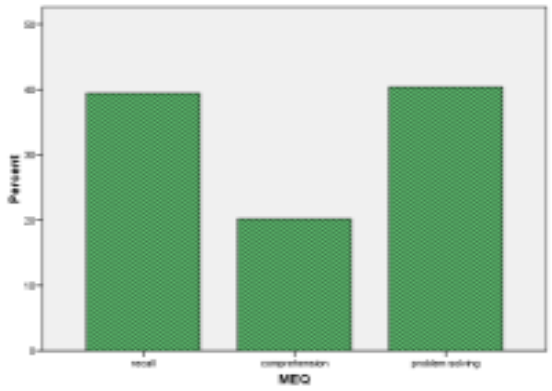
Results The MEQ included in the evaluation had 104 stems. Their distribution according to the levels was as follows: Level I: 41 (39.4%); Level II: 21 (20.2%); Level III: 42 (40.4%) (k=0.195). Among the selected questions, 34% were from Final exams and 66% were from mid-terms. Their distribution by specialty was as follows: Cardiology 20%; Hematology and Rheumatology 12% each; Pulmonology and Nephrology 8% each; and Infectious diseases 4%. The MCQs distribution according to levels was: Level I: 14 (28%); Level II: 6

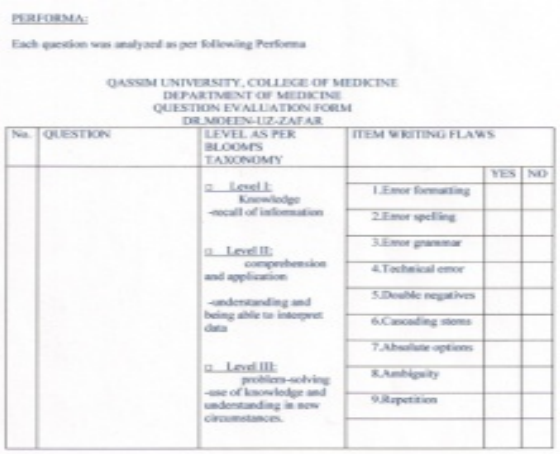
(12%); and Level III: 30 (60%) (k=0.606). Among the MCQs, 80% of the questions were from Final exams and 20% were from mid-terms. Their distribution according to specialty was: Cardiology 23%; Infectious disease and Pulmonology 17% each; Neurology 16%; Rheumatology 15%; Nephrology 8% and Gastroenterology 21%. 'Item writing flaws' were found in 16% of MEQs and 12% of MCQs.

% of MCQs addressing different levels of cognitive domain



% of MEQs addressing different levels of cognitive domain





PERFORMA:

Each question was analyzed as per following Performa

QASSIM UNIVERSITY, COLLEGE OF MEDICINE
DEPARTMENT OF MEDICINE
QUESTION EVALUATION FORM
DR.MOEEN-UZ-ZAFAR

No.	QUESTION	LEVEL AS PER BLOOM'S TAXONOMY	ITEM WRITING FLAWS		
				YES	NO
		□ Level I: Knowledge -recall of information	1.Error formatting		
			2.Error spelling		
		□ Level II: comprehension and application -understanding and being able to interpret data	3.Error grammar		
			4.Technical error		
			5.Double negatives		
			6.Cascading stems		
			7.Absolute options		
		□ Level III: problem-solving -use of knowledge and understanding in new circumstances.	8.Ambiguity		
			9.Repetition		

Discussion The result of this study show that MCQ is superior to MEQ as a test of problem solving skills as 60% of MCQ tested problem solving skills whereas only 40% MEQ addressed that level. The results comply with Edward JP *et al* [4], and conflict with those of Irwin WG *et al* [5]. The two confounders that played a major role in deciding the level of cognitive domain were type of examination (final or mid-term) and the specialty of the question. It is also inferred that constructing an MEQ might be technically more difficult than MCQ as item writing flaws in MEQ were 16% as compared to 12% in MCQs. We conclude that MCQ is superior to MEQ in testing Level II of cognitive domain i.e. problem solving. However, the teaching staff has to be well trained in formulating the questions, more so for MEQ to avoid item writing flaws.

References

1. Ebstein RM. Assessment in Medical educatiobn.N Engl J Med 2007;356:387-396.
2. Bloom BS(ed).Taxonomy of educational objectives. 1956 Longman group. UK.
3. Anderson LW, Krathwol DR (2001). A taxonomy for learning teaching and assessing. Longman: New york NY
4. Edward JP, Peter JD. Assessment of higher order cognitive skills .BMC Medical education 2007, 7:49:10
5. Irwin WG, Bamber JH. The cognitive structure of MEQ. Med educ. 1982 Nov: 16(6)

Community Medicine

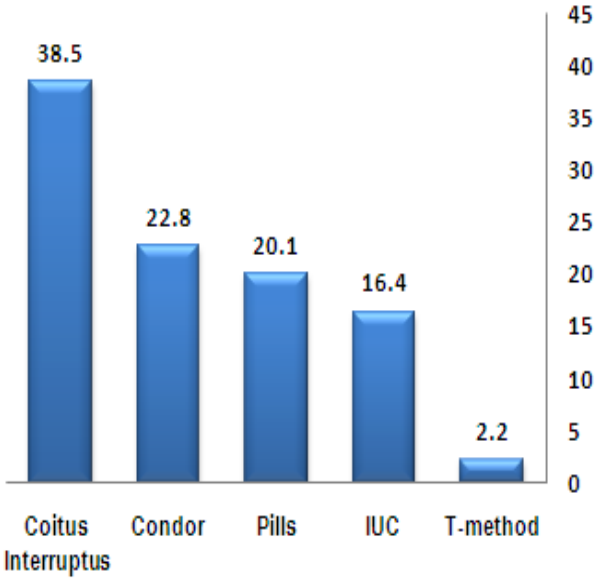
Family planning: knowledge, attitudes and practice among married couples

Faiza Shaukat, Zahid Naeem and Zubair Ahmed

Introduction World Health Organization (WHO) reports that an estimated 94% of world's population lives in countries with policies that favor family planning. In spite of these policies, however, a significant proportion of couples in the reproductive age do not use adequate measures of fertility regulation. Pakistan's current population is approximately 170 million, almost 2.3% of world's total population, making it the 6th most populous country of the world. Each year another 3.2 million children are added to this number. If this rate continues Pakistan's population would reach 222 million by the year 2020. This study was conducted to assess the knowledge, attitudes and practices of married couples regarding family planning in Baltistan, which is a remote rural area in the mountainous North of the Gilgit-Baltistan province of Pakistan.

Methods This was a cross-sectional survey, carried out among married couples with female partner of childbearing age. A study questionnaire was designed and pretested. Responses were obtained from 200 conveniently selected married couples (females: 152, males: 48), which reported to the government's primary health care facilities for any reason. Informed consent was obtained from all study participants.

Results A majority of study participants (67%) were of the view that rapidly increasing population is a problem affecting socioeconomic development of the country. However, there was a wide gap between knowledge and practice of family planning. The contraceptive prevalence rate (CPR) of Baltistan is 8.5%, which is much lower from the national average CPR of Pakistan. A little over half of the non-users of family planning considered it against the religion, while fear of side effects and inability to use a certain method was the reason for not using among 18% of non-users. Moreover, 10% of couples did not use family planning because of the opposition from family or spouse.



Percentage distribution of contraceptive users by currently used method.

Table: Reasons for not using family planning

	Reasons	Percent
1	Against religion	**51%**
2	Want more children	**21%**
3	Fear of side effects	**11%**
4	Methods inconvenience	**7%**
5	Wife/Husband opposes	**6%**
6	Family pressure	**4%**
	Total	**100%**

Discussion Findings of this study regarding knowledge of contraception methods are consisted with the findings from national and provincial

surveys in Pakistan. The knowledge of at least one contraceptive method in Pakistan has increased from 70% in 1990-91 to 85% in 2004. It is interesting to note that knowledge for coitus interrupts was found 36% and this method is also persistently reported to be well known in previous surveys. Attitude regarding age at marriage for boys and for girls is important and has impact on fertility. In a study carried out by National Institute of Population Studies (NIPS), a majority of the respondents were of the view that age for marriage for boys should be 20-24 years and for girls it should be 15-19 years. Early marriage of girls is very common in Pakistan and in this study, 36% respondents approved early marriage below 18 years.

We conclude that the important reasons for not using contraceptive measures were that family planning was considered against religion, beside illiteracy, poverty and poor communication. Efforts are required to provide better information about and access to modern family planning methods, particularly in the remote, rural areas of Pakistan.

References

1. Govt of Pakistan, District Census Report of Baltistan, Islamabad: Statistical Division, Population Census Organization, 2008
2. Government of Pakistan. Pakistan Economic Survey 2007-08 Islamabad Finance Division
3. Hakim Abdul, Demographic situation and its Socio Economic Implications on Pakistan, NIPS, Islamabad

Prevalence of Hearing Impairment in School Children

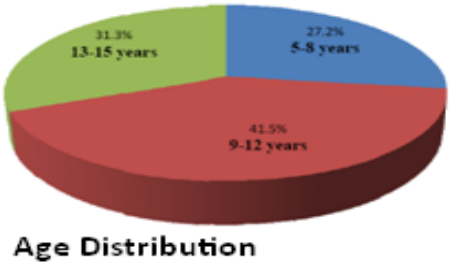
Tahir Hussain, Abdullah A. Alghasham and Muhammad Raza

Introduction Hearing impairment is a handicap which can be corrected therapeutically and can be helped with amplification. It is usually associated with a loss of communication [1] and in the young children it will also impair the normal development of speech and language [2]. The poverty of language in school children also affects reading skills and diminished functional status [3]. Early childhood hearing impairment (HI) may have a negative effect on educational outcome and employment in adulthood[4]. Persons with HI are likely to have lower family incomes, less educated and unemployed [5]. Early identification of HI followed by a timely and effective intervention is necessary to minimize its negative effects on the development of cognition, psychological and verbal communication skills [6].

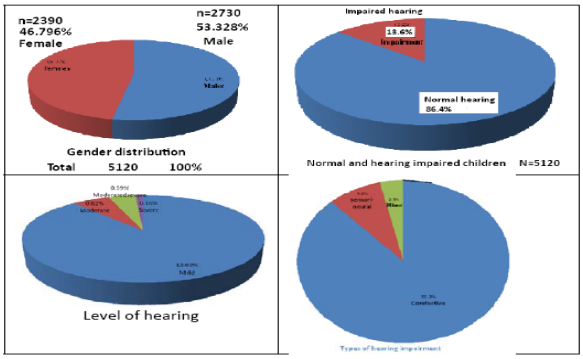
The main objective of this study was to determine the hearing level in Karachi's school children between ages 5-15 years, to determine the prevalence of HI and to identify its causes of among these children.

Methods A survey was conducted during 2008-2009 in 170 schools of Karachi. The prevalence of HI in Karachi's school children between the ages of 5-15 years of both genders and studying in normal schools was estimated. There are 0.629 million children registered in 2,988 schools in Karachi. From each school, 30 children from different classes were randomly selected with total of 5,120 children in the sample. Hearing assessment was carried out using standard procedure with a portable audiometer. The threshold of hearing was measured at 250, 500, 1000, 2000 and 4000Hz, respectively, and data was recorded. Children were labeled as hearing impaired if they did not respond to tone in the range of 25 dB and below, in any frequency tested as per WHO protocol [7]. Children with HI were subjected to otoscopy. Later on, parents of children having HI were interviewed regarding ear discharge, family history of HI, drug history, noise exposure, and parent's consanguinity.

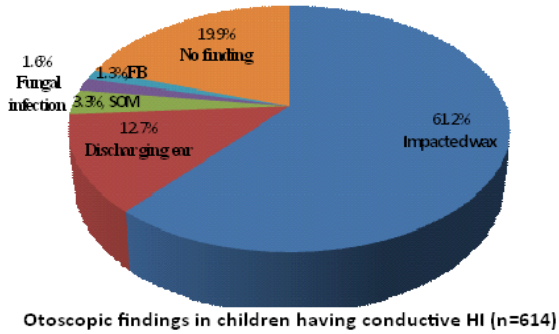
Results Among our sample of 5,120 children, 2,730 (53.3%) were male and 2,390 (46.8%) were female (male to female ratio 1.4:1). Most of the children (n=4,424; 86.4%) were found to have normal hearing i.e. < 25 dB, and 696 (13.6%) had some degree of hearing loss i.e. hearing threshold > 25 dB. There was no difference in prevalence of HI between males (14.1%) and females (13.0%). Among the 696 children who had HI, 616 (12.0%) had mild hearing loss (26-40 dB); 42 (0.8%) had moderate hearing loss (41-50) dB; 30 (0.6%) had moderately severe hearing loss (51-70 dB); and 8 (0.2%) children had severe hearing loss (71-90 dB). No child was profound deaf. Among the hearing impaired children most (88.2%) had conductive hearing loss, 58 (8.3%) had sensorineural hearing loss and 24 (3.5%) children had mixed type of hearing loss. A majority (61.2%) of children with conductive hearing loss had impacted wax in one or both ears; 78 (12.7%) children had unilateral or bilateral discharging ears suggestive of chronic suppurative otitis media. The less common findings were fungal infection (1.6%) and foreign body (1.3%). About 20% children had no positive finding on otoscopic examination.



Age Distribution



Percentage distribution of children by gender and level of hearing



Otoscopic findings in children having conductive HI (n=614)

Discussion In Karachi, HI in school children is 13.6%. Impacted wax was found to be the most common problem which can be very easily managed. The next common finding was history of ear discharge and it showed a very significant cause of conductive HI. We found that assessment of school children is useful to identify manageable HI. We recommend that ear hygiene among school children should be promoted. Similarly, ear infections should be promptly diagnosed and treated to avoid complications.

References

1. Davis A, Int-J-Pediatr. Otorhinolaryngol. 1999 Oct 5; 49 suppl1: S51-4.
2. Parving A. Int. J. Pediatr. Otorhinolaryngol. 1999 Oct 5; 49 suppl 1: S 287-92.
3. Keller BK. J. Am. Geriatr. Soc. 1999 Nov; 47 (11): 1319 – 25.
4. Huttunen KH, Sorri MJ. Scand Audiol Suppl. 2001; (52): 106-8.
5. Blanchfield BB, Feldman JJ, DunbarJL, Gardner EN. J Am Acad Audiol. 2001 Apr; 12(4): 183-9
6. Gopal R, Hugo SR, Louw B. Int. J Pediatr Otorhinolaryngol. 2001 Feb; 57(2): 99-113

7. WHO, The Prevalence of Ear and Hearing Disorders Protocol, WHO Geneva, 1999

Child labor in relation to poverty

Zahid Naeem, Faiza Shaukat and Zubair Ahmed

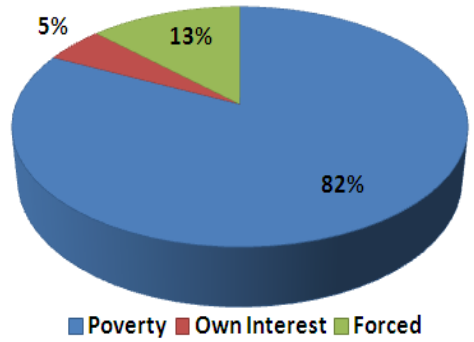
Introduction Child labor is recognized as the worst form of abuse and exploitation of children. The Convention on rights of child (CRC) urges the governments to take effective measures for its eradication. Child labor is pervasive problem in developing countries. Africa and Asia account for over 90% of total child employment. The International Labor Organization (ILO) estimates that number of working children are about 250 million in the developing countries, of whom at least 120 million are working full time. Of these working children 61% are in Asia, 32% in Africa and 7% in Latin America. In Pakistan about eight million children are working in different occupations and two third of them are employed full time. The fundamental rights of child as survival, education, protection and development are grossly violated by child labor. The root cause of child labor is extreme poverty which forces the parents to employ their children for some extra money for daily living. This study was designed to collect information on dimensions of working children and to establish relation between poverty and child labor in Pakistan.

Methods A cross-sectional study was conducted to correlate poverty and child labor. The study was conducted in the Malir district of Karachi, Pakistan, between April-July 2008. By systematic random sampling, 200 children between ages of 5 -15 years were selected from among the working children in the district. A structured questionnaire was designed, including questions about the child, his family, nature of work and workplace and socio economic conditions. Informed consent was obtained from parents of working children.

Results The mean age of children in the sample was 10.5 years; the male to female ratio was 5:1. Twenty four percent of children were collecting papers from streets and garbage dumps; 21% were working in auto workshops as helping hands, while at the same time learning some form of technical skills; 16% were street children doing car washing or selling newspapers and other items. Besides these, some children worked in homes as house servants, did shoe polishing or worked in small businesses in the neighborhood. A majority of girls worked either as house servants or picked paper and other stuff from garbage dumps. About 83% children belonged to poor families.

Table 1 Distribution of children by occupation

Type of Work	Number	Male	Female
Street Work	32	26	**6**
Auto workshops	42	42	**0**
Hotels	24	24	**0**
Domestic Work	28	16	**12**
Paper Collection	48	38	**10**
Shops/ Stores	20	16	**4**
Shoe Polishing	6	6	**0**
Total	200	168	**32**



Stated reasons for child labor

Discussion In this study, we found that children work mainly due to poverty. Poor parents are forced to send their children to

work instead of school. Poverty reduction is the key to reducing child labor. The government should provide some compensation to poor parents for sending their children to school.

References

1. Baig. L. Child labor a reality: results from a study of a squatter settlement of Karachi, JPMA 2002;52(11):507-10
2. International Labor Organization Working out of poverty, Report of the Director General to the 91st ILO Conference., Geneva

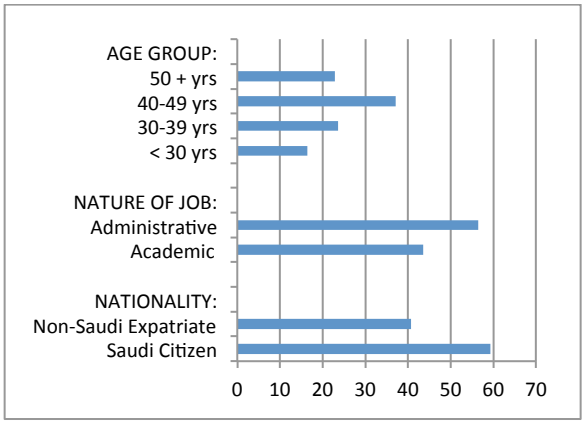
Prevalence of metabolic syndrome among Qassim University personnel in Saudi Arabia

Issam Barrimah, Abdul- Rahaman Al Muhaimeed, Hani Al Shobaily and Farid Midhet

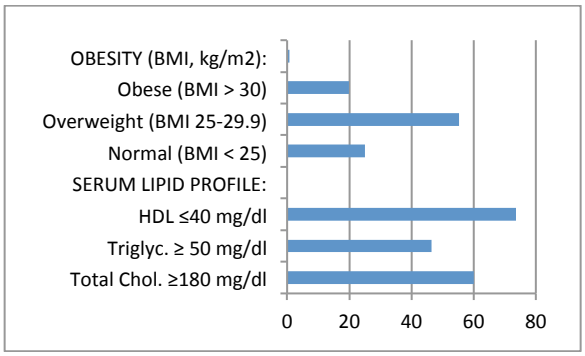
Introduction Metabolic syndrome represents the presence of a combination of interrelated risk factors including central obesity, insulin resistance, dyslipidemia and hypertension. Subjects with metabolic syndrome have substantially increased risk for developing type 2 diabetes mellitus and cardiovascular diseases (CVD). Also, the overall mortality is higher among patients with metabolic syndrome, particularly the mortality associated with CVD. This increase in CVD disease risk appears to be independent of other important and potentially confounding factors such as smoking and elevated low-density lipoprotein cholesterol (LDL-C) levels. The adverse effects of metabolic syndrome are manifested across the whole spectrum of blood glucose level status (i.e., patients having normal blood glucose levels, those having impaired fasting blood glucose and those with frank diabetes mellitus).

Methods A cross sectional study was conducted that included all male staff of different ages and careers in Qassim University. All staff members were invited to participate; however, 560 individuals participated in this study with a response rate of 85%. For all participants, the data were collected on socio-demographic characteristics, past history of or currently taking medication for diabetes or hypertension, smoking habits, physical activity, and the measurements necessary to identify metabolic syndrome.

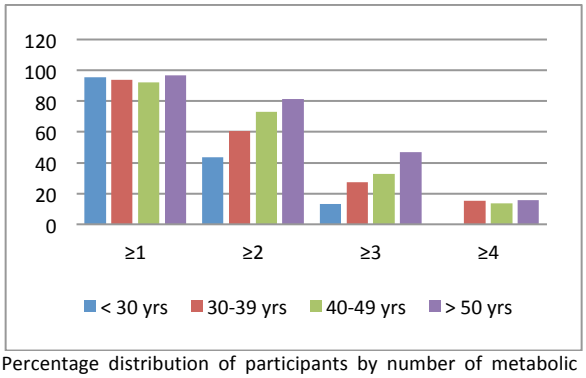
Results Prevalence of metabolic syndrome was 31.4%. The prevalence was found to show a steady increase with increasing age, BMI and serum cholesterol**.** General obesity measured by BMI was the most common component associated with the syndrome where 75% of participants suffered from overweight and obesity. Participants with high-density lipoprotein below protective level constituted 73.6%, while those with total cholesterol and triglyceride above clinically normal level constituted 60.0% & 46.4% respectively. Fasting plasma glucose and hypertension was the least common**.** After adjustment, factors found to be associated with metabolic syndrome were being a Saudi national, smoking, not doing regular exercise, being obese, having total serum cholesterol above 180 mg/dl, and age groups above 40 years.



Prevalence (%) of metabolic syndrome by socio-demographic characteristics



Clinical characteristics of study participants



Percentage distribution of participants by number of metabolic syndrome criteria and age-group

Discussion Almost a third of the university personnel have metabolic syndrome and therefore they are at higher risk for both cardiovascular disease and diabetes mellitus. The number of metabolic syndrome criteria, and, therefore, the risk of acquiring chronic diseases, increases with age. Similar studies are required among a wider range of subjects to assess the scope of the problem in Saudi Arabia.

References

1. Al-Nozha MM; Al-Khadra A; Arafah MR; Al- Maatouq MA; Khalil M; Khan NB; et al. Metabolic Syndrome in Saudi Arabia. SMJ 2005, vol. 26, no12, pp. 1918-1925
2. Al-Qahtani DA, Imtiaz ML: Prevalence of metabolic syndrome in Saudi adult soldiers. Saudi Med J Vol.26(9): 1360-1366
3. Ford ES, Giles WH, Dietz WH: Prevalence of metabolic syndrome among US adults: finding from the Third National Health and Nutrition Examination Survey. JAMA 287: 356-359, 2002