Cardiovascular risk factors in Kuwaiti adults with type 2 diabetes

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Type 2 diabetes is associated with a marked increased risk for cardiovascular disease (CVD) morbidity and mortality.[1] The United Kingdom Prospective Diabetes Study (UKPDS) identified several modifiable risk factors associated with CVD in patients with type 2 diabetes.[2] These factors were increased concentrations of low density lipoprotein cholesterol (LDL-C), decreased concentrations of high density lipoprotein cholesterol (HDL-C), raised blood pressure, hyperglycemia, and smoking. The American Heart Association (AHA) and the American Diabetes Association (ADA) have published guidelines for cardiovascular disease prevention.[3] The recommended targets for glycosylated hemoglobin (HbA1c) and blood pressure is <7% and <130/80 mm Hg. The optimal level for triglycerides and LDL-C is <1.7 mmol/l and <2.6 mmol/l. The optimal HDL-C levels for men and women are >1.1 mmol/l and >1.4 mmol/l. Cardiovascular disease is the first leading cause of death in Kuwait and diabetes is a major public health problem.[4] The aim of this study was to determine the prevalence of CVD risk factors in Kuwaiti adults with type 2 diabetes according to UKPDS factors and ADA targets.

All Kuwaiti adults (aged ≥20 years) who presented to the diabetes outpatient clinic at Al-Sabah Hospital, Kuwait between October 2000 and March 2005 were screened for CVD risk factors. The inclusion criteria were type 2 diabetes based on the treatment with diet alone or; treatment with diet and oral antihyperglycemic agent(s) or; treatment with diet and insulin provided that insulin was prescribed at least one year after diagnosis. Other data collected included age, gender, duration of diabetes, and body mass index (BMI). Hypertension was defined as history of hypertension or systolic and/or diastolic blood pressure on examination was ≥140/90. Subjects were classified as normal-weight if BMI =18.5–24.9, overweight if BMI=25-29.9, and obese if BMI≥30. Glycemic control was diagnosed as good, acceptable, or poor when HbA1c% was <7.0, 7.0-8.0, and >8.0. Glycosylated hemoglobin was performed using turbid metric inhibition immunoassay (Roche HB1c II kit, Indianapolis, USA). Serum cholesterol and triglycerides were measured by the enzymatic method. History of CVD was defined as history of angina with ECG changes, positive stress thallium scan or dobutamine echocardiogram, history of nonfatal myocardial infarction, coronary artery bypass grafting, percutaneous coronary intervention, or nonfatal stroke.

Data were analyzed using the SPSS program. Data are presented as means ± SD, or as numbers and percentages. The χ^2 test and ANOVA were used as appropriate. A p-value of <0.05 was considered to be statistically significant. Multiple linear regression analysis was performed to determine the independent effects of the studied variables.

During the study period, a total of 170 patients were screened (113 were women). Mean age was 49.6 ± 10.5 years and mean disease duration 8.0 ± 7.1 years. Mean BMI was 33.3 ± 7.5 kg/m^2 and mean HbA1c level 9.4 ± 2.4%. Mean systolic blood pressure and diastolic blood pressure was 135.8 ± 22.1 and 81.4 ±10.4 mm Hg. Mean level of LDL- and HDL-C was 3.7 ± 1.0 and 1.2 ± 0.3 mmol/l. The majority (59.3%) of patients were hypertensive, 26.3% were overweight, and 65% were obese. The majority (64.1%) of patients had poor glycemic control whereas 16.2% had good, and 19.8% had acceptable levels. Only 11.8% of the patients received lipid lowering therapy, and 45.7% were given antihypertensive medication. History of CVD was present in 10% of the patients. Hypertension was more prevalent among women compared to men (69.6% versus 38.2%, p=0.0001). The overall prevalence of history of smoking was 13.3%, which was solely prevalent among men (40%). The BMI, systolic and diastolic blood pressure, and HDL-C were significantly higher in women than men (35.3 versus 29.4; 140.4 versus 126.5 mm Hg; 83.4 versus 77.2 mm Hg; 1.3 versus 1.1 mmol/l, p=0.0001). The percentage of patients with levels of LDL-C, HDL-C, systolic and/or diastolic blood pressure, and HbA1c outside the target levels according to the studied variables are summarized in **Table 1**. Overall, 86.2% and 63.2% of the patients had LDL-C and HDL-C outside the target levels. In addition, 55.3% of the patients had levels of LDL and HDL-C combined out of the targets. There was no difference in the prevalence of elevated LDL-C by the studied variables. However, there was an increasing trend for lower HDL-C levels as age advanced. Elevated systolic or diastolic blood pressure and elevated HbA1c values occurred in 84.6% and 83.8% of the patients. Although elevated systolic and/or diastolic blood pressure occurred more often in women than men, BMI was the independent factor for elevated blood pressure. Male gender and those who were treated with sulfonylurea and/or insulin were the independent factors associated with elevated HbA1c. Although history of smoking was higher among those with lower BMI, regression analysis

revealed that male gender was the independent factor. Approximately 82% of the patients had multiple (≥3) cardiovascular risk factors, whereas only 2.7% of the patients had one CVD risk factor. Furthermore, 80% of patients with newly diagnosed diabetes had multiple CVD risk factors. Male gender and being overweight or obese were the independent factors associated with high prevalence of multiple CVD risk factors, whereas among the modalities of therapy, metformin was associated with the least prevalence rate of CVD risk factors.

Cardiovascular disease accounts for up to two-thirds of all deaths in the diabetic population.[1] Multifactorial intervention using comprehensive risk reduction and appropriate therapy of cardiovascular risk factors has reduced mortality in type 2 diabetes.[5] The findings of this study showed that the prevalence rate of CVD risk factors was very frequent in Kuwaiti adults with type 2 diabetes. The most prevalent CVD risk factor in our patients was elevated LDL cholesterol, affecting more than 85% of our patients. The UKPDS has shown that increased concentrations of LDL cholesterol were a major risk factor for CVD, and there was 57% increased risk for every increase of 1 mmol/l in LDL cholesterol.[2] Clinical trials have shown that lowering LDL cholesterol by 33-40% with statins will reduce the risk of major CVD events by 31-37% in patients with diabetes.[3] Uncontrolled systolic and/or diastolic blood pressure occurred in approximately 85% of our patients. The UKPDS has also shown that increased blood pressure was also a major risk factor for CVD, with a 15% increased risk for every 10 mm Hg increase in systolic blood pressure.[2] Randomized clinical trials have demonstrated that lowering blood pressure ≤140/90 was associated with less cardiovascular events.[3] Uncontrolled hyperglycemia occurred in approximately 84% of our patients. The UKDPS has shown that for each 1% increase in HbA1c, there was an 11% increase in the relative risk of CVD for patients with type 2 diabetes.[2] However, currently, there is no conclusive evidence that tight glycemic control will reduce CVD

Table 1 - Percentages of Kuwaiti adults with type 2 diabetes (n=170) with cardiovascular risk factors out of the targets.

Variable	LDL-C (≥2.6 mmol/l)	HDL-C (≤1.1 for men; ≤1.4 mmol/l for women)	Systolic and/or diastolic blood pressure (≥130/80 mmHg)	HbA_{1c} (≥7%)	Current Smoking
All	86.2	63.2	84.6	83.8	13.3
Gender					
Male	90.6	54.7	73.2	92.9	40.0
Female	83.8	67.7	90.3	79.3	0.0
P-value	0.252	0.114	0.004	0.024	0.0001
Age (years)					
≤ 40	76.7	56.7	84.8	90.9	24.2
41-50	86.4	69.5	81.3	80.6	14.3
51-60	91.9	45.9	82.6	78.3	9.1
>60	88.5	80.8	96.2	92.3	3.8
P-value	0.332	0.021	0.339	0.249	0.113
Duration (years)					
Newly diagnosed	84.0	64.0	73.1	69.2	23.1
≤ 5	91.3	60.9	85.1	73.3	8.7
6-10	80.0	65.7	90.2	95.1	15.4
>10	87.0	63.0	85.5	90.9	10.9
P-value	0.520	0.976	0.296	0.003	0.609
BMI (kg/m²)					
<25	92.9	64.3	57.1	78.6	35.7
25-29.9	89.7	53.8	73.8	90.2	24.4
≥30	83.2	67.4	92.2	80.4	6.8
P-value	0.446	0.335	0.0001	0.335	0.001
Diabetes treatment					
Diet only	90.9	69.7	73.5	64.7	9.1
Sulfonylurea	88.4	53.5	84.4	90.9	28.3
Metformin	64.3	35.7	78.6	50.0	7.1
Combination oral	87.0	65.2	80.8	96.2	8.0
Insulin only	85.7	81.0	93.3	93.3	10.7
Insulin + oral	88.9	72.2	100.0	94.7	0.0
P-value	0.256	0.065	0.098	0.0001	0.017

LDL-C - low density lipoprotein cholesterol, HDL-C - high density lipoprotein cholesterol, HbA_{1c} - glycosylated hemoglobin, BMI - body mass index

events in type 2 diabetes.[3] More than half of our patients had low HDL cholesterol. The UKPDS study has shown that a decreased concentration of HDL cholesterol was an independent cardiovascular risk factor. There was a 15% decreased risk for every 0.1 mmol/l increase in HDL cholesterol concentration.[2] Studies on fibrates have shown that increasing HDL cholesterol along with decreasing LDL cholesterol and triglycerides had a favorable effect in reduction of cardiovascular events in patients with type 2 diabetes.[3] The UKPDS has shown that smoking is another major modifiable cardiovascular risk factor for patients with type 2 diabetes and smokers had a 41% increased risk for CVD.[2] Studies on smoking cessation demonstrated a reduction in mortality rate including CVD deaths, but these data have not been reported for patients with diabetes.[3] The present study showed that multiple CVD risk factors were prevalent among patients with type 2 diabetes. Studies have demonstrated that intensive multifactorial intervention in patients with multiple CVD risk factors using multiple drug combinations reduced the risk of cardiovascular morbidity and mortality among patients with type 2 diabetes mellitus.[5] Limitations of our study include its small size and being carried out in one center.

In conclusion, Kuwaiti adults with type 2 diabetes have a high prevalence of cardiovascular risk factors and thereby they are at high risk for cardiovascular morbidity and mortality at an early age. The findings of this study pose an urgent health challenge to control these modifiable risk factors. A population-based study is needed to verify the high prevalence of cardiovascular risk factors among Kuwaiti adults with type 2 diabetes.

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Distribution of hepatitis C virus genotypes and its response to treatment in Pakistani patients

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Hepatitis C virus (HCV) infection is a major cause of chronic liver disease (CLD) in Pakistan and has surpassed hepatitis B as the single most important cause for cirrhosis and hepatocellular carcinoma (HCC) in Pakistan.[1] Currently, HCV can be classified into 6 main genotypes. The available HCV genotype local data do not represent the genotypes prevalent in the entire country. At present, the main treatment for chronic hepatitis C (CHC) is based on a combination of standard or pegylated interferon (IFN) and ribavirin, although until the late 1990's IFN monotherapy was the only therapy available.[2] The HCV genotype 3 infection is considered to be highly responsive to IFN and ribavirin therapy, with more than 70% reported rates of sustained virological responses (SVR) in different clinical trials; these reports are, however, mainly from western countries.[2] There is paucity of clinical data regarding the treatment of CHC with standard IFN monotherapy and combination treatment from Pakistan. The aims of this study were to determine the HCV genotype prevalence and its treatment results in the Pakistani population.

In this study, we determined the distribution of HCV genotypes in 805 samples received at the Aga Khan University Hospital (AKUH) clinical laboratory from different parts of country. These samples were collected from 1998-2006 by a non-probability convenient sampling technique, from all 4 provinces of the country namely, Sind, Punjab, Baluchistan, and North West Frontier Province. The HCV genotype was determined using line probe Inno-LiPA HCV II assay (Innogenetics, Ghent, Belgium). In the second part of our study, we recorded the comprehensive data of IFN based treatment in 291 HCV genotype 3 patients who were able to complete the full course of therapy and follow up from 1998-2006. Monotherapy (IFN alone) was given to 83 patients who visited our clinics in the late 1990's when it was the only treatment option available, while combination treatment (IFN +

ribavirin) was given to 208 patients from 2000 to 2006. The demographics and biochemical parameters of these patients were recorded before the start of treatment and during follow ups in our hospital outpatient CHC database. The responses to treatment were observed at 12 weeks defined as early virologic response (EVR), at the end of treatment defined as end treatment response (ETR), and 24 weeks after stopping treatment defined as SVR with the help of HCV RNA polymerase chain reaction (PCR) qualitative assay. Patients were eligible for treatment if they had CHC diagnosed on the basis of raised aminotransferases for at least 6 months, positive serum antibodies to HCV, detection of HCV RNA by PCR, biochemical parameters, ultrasound of abdomen and/or a liver biopsy. A scoring system that ranked inflammation and fibrosis by Desmet et al,[3] on liver biopsy was used; scores could range from 0-9, with higher scores indicating severe abnormalities. Liver histology was available in 230 out of 291 study subjects before the start of treatment. An approval of the Ethics Review Committee (ERC) of our hospital was granted before collecting the data. The data recorded for treatment of CHC in our study is purely clinical data, and not clinical trial data as reported previously.[2] In our study, all patients in the combination therapy were given IFN alfa-2b in dose of 3 MU thrice a week subcutaneously along with ribavirin 1000 mg (body weight <75 kg) or 1200 mg (body weight >75 kg) per day in divided dosages. Biochemical and hematologic tests were regularly performed during each clinic visit. Serum HCV RNA qualitative analysis was determined before treatment, during treatment at weeks 12 (for assessing EVR) and 24 (for ETR); and finally at 24 weeks (for SVR) after stopping therapy. The HCV genotype was checked before the start of treatment in all patients. Statistical analysis was performed using SPSS version 11.0 for Windows (SPSS Inc., Chicago, Illinois, U.S.A.) Descriptive analysis is reported as frequency, like number with percentages for genotype and means are reported. For the analysis, chi-square test and Fisher's exact test were used where appropriate for categorical variables, while the independent sample t-test was applied for numerical variables to compare the 2 groups. A $p<0.05$ was taken as significant. To identify independent predictors of sustained virological response, we used multivariable logistic regression analysis.

The results of our study suggested that genotype-3 is found in 708/805 (87.8%) patients, followed by genotype one in 64 (7.9%), genotype 2 in 10 (1.2%), genotype 4 in 9 (1%), genotype 6 in 7 (0.8%), and genotype 5 in 2 (0.2%). The genotype was untypable in 9 (1%) patients. There were 439 (54.5%) males and 366 (45.5%) females. The mean age of our patients was 40±10 years; the mean age of patients with genotype-3 was 39±10 years, while the mean age of genotype non-3 was 42±8 years, $p=0.012$. The distribution of genotype 3 was uniform in all 4 provinces of Pakistan. A total of 291 patients, 101 females (35%) and 190 males (65%) completed CHC treatment. The mean age was 42±10 years, and ranged from 15-70 years. Monotherapy (IFN alone) was given to 83 patients, as this was the standard of care in the initial part of our outpatient database entry. Combination treatment (IFN and ribavirin) was completed by 208 patients. The SVR to combination therapy was achieved in 130/208 (62.5%); there were 21/208 (10.1%) relapsers (RR), and 57/208 (27.4%) non-responders (NR). Monotherapy achieved SVR in 25/83 (30.1%), RR in 15 (18.1%), and NR 43 (51.8%) patients. Combination therapy achieved a higher SVR compared to monotherapy (62.5% versus 30.1%, $p=0.005$). Regardless of monotherapy or combination therapy, patients' age of <35 years showed an SVR in 72% (54/75) as compared to age >35 years, with an SVR in 46.8% (101/216), $p=0.0001$. In the combination therapy group, the maximum SVR was achieved in patients aged 15-30 years (27/33, 81.8%) followed by 31-45 years (71/110, 64.5%), and >45 years (32/65, 49.2%). The same trend of better response was seen in the monotherapy group, in which the maximum SVR was seen in patients age 15-30 (3/6, 50%) followed by 31-45 years (13/37, 35%) and >45 years (9/40, 22%). Liver biopsy grades and stage distribution suggested that most patients belong to grade and stage II and III. Most of the RR and NR patients in both groups (monotherapy and combination therapy) had advanced fibrosis on liver biopsy as compared to those with SVR, where most of the patients had stage I and II fibrosis. To control for potentially confounding variables, we utilized a multiple logistic regression model controlled for gender, pretreatment albumin, pretreatment prothrombin time, HCV PCR at the twelfth week of treatment and fibrosis stage in the pretreatment liver biopsy sample. The SVR was significantly more likely to occur in those with serum albumin >3.6 at the start of treatment, combination therapy, and stage of fibrosis <2 as shown in **Table 1**.

This is one of the largest reports of HCV genotypes from Pakistan, which shows predominance of genotype 3 (87%). In previous small studies, genotype 3 has been reported to be common in Pakistan.[4] In our study, samples were collected from all over the country and therefore it is a true depiction of HCV genotype in Pakistan. A wide variety of HCV genotypes have been reported from South East Asia, including genotype 1a, 1b, 1c, 2a, 2b, 3a, and 3b, as well as types 6a. The HCV genotype 1 predominates over genotype 3 in southern India,[5] while Bangladesh has the same HCV genotype-3 dominance. As already reported by other studies,[3,4] we found that combination therapy with IFN and ribavirin

Table 1 - Factors predictive of sustained viral response on multiple logistic regression analysis.

Variable	OR	95% CI	*P*-value
HCV–PCR (12 weeks)			
Undetected			
Detected	25.7	12.4 - 53.1	0.0001
Therapy			
Combination therapy			
Mono-therapy	3.0	1.4 - 6.6	0.005
Albumin (pre-treatment)			
≥3.6			
<3.6	2.7	1.1 - 6.6	0.032
Liver biospy stage			
Stage ≤2	-	-	-
Stage 3 or 4	2.5	1.1 - 5.7	0.022

HCV - hepatitis C virus, PCR - polymerase chain reaction, OR - odds ratio, CI - confidence interval

is better than monotherapy. We found an SVR of 62.5% and 30% in our patient population with combination therapy and monotherapy. The main reason for a better response in our population is the predominance of genotype 3 in Pakistan as discussed. Most of these studies have reported the results of combination therapy in a clinical trial setting, but our study shows the results of purely clinical data in a hospital outpatient clinic setting. The patients in a clinical trial are usually homogenous, selective, and ideal for the treatment, but in the outpatient clinic setting, a heterogeneous population is encountered. As such, the results can be different from what we see in a trial setting. We found that younger patients age (<35 years) had a better sustained response (78%) as compared to those with age >35 years (57%); this effect was found in both monotherapy and combination treatment. Western literature has also reported that patients with HCV genotype 3 who are younger had a better response.[2] We identified 3 significant factors by multivariate analysis that were predictors of a favorable response, which were HCV RNA at 12 weeks, pre-treatment serum albumin and liver fibrosis stage <2, which are also reported in the literature. However, pretreatment serum albumin has never been reported to predict a favorable response as in our study. Finally, our study clearly demonstrated that stage of fibrosis <2 is also an independent predictor of sustained response as compared to advanced stage. A systematic review has also endorsed our findings that advanced stage of fibrosis and cirrhosis on initial liver biopsy is associated with a modestly decreased likelihood of sustained viral response to treatment.[2,3]

In conclusion, this is one of the largest, countrywide analyses showing that HCV genotype 3 is predominant in Pakistan. The overall treatment response rate was found to be 62% in HCV genotype-3 patients. Factors predictive of response to standard treatment are fibrosis stage <2, initial serum albumin >3.6 gm/dl, and negative HCV PCR at week 12 of treatment. However, younger patient age (<35 years), has an excellent rate of SVR (78.5%) to combination therapy. All these aspects are important when counseling patients in outpatient clinic settings on treatment outcomes. The study limitation in the second part of the study was that it was not a randomized control trial of interferon and ribavirin. Further research can be helpful to confirm the results of our study in chronic hepatitis C patients.

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Detection and quantification of cytomegalovirus in bone marrow transplant recipients by real time PCR and pp65 antigenemia

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Cytomegalovirus (CMV) infections are common and usually asymptomatic in healthy children and adults; however, the incidence and spectrum of disease in immunocompromised hosts establish this virus as an important human pathogen.[1] In immunocompromised patients, diagnosis of CMV infection depends upon

the revealing of CMV viremia. Cytomegalovirus antigenemia test and detection of CMV genome in blood show viremia.[2] The detection of CMV pp65 lower matrix protein in blood leukocytes allows a very sensitive and specific detection of CMV. However, pp65 antigenemia has some important pitfalls since it is a completely manual test, requiring the immediate processing of specimens, technical skills, and training for the final reading of the assay.[3] The polymerase chain reaction (PCR) is more sensitive than pp65 antigenemia, but qualitative PCR data are of little clinical value because they do not discriminate latent CMV infection from replicating infection.[4] However, the level of CMV DNAemia plays a critical role in the pathogenesis of CMV disease. It is considered a major risk factor for the development of CMV disease and has been shown to predict CMV disease in transplant patients.[5] Real time PCR has greatly improved precision in DNA quantitation due to the fact that threshold cycle (C_T) values observed when PCR is still in phase is a more reliable measure than an endpoint measurement of the amplified PCR product.[6] In this study, our aim was to compare the real time PCR and antigenemia test in bone marrow transplant recipients for detection of CMV.

Patients undergoing allogeneic or autologous peripheral blood stem cell transplantation (PBSCT) in our center between November 2006 and September 2007 were enrolled in this study. All patients gave their informed consent to participate to the study. All patients and donors were screened for CMV IgG before transplantation. Allogenic PBSCT patients have been monitored weekly by CMV antigenemia test and real time CMV PCR, from the week preceding transplantation to 100 days post-transplantation. Also, autologous recipients have been monitored weekly from time of engraftment until 60 days post-transplantation for CMV reactivation with same method. Cytomegalovirus antigenemia and PCR may also be performed at any time on clinical indications. In CMV antigenemia test, pp65 (matrix protein) presence was investigated with indirect immunofluorescence method (CINA kit, Argene-Biosoft, France) according to the manufacturer's instructions. For CMV antigenemia test, the blood samples were taken to the tubes with ethylenediaminetetraacetic acid (EDTA). The blood samples were studied with erytrocyte lysis and the leukocytes were sticked for the lam approximately $2x10^5$ cells/spot. Polymorphonuclear leukocytes stained such as apple green with fluorescein isothiocyanate were counted. If 2 or more green fluorescent cells were seen in whole area the test was evaluated as positive. Cytomegalovirus DNA was examined with real time PCR. Viral DNA was extracted from serum using the QIAamp Min Elute kit (Qiagen, Germany) according to the manufacturer's instructions. Real time PCR based on Taqman chemistry was performed by Fluorion CMV QNP 2.1 Real time PCR Kit (Iontek, Turkey) with primers and probe specific for the CMV glycoprotein B gene. Ten microliters of extracted DNA were added to the plate containing 15.25 µL of the reaction mixture. Approximately, 0.25 µL internal control was added to the reaction mixture. Polymerase chain reaction was performed under the following conditions: after 13.30 minutes at 95^0C, the samples were submitted 50 cycles, with each cycle consisting of a step at 95^0C for 30 seconds, followed by step at 54^0C for one minute and 30 seconds. Amplification and detection were performed by ICycler detection system (Biorad, USA). No amplification results were reported as <500 copy/mL. The results between 500-5000 copy/mL were reported as <5000 copy/ml. The quantification range of real time PCR was 5×10^3 - 10^6 copy/mL. The results greater than $5x10^6$ copy/mL were reported as >5x106 copy/mL. Qualitative results were analyzed with the Mc Nemar's Chi-square test. Quantitative results were analyzed with Spearman's test.

Fifty-four patients undergoing PBSCT were included in the study period. Forty-six of the patients had allogenic PBSCT and 8 autologous PBSCT. Forty of them (74 %) were male, 14 (26 %) were female. Median age was 26 (range, 16-58 years). In pre-transplantation setting, all patients and donors were CMV-IgG seropositive. After PBSCT, CMV antigen and CMV DNA were investigated in total of 450 samples. Antigenemia test could not be evaluated in 30 samples as there were insufficient polymorphonuclear leukocytes. Five of these 30 specimens were found CMV DNA positive. Cytomegalovirus antigen and CMV DNA were found negative in 334 (79.5%) of 420 samples. Cytomegalovirus antigen and DNA were found positive in 22 (5.2 %) of the samples, 25 samples (6%) were antigenemia positive but CMV DNA negative, 39 samples (9.3 %) were CMV DNA positive, but CMV antigenemia negative (**Table 1**). When we analyzed the results of pp65 assay and PCR data in 420 specimens statistical difference was not found between tests (p>0.05). The number of pp65 positive cells and the number of log10 genome copies per capillary tested among the positive results with both techniques were correlated (rho=0.487 p=0.02). Griscelli et al[7] had found antigenemia test negative in 66 of 128 samples, which were found positive by real-time PCR method. Mengelle et al,[8] compared results obtained using real time Light cycler TM quantitative PCR and the pp65

Table 1 - The results of real time polymerase chain reaction (PCR) and antigenemia assays.

Results	Real time PCR positive	Real time PCR negative	Total
Antigenemia positive	22	25	47
Antigenemia negative	39	334	373
Total	**61**	**359**	**420**

antigen assay on samples collected from recipients of solid organ transplants, they found correlation between the number of pp65 positive cells and the DNA copy number. The statistical results of our study and Mengelles' study were similar. Good correlations were also found between real time PCR and pp65 antigen assays for CMV in other studies.[3] As a result, real time PCR is useful for detection and quantification of CMV DNA. Cytomegalovirus antigenemia and CMV DNA PCR assays should be performed together for the diagnosis of CMV disease in bone marrow transplant recipients.

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Motorcycle-related foot injuries in children. *A growing menace*

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Trauma of the feet leading to amputations in the growing children is a serious injury, which causes permanent damage and long-term disability. Children are more prone to such injuries due to their smaller extremities and can easily be caught in the line of traumatic force. The traumatic amputations of the skeletally immature of either digit, partial or whole foot leads to stunted or painful overgrowth requiring repeated surgeries and limb shortening. In the western countries, these injuries are common due to lawn mowers and exercise equipment and in the eastern countries it is rare to find these injuries. Bicycle-related injuries are universal in school going children and usually feet are involved.[1] Motorcycle injuries are always devastating whether it is young or old, but in Saudi Arabia such injuries are becoming common.[2] A review of the literature did not reveal any reports regarding the pattern and the nature of foot injuries due to motorcycle accidents, particularly in non-drivers. This study was carried out with the aim to describe the pattern of foot injuries in children, which occurred while they were passengers on motorcycle rides. King Fahad University Hospital is a hospital attached to the teaching institution of College of Medicine, King Faisal University, and serves the inner city population of Al-Khobar and the surrounding suburbs. The hospital is also a tertiary care center of the region.

The inclusion criteria of the review were children ≤12 years with foot injuries due to motor cycle accidents, and referred to orthopedic department from the emergency room between January 2005 and December 2007. Children who presented to the out patients department were excluded. Patients and their medical charts were reviewed. The data of age, gender, site of injury, extent of injury, injury diagnosis, a brief note describing the

accident, primary treatment and final outcome was recorded. All children had assessment of the injuries under general anesthesia and debridement was carried out as required. The injuries were described as phalangeal, interphalangeal, and metatarso-phalangeal and traumatic amputation was described as a traumatic loss of a limb, organ or part. The data was entered into database and analyzed. The Research Committee of the College of Medicine, King Faisal University, Dammam, approved the study. There were 8 males and 4 females with a mean age of 3.83±2.14 (2-9 years). All children were silent riders and were sitting on the lap of the drivers. Right side was involved in 9 children and the left in 3 children. In the majority of children MTP joint (8/12) was the site of the injury. Big toe was involved in 2 and multiple phalanges in 2 children (**Figure 1**). A total of 16 fractures of phalanges were treated and 14 tendons were repaired. Primary amputation was carried in 4/12 patients and re-implantation was attempted in 2 children. Children who had primary amputation were over the age 5 years, and 2 of the children had the digits lost at the site of the accident. Debridement and closure was the most common procedure performed, and was successful (**Figure 2**). One re-implantation failed and developed gangrene of toe, which was later amputated. Six (50%) recovered and had no residual deformities. One child required multiple surgeries after a flap failed and still complains of persistent pain and residual deformity. The average follow up was for 18.25±9.78 months. In this study, 12 children had motorcycle related injuries to the foot of which 5 children had more than a digit amputated. Some children were sitting on the laps of their elders while riding on the motorcycle. Majority of the children were boys and the youngest was 2 years. Hostetler et al,[3] reported bicycle related amputations of 8.1% and 9.8% in 3-5 years and 6-12 years age respectively. We evaluated only foot injuries and 50% resulted in amputations of digits involving metatarso-phalyngeal and phalangeal joints. Reports in the literature indicate that injuries to big toe and amputations are common due to bicycles particularly in passengers. In our study even though children were sitting on the front of the motorcycle still 2 of the 12 children had their toes amputated. Mine et al[4] reported injuries in 26 children due to bicycles and none of them had any fractures or digit amputations. Subramanyam[5] described that the toe is usually get caught in the chains/gear of the rear wheel, but in our patients the mechanism was different. The children sat on the lap of their elder where he is not suppose to be there and their feet got caught in the spoke of the front wheel. There is little information in the medical literature in general and particular from Saudi Arabia regarding motorcycle injuries in the young silent riders. It is recommended that a prospective study is the call of the time to assess the prevalence and severity of such injuries in the region. Bicycle injuries were reported over 60 years and improvement in the management has occurred, but we have not given enough attention to the prevention of such injuries. Unless surgeons who take care of such devastating injuries should actively participate in the prevention methods.

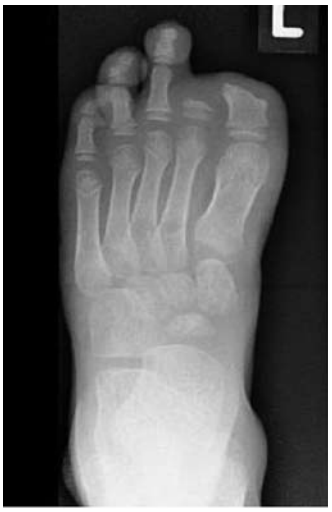

Figure 1 - Radiograph showing amputated left first and second toes.

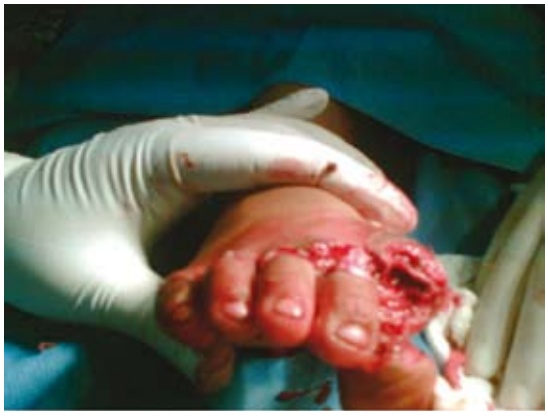

Figure 2 - Clinical picture of amputated big toe.

In conclusion, we believe that digit amputations due to motorcycles in the silent riders are not uncommon in eastern province of Saudi Arabia. We believe that advice on prevention to the parents need to be given to avoid small children on their laps while driving the motorcycles and secondly to push for a protective cover on the front wheel.

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