Brief Communication

Comparison of branched DNA and real-time polymerase chain reaction methods in quantitation of hepatitis B virus DNA

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Hepatitis B virus (HBV) is a major causative agent of chronic hepatitis, and can cause liver cirrhosis and hepatocellular carcinoma. Serological assays are not sufficient for the diagnosis of HBV infection. Measurement of HBV DNA levels are routinely used to identify infectious chronic carriers, and to predict and monitor the efficacies of antiviral treatment regiments.[1] A number of commercial assays are currently available for the quantification of HBV DNA in patient serum or ethylenediamine tetraacetic acid-plasma, including hybridization, signal, and target amplification based technologies.[2,3] The Quantiplex™ HBV DNA branched DNA assay (Versant 3.0, Bayer, Germany) is a signal amplification assay based on branched DNA (bDNA) technology.[3] Real-time polymerase chain reaction (PCR) quantitation is based on the evaluation of the threshold cycle (C_T) when amplification of a PCR product is first detected. The higher the starting copy number, the sooner amplified product is detected.[4] Our aim in this study was to compare bDNA (Versant 3.0, Bayer, Germany) and real-time PCR (Fluorion Iontek, Turkey) assays for quantitation of HBV DNA in sera of patients.

The study was carried out in Erciyes University Medical Faculty, Department of Microbiology, Laboratory of Virology, Erciyes, Turkey in May 2005. Blood samples collected from 220 chronic hepatitis B patients were included in the study. The sera of the patients were kept at -20°C until the study. All of the sera were from Turkish patients. The HBV DNA in sera was investigated by the bDNA assay according to the manufacturer's instructions. The quantification range of the bDNA assay was 2 x 10^3-10^8 copy/mL. The results greater than 2 x 10^3 copy/mL were accepted as positive. If there was no virus detection, it was reported as <2 x 10^3 copy/mL. The results greater than 10^8 copy/mL were reported as >10^8 copy/mL. The HBV DNA in sera was investigated by the real-time PCR assay according to the manufacturer's instructions. Viral DNA was extracted from 200 µL of serum using the QIAmp MinElute kit (Qiagen, Germany). Reaction mixture was prepared by Fluorion HBV QNP (Quantification probe) Version 2.0 (Iontek, Turkey). Five microliters of extracted DNA were added to the plate containing 20 µL of the reaction mixture. The amplification profile was performed as follows: 30 minutes at 95°C, 30 seconds at 95°C, 90 minutes at 54°C with 50 cycles, and 10 seconds at 22°C. Amplification and detection were performed by ICycler detection system (Biorad, USA). The quantification range of real time PCR was 10^3-10^7 copy/mL. The analytical detection limit for real-time PCR was 200 copy/mL. No amplification results were reported as <200 copy/mL. The results between 200-1000 copy/mL were reported as <1000 copy/mL. The results greater than 10^7 copy/mL were reported as >10^7 copy/mL.

Pearson's correlation coefficient was used to assess the strength of the linear association between the log-transformed values of real-time PCR and bDNA assay. A 2-tailed *p* value of less than 0.05 was considered to indicate statistical significances. Furthermore, consistency of the differences between the $\log_{10}$ quantitative values obtained from Versant 3.0, and real-time PCR was assessed according to the analysis as proposed by Bland and Altman.[5]

The HBV DNA was investigated by real-time PCR and bDNA assay in 220 sera. The results below or above the detection limit of each assay were excluded from calculation. Seventy-one samples (32.3%) were detectable by both real-time PCR and bDNA tests within their quantitation ranges. When quantitative results were compared, significant correlation was

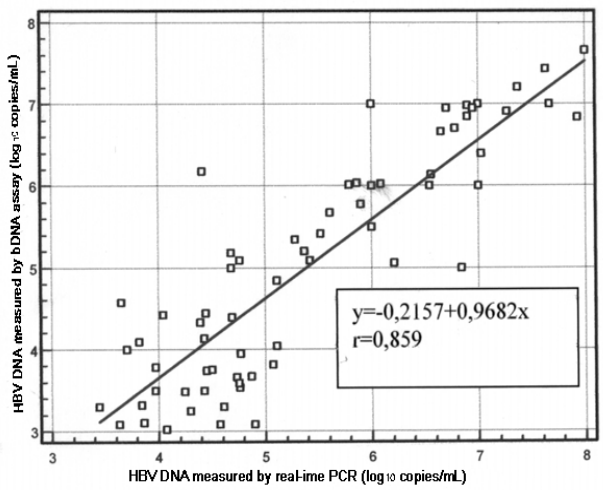

Figure 1 - Correlation plot of $\log_{10}$ hepatitis B virus (HBV) DNA values measured by the branched DNA and real-time polymerase chain reaction tests. y = regression equation dependent variable, r = correlation coefficient

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found between the results of real-time PCR and those of the bDNA assay (r=0.859, $p<0.001$) (**Figure 1**). Bland Altman analysis indicated no difference between the 2 assays. Of the 220 tested samples, 124 (56.4%) by bDNA test and 120 (54.5%) by real-time PCR test had HBV DNA levels either above or below the manufacturer's recommended detection ranges. There seems to be no difference between the 2 tests in terms of retesting.

Yao et al[6] found a good correlation between results obtained from clinical samples analyzed by Versant 3.0, and the comparative HBV DNA quantitative assays (Versant 1.0, Digene Hybrid capture II, Cobas Amplicor). A significant correlation was also found between the results of bDNA assay and real time PCR.[7] We conclude that quantitative HBV DNA results obtained by real-time PCR and bDNA tests as compared in this study have significant correlation. A larger study with a large series of samples should be carried out in the future to confirm these results.

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Detection of Salmonella species isolated from clinical specimens by serotyping and polymerase chain reaction

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Typhoid fever caused by *Salmonella typhi (S. typhi)* remains an important public health problem in many parts of the world. Rapid and sensitive laboratory methods for diagnosis of typhoid fever are essential for prompt and effective therapy. Although several serological assays for detecting *S. typhi* antigens or antibodies have been used for their speed and simplicity, no non-culture tests for typhoid fever have repeatedly been shown to be highly sensitive and specific. The classical and the most commonly used serological method, the Widal test, is particularly unreliable with the single titers in endemic areas. Confirmation of typhoid fever requires the identification of *S. typhi* in clinical specimens. The *S. typhi* can be isolated from more than 90% of patients with typhoid fever if blood, stool, rose spots, and bone marrow aspirates are all cultured.[1] The clinical usefulness of the culture method is further restricted as it takes at least 2 days until the identification of the organism. Rapid and sensitive laboratory methods for diagnosis of typhoid fever are essential for prompt and effective therapy. The identification of *Salmonella* species by serological methods has problems due to cross-reaction of antigens on species. Although several serological assays for detection of *S. typhi* antigens or antibodies have been used for their speed and simplicity. Many molecular techniques such as the polymerase chain reaction (PCR), ribotyping, and extraction of whole-cell proteins of bacterial cells by sodium dodecyl sulfate polyacrylamide gel electrophoresis have been used for identification and typing purposes of *Salmonella* species.[2-5] Previously, a DNA probe specific to the Vi antigen of *S. typhi* had been used to detect the organism in the blood of patients with typhoid fever.[1] The flagellar antigen of *S. typhi* (H1-d) is encoded by a 1530 bp DNA sequence.[1] Although flagellar antigen is not a structure specific to *Salmonella* species, and d-antigen is also present in many *Salmonella* species other than the *S. typhi*, the flagellin gene of *S. typhi* has unique nucleotide sequences in the hypervariable region of the gene.[1] These findings suggested that the PCR test, based on the unique sequence in the flagellin gene of *S. typhi*, could be used to detect *S. typhi*, specifically in the clinical specimens. The aim of this study is to detect flagellin gene in typhoidal *Salmonella* species for identification purpose, and to differentiate them from other Enterobacteriaceae species.

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Bacterial strains. In this study, 98 clinical isolates of *Salmonella* isolates that were previously collected from laboratories of Hamadan Medical Centers, Hamadan, Iran, and have been serotyped. Four reference strains of *Salmonella* species (including *S. typhi* Persian type culture collection [PTCC] 1609, *S. para typhi A* PTCC 1230, *S. para typhi B* PTCC 1231, *S. para typhi C* PTCC 1232), 5 Enterobacteriaceae species (including *Escherichia coli* PTCC 1222, *Enterobacter aeruginosa* PTCC 1221, *Citrobacter freundii* PTCC 1600, *Klebsiella pneumoniae* PTCC 1053, *Serratia marcescens* PTCC 1111) were used for the detection of the sequences of flagellin gene by PCR. The *S. typhi* PTCC 1609 was used as a positive control. Blood specimens (3 ml) were obtained before antibiotic therapy from patients with typhoid fever who were admitted to the Sina Hospital of Hamadan, Hamadan, Iran, consecutively during the period from December 2003 to March 2004. All cases were confirmed by blood culture. Informed consent was obtained from the patients.

PCR detection. For detection of the sequences of flagellin gene of reference strains and clinical isolates, the primers of ST_1 (5'-ACT GCT AAA ACC ACT ACT-3') and ST_2 (5'-TTA ACG CAG TAA AGA GAG-3') were amplified. Oligonucleotides ST_1 and ST_2, which were used in the first round of the PCR to amplify a 458-bp fragment, correspond to nucleotides 1072 - 1089 and 1513-1530, in the flagellin gene of *S. typhi*.[6] Chromosomal DNA of *Salmonella* strains and other organisms were extracted, as previously described.[1] After centrifugation at 3,000 x g of 0.5 ml of an overnight Luria broth culture, the resulting pellet was resuspended in 75 µl of 50 mM Tris-hydrogen chloride buffer (pH 8.0) containing 0.9% glucose, 250 mM ethylenediamine tetraacetic acid (EDTA), and 140 µg of lysozyme. The reactants were incubated at 37°C for 30 minutes. After which, a 300 µl of 50 mM sodium chloride containing 1% sodium dodecyl sulfate and 800 µg of proteinase K were added, and the incubation was continued for an additional of 120 minutes. The procedure was followed by phenol-chloroform-isoamyl alcohol (25:24:1) extraction. The DNA was precipitated by the addition of absolute ethanol and harvested by centrifugation. The PCR reaction mixture contained 2 µg of extracted DNA, 25 pmol (each) of primers, 200 µM (each) Deoxyribonucleotide triphosphate (dNTP), 0.5 U of Taq DNA polymerase (Boehringer Mannheim, Mannheim, Germany), and the standard PCR buffer (10X) in a final volume of 25 µl amplification in an automated DNA thermal cycler (Eppendorf, Germany) consisted of 40 cycles at 94˚C for 1 minute, 57˚C for 1 minute and 15 seconds, and 72˚C for 3 minutes. For detection of PCR product 10 µl of the PCR products was electrophoresed on a 1.5% agarose gel for 60 minutes at a constant 80V, with Tris-Borate-EDTA buffer (1X). Molecular size makers (1 kb DNA ladder, Bethesda Research Laboratories, United Kingdom) were run concurrently. The gels stained with ethidium bromide were examined under ultra violet illumination for the presence of a 458 bp fragment.

Specificity of the PCR. To verify that primers used in this study were specific for *S. typhi*, the PCR was carried out with DNA from 4 *Salmonella spp.* and 5 other organisms. With the single round of PCR with ST_1 and ST_2, amplification products of the expected size (458 bp) were seen only from the extracts of *S. typhi* strains but not from the extracts of other organisms, both on the agarose gel electrophoresis. The amount of template DNA from various bacteria was 4 ng (corresponding to 106 organisms).

Serotyping. Out of 98 *Salmonella* isolates, 69 strains (70%) were typhoidal *Salmonella* species and 29 (30%) were non-typhoidal *Salmonella* species. From typhoidal *Salmonella*, 43 (62.3%) strains were *S. typhi*, 13 (18.8%) *S. para typhi B*, 11 (16%) *S. para typhi C*, and 2 (2.9%) *S. para typhi A*. From non-typhoidal *Salmonella,* 17 (58.6%) strains were *S. typhimurium*, 3 (10.3%) *S. enteritidis* and *S. chlorosis*, *S. arizonae* each 2 (6.9%), *S. infantis*, *S. havana*, *S. lexington* and *S. virchow* each 1 (3.4%). One isolate was not identified by serotyping. A total of 43 isolates of *S. typhi* (19 strains isolated from blood specimens and 24 from other specimens) were subjected to PCR.

PCR results. The PCR products of *Salmonella* species showed reasonable results to differentiate these strains. With the single round of PCR with ST_1 and ST_2 primers, amplification products of the expected size (458 bp) were seen only from the extracts of *S. typhi* strains but not from the extracts of other organisms. All reference strains of *S. typhi* and 41 (95%) strains of cillotically isolates had flagellin gene, and produced 458 bp fragment. With the first round of PCR on DNA from 2 strains of *S. typhi* isolated of fecal specimens with culture and serotyping-confirmed typhoid fever before antibiotic therapy, no amplification products were seen on the gel.

Typhoid fever caused by *S. typhi* remains an important public health problem in many parts of the world. The classical and the most commonly used serological method, the Widal test, is particularly unreliable with the single titers in endemic areas.[1] Since it is often difficult to obtain bone marrow aspirates in many endemic areas, in most cases, only blood specimens have been cultured. Blood culture, however, can detect only 45-70% of patients with typhoid fever, depending on the amount of blood sample, the bacteremic level of *S. typhi*, the type of culture medium

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and the length of incubation period.[1] Detection of the nucleotide sequences of flagellin gene of *S. typhi* has been used for identification purpose of these species. In our study, PCR with ST_1 and ST_2 was highly specific in detection of the *S. typhi* DNA. In view of the practical use of PCR, however, this finding is thought to be not critical as the PCR technique can be used to reinforce the clinical diagnosis of typhoid fever in patients with suspected clinical features of typhoid fever, such as high fever, leukopenia, hepatosplenomegaly, and so on, but with negative cultures. Non-typhoidal *Salmonella* is an uncommon etiologic agent of gastroenteritis, which can be clearly differentiated from typhoidal *Salmonella* on the basis of clinical findings.

Furthermore, the detection of non-typhoidal *Salmonella* in blood specimen would be impossible with this PCR technique even if it is present, as it is subjected to be amplified by only the second round of reaction (40 cycles) with ST_1 and ST_2. As shown in the sensitivity test for *S. typhi* DNA, the single round reaction is insufficient to detect a small number of organisms in clinical specimens.

In conclusion, our results showed that the PCR technique, which amplified fragments of flagellin gene of *S. typhi* in the blood specimens, could be directly detected on the gel. For the practical use of the PCR as a diagnostic test, the gel electrophoresis should be sufficient to detect amplification products without the aid of hybridization methods, which take at least 2 days with the use of radiolabeled probes. The whole procedure to identify *S. typhi* DNA in the blood by agarose gel electrophoresis took only 16 hours, demonstrating the PCR to be a simple, specific, and rapid method for the early diagnosis of typhoid fever.

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The perioperative renal function in cyanotic versus acyanotic children undergoing desflurane anesthesia for open-heart surgery

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There is a relatively little data on perioperative renal function in children with cyanotic congenital heart disease undergoing open-heart surgery.[1,2] This study aims to investigate the perioperative renal function in cyanotic versus acyanotic children undergoing desflurane anesthesia for open-heart surgery. Renal function was assessed through the use of the clinical laboratory markers of kidney function, electrolytes, and by a subclinical, sensitive marker of tubular damage. After the ethics committee approval and the written parental consent were obtained, 20 patients with cyanotic and acyanotic congenital cardiac anomalies were included in this study. Patients with renal or hepatic failure, a history of diabetes mellitus, heart failure, acute or chronic pulmonary disease, a previous operation due to cardiac pathology, on medication, or patients with anomalies of other organ systems related to cardiac anomalies were excluded from the study. Patients who had undergone revision surgery, patients receiving ventilatory support for more than 24 hours, and those who required peritoneal dialysis, hemodialysis or ultrafiltration intra- or postoperatively, and patients with pre- or postoperative inotropic support.

The patients were divided into 2 groups according to the oxygen saturation (O^2 Sat) measured by pulse oximetry: the acyanotic group (O^2 Sat ≥85%) and the cyanotic group (O^2 Sat <85%). Patients were premedicated with midazolam (0.5 mg kg^{-1} intranasal) on operation day, 30 minutes before the operation. Anesthesia was induced with thiopental 5 mg kg^{-1}, morphine hydrochloride 0.1 mg kg^{-1}, and vecuronium bromide 0.1 mg kg^{-1} was used for muscle relaxation.

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Morphine and vecuronium were added when necessary. Anesthesia was maintained with desflurane at 4-6% together with 3 L/min nitrous oxide and 3 L/min oxygen using a semiclosed circle system with a soda lime canister before cardiopulmonary bypass (CPB) and 2-4% during CPB. The depth of anesthesia was monitored with bispectral index and an acceptable anesthetic depth (between 30-50) was achieved by titration of desflurane. The same anesthesia and surgery group performed all the operations. Data regarding the hemodynamic parameters, urine output, blood and blood product requirements, diuretic (furosemide, mannitol) use, total amount of drainage as well as serum and urine electrolytes were recorded. To detect subclinical and transient renal dysfunction after CPB, the N-acetyl-ß-D-glucosaminidase ([NAG] a sensitive marker of proximal lysosomal tubular damage)[3], was measured. For the statistical analysis, chi-square test, unpaired test, analysis of variance with repeated measures, post hoc Scheffe's test, Mann-Whitney U test, or the Kruskal-Wallis test were used when appropriate.

There were no differences between the study groups regarding demographic and clinical data (**Table 1**). Four patients in the acyanotic group had atrial septal defect, whereas 6 had ventricular septal defect. In the cyanotic group, 2 patients had a total anomalous pulmonary venous connection, whereas 8 had tetralogy of Fallot. They all underwent total corrective intervention. There were no differences in plasma sodium, calcium, chloride levels, blood urea nitrogen concentration, uric acid levels, and fractional uric acid excretion between the 2 groups. Serum inorganic fluoride levels were less than 0.02 ppm in 18 patients (9 patients in each group). None of the patients had toxic levels of inorganic fluoride. Urinary inorganic fluoride levels were less than 0.02 ppm at various sampling times in 6 patients (3 patients in each group). In all patients, there was a general declining trend of electrolyte levels from the baseline as the dilution is due to the fluid replacement and the priming solution of the CPB. In the cyanotic group, the serum potassium levels have increased (during, and at the end of the CPB), while the urinary potassium levels (during CPB), and sodium levels (at the end of the operation) have decreased more than the cyanotic group. All these changes in the electrolyte levels suggest a change in renal tubular electrolyte management in cyanotic children.

Table 1 - Comparison of the acyanotic and the cyanotic groups, n=10 (values are mean±SD).

Demographics	Acyanotic	Cyanotic
Gender		
Male	7	5
Female	3	5
Age (Months)	67.8±37.5	65.4±40.2
Body weight (kg)	17.6±5.9	20.5±10.5
Oxygen saturation (%)	95±3*	78±7*
Preoperative hemoglobin (g dL^{-1})	11.9±1.2*	16.5±3.4*
Duration of operation (minute)	185±43	214±33
CPB time (minute)	67±26*	90±16*

SD - standard deviation, *p<0.05 - between the 2 groups, CPB - cardiopulmonary bypass.

Another major finding supporting the renal tubular damage was the levels of urinary NAG at the end of the operation, which were higher in the cyanotic group than the acyanotic group. We measured NAG as the standard marker of renal damage, as creatinine and creatinine clearance are not sensitive enough to detect discrete changes in the renal function.[3] The more pronounced alteration in kidney function in cyanotic patients may be the further decrease of renal plasma flow of CPB in already decreased renal plasma flow due to chronic cyanosis.[1,2] The capillaries of the peritubular plexus provide the tubular apparatus, which is most vulnerable to ischemic damage. To maintain normal blood flow in the peritubular capillaries as there is an increased resistance to flow of viscous blood in patients with elevated hematocrit, a high intravascular pressure is needed, which makes these patients easily susceptible to renal injury during CPB.[2]

In another study of 34 patients weighting less than 10 kg, repeated analysis of urine, blood, and plasma viscosity were performed only during CPB. Polyuria and proteinuria that appeared during CPB indicated an elevated transglomerular filtration gradient, which recovered within 24 hours. Similar to our results, the appearance of NAG in the urine and elevated excretion of sodium, were additionally indicative of mild tubular damage.[4] The plasma creatinine concentration has been validated as clinically important for renal function.[5] The serum creatinine level was higher, while urinary creatinine was lower in the cyanotic group than the acyanotic group after CPB. However, the urinary creatinine concentrations showed similar trends in both groups; the urinary creatinine levels decreased during and after CPB compared to the baseline.

Patients with cyanotic heart disease undergoing desflurane anesthesia for open-heart surgery have developed significantly more renal tubular dysfunction compared with acyanotic patients. These transient alterations in kidney integrity were not detected with standard measures of kidney function such as the serum creatinine concentration. We conclude that the

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renal function should be monitored more closely for electrolyte changes, and the renal tubular damage in cyanotic children undergoing desflurane anesthesia for open-heart surgery.

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Cardiopulmonary resuscitation report on Utstein template from eastern Saudi Arabia

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The outcome of cardiac arrest and cardiopulmonary resuscitation (CPR) is dependent on critical interventions, particularly early defibrillation, effective chest compressions, and assisted ventilation. The CPR and advanced life support (ALS) have been used to improve outcome for in-hospital patients with cardiac and respiratory arrest. Characterization of in-hospital cardiac arrests has been limited by the lack of consistent data collection and analysis. The Utstein style guidelines for uniform data reporting of cardiac arrests and resuscitation have recently been reviewed and updated.[1] The revised template is suitable for recording in the resuscitation attempts in both adults and children. The Utstein style definitions and reporting templates have been used in published outcome studies of cardiac arrest.[2,3] Outcomes from arrests of these types have not been reported from the Kingdom of Saudi Arabia (KSA) on Utstein style. In our study, we have used the Utstein template-based form to record cardiac arrest data at out hospital. We have analyzed the demographic characteristics of the patients and the possible event variables that may influence the outcomes. The aim of the study is to describe the outcome of patients suffering in-hospital cardiac arrest according to the Utstein template.

The study is conducted at the King Abdul-Aziz National Guard Hospital in the eastern region of KSA. Resuscitation records are generated in all instances where the CPR team is summoned, and documentation is maintained as part of both patients' official medical records and the CPR committee's archives. During the study period, there was a designated resuscitation team available around the clock. The team is dispatched by overhead pager, beeper, or both. All hospital staffs are trained in basic life support and advance cardiac life support at a regular basis. The medical personnel and the nurses have also received training in manual defibrillation. We examined all records of cardiac or respiratory arrest that occurred from January 2003 and June 2006. We then retrieved the full inpatient medical records and abstracted data. We used the Utstein template[1] to record the patient's demographics and co-morbidities, the date, time, location, and nature of the event, whether or not it was witnessed, the patient's 'Do Not Attempt Resuscitation' (DNAR) status and the initial cardiac rhythm. The duration of the resuscitation attempt and the treatment given were also recorded. Immediate survival (Return of Spontaneous Circulation [ROSC]) and at hospital discharge, together with Cerebral Performance Category (CPC), was also obtained. Only those patients who had a "true arrest" (defined as requiring one or more of manual CPR, intubation or defibrillation) were included in our study. The first arrest event was recorded for patients who had multiple cardiac arrests. The study protocol was approved by the Hospital's Regional Committee.

The hospital has a DNAR policy, and the decision was stated in the hospital record. The CPC is a way to measure the neurological outcome. A score of one represents no neurological impairment, while a score 4 represents poor neurological outcome, dependent on others for activities of daily living. Statistical analysis was performed using the Statistical Package for Social

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Sciences version 10 for Windows. Descriptive statistics were used to summarize the data and to describe the distribution of patients. The chi-square test was employed to assess association between categorical variables, and Mann-Whitney test for continuous data. We analyzed univariable associations between hospital site, gender, and the type of arrest with the survival after arrest. We also examined whether survival varied significantly by the type of cardiac arrest (shockable versus non-shockable rhythms). Logistic regression was performed to identify independent variables associated with outcome of resuscitation. These variables included age, sex, duration of arrest, initial rhythm whether the arrest was witnessed or not, and hospital location. We determined the final logistic regression models by backward selection of the variables, maintaining those that were significant at a p value of 0.05 or less.

There were 374 records of in-hospital cardiac or respiratory arrest during the study period. Of these, 125 arrests were resuscitated. It included 77 males and 48 females. Seventeen (13.6%) were the pediatric patients, while 73 (58.4%) were above the age of 60 years. Asystole was the most frequent arrest type, and the pulse-less electrical activity was the second. Almost two-thirds of the patients were admitted due to cardiac related diagnoses. Nearly half of the patients had a history of coronary artery disease, one-third had a history of myocardial infarction, and two-fifth had a history of diabetes. The mean time interval from cardiac arrest to start of CPR was 2±3.5 minutes (min), and the mean interval from initiation of CPR to first DC shock was 8±3 min. Thirty-nine percent of cases occurred in the intensive care unit (ICU), 29% in the wards, 21% in the emergency department (ER), and 10% in the neonatal (N) ICU. The rates of hospital discharge were 28.5% ICU, 19.5% ward, 15.5% ER, and 42% NICU. The ROSC and survival to hospital discharge was significantly associated with the initial cardiac rhythm. Although the initial cardiac rhythm was more often non-fibrillation/ventricular tachycardia (VF/VT) than VF/VT [74% (84/114) versus [vs] 26% (30/114)], their frequency is almost reversed among those who have ROSC [38% (32/84) vs 67% (20/30)] (p=0.02), and among those who survived to hospital discharge [15.4% (13/84) vs 46.7% (14/30)] (p=0.04). The mortality rate at hospital discharge was significantly higher in patients whose initial cardiac rhythm was non-VF/VT (85%) (p=0.001). The mean CPR duration was 19.9±13.1 min. It was 12.5±9.2 min for patients who achieved ROSC as opposed to 26.2±12.7 min for patients who did not achieved ROSC (p=0.01). Similary, the duration of CPR for patients who survived to hospital discharge was 10.8±9.8 minutes, as compared to 22.8±12.8 minutes for patients who did not survive to hospital discharge (p=0.01). Both ROSC and survival to hospital discharge decreased with the increase in the duration of CPR. No patients survived to discharge with CPR lasting more than 20 minutes. Of the 111 patients whose arrest was witnessed, 47.0% (95% CI, 39.8-56.8%) could be resuscitated, and 26.0% (95% CI, 15.8-30.1%) survived to hospital discharge. Of the 14 patients whose arrest was not witnessed, 35.7% (95% CI, 13.8-30.3%) could be resuscitated, only one patient survived to discharge. Overall, 45.6% of the patients could be resuscitated, and 24.0% survived to hospital discharge, as shown in **Table 1**. The outcomes were similar in both pediatric and adult patients. Of the 30 patients who survive to discharge, 10% experienced a significant decrease in function after their arrest that they were no longer deemed able to fully care for them. We found no significant association between either age or sex and the outcome. The CPR duration (p=0.01), shockable rhythm (p=0.02) and witnessed cardiac arrest (p=0.02) were found to predict both the ROSC and survival to hospital discharge.

During the study period, there were 97,429 patients admitted in the hospital, and 344 patients died during the hospital stay. The resuscitation team was alerted 125 times. The ultimate goal of resuscitation is to improve the survival rate with good neurological outcome. Immediate survival and discharge rates of CPR attempted at our hospital demonstrate similarities with the global data. The main factors influencing discharge survival were the CPR duration and witnessed cardiac arrest. During the 40 years after the introduction of the modern CPR,

Table 1 - Index event characteristics of patients who received cardiopulmonary resuscitation.

Characteristics	n (%)
First pulseless rhythm	
Asystole	48 (42.1)
Pulseless electrical activity	36 (31.6)
Ventricular fibrillation	18 (15.8)
Pulseless ventricular tachycardia	12 (10.5)
Return of spontaneous circulation	
Overall	52 (45.6)
Asystole	13 (27.1)
Pulseless electrical activity	19 (52.7)
Pulseless ventricular tachycardia	7 (58.3)
Ventricular fibrillation	13 (72.2)
Survival to hospital discharge	
Overall	30 (24.0)
Asystole	5 (10.4)
Pulseless electrical activity	8 (22.2)
Pulseless ventricular tachycardia	5 (41.6)
Ventricular fibrillation	9 (50.0)
Respiratory arrest	3 (27.3)

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there have been major developments and changes in the performance of resuscitation. Utstein reports of in-hospital cardiac arrest showed survival that range from 8 - 26%. In our current work, the immediate survival rate was 45.6%, and discharge rate of CPR was found to be 24%. In our study, age, gender, and etiology did not influence immediate survival and discharge rate. However, it has been demonstrated that a decrease in survival rate associated with increased age was not related to a certain age limit, rather to the presence of co-morbidities with increased age. We found that 60% of the resuscitations were started on patients 60 years or older. Resuscitation should not be considered futile just on the basis of old age, since 29% in the age group >75 years were discharged alive. Thus, the patients' co-morbidity and status before the arrest must be taken into consideration.

The site of resuscitation has no bearing on survival. It has been suggested that the primary etiology rather than the cardiac arrest site was influential on survival. The interval from the time of arrest to the start of resuscitation, the duration of arrest, and the type of primary arrhythmia occurring during arrest were shown to be an independent risk factors affecting immediate and discharge survival.[4] Duration of cardiac arrest seems to have a negative correlation with the initial response, and we found significantly better survival if the arrest period was less than 20 minutes. We found that patients with shockable rhythms (67%) had higher immediate survival rates than patients with non-shockable rhythms (38%), which is consistent with the literature.[4] The primary arrhythmia was one of the most significant predictors of immediate survival.

Currently, the most important and effective way of treating VF/VT is early defibrillation.[5] In our study, initial resuscitation as well the discharge survival was significantly improved in patients who were defibrillated. In most of the patients in whom there was no response to defibrillation, the site of arrest was in the ward where defibrillators were not readily available. The mean time from arrest to defibrillation was significantly shorter in survivors than non-survivors (3.2±2 vs 8.8±2.5 min). We know that when the time lag to defibrillation is less than 3 min, survival was increased to 38% from 21%. All this, emphasize the importance of early defibrillation. There is an evident need to improve the outcome of patients suffering cardiac arrest on the wards. An important step is to reduce the time interval to defibrillation. We believe that our DNAR policy results in resuscitation attempts in only third of the patients who died during the hospital stay. The DNAR decisions affect the survival rates, as the exclusion of the DNAR patients from the study has led to a 15% increase in survival to hospital discharge.

In conclusion, the survival rate (hospital discharge) for cardiac arrest among patients for whom the resuscitation team was called was 24%. This rate of survival is in par with other Utstein reports of in hospital cardiac arrest ranging from 8 - 26%. A combination of factors can contribute to this, including a functional DNAR policy, an efficient and competent resuscitation team, and well-trained hospital staff in CPR. There is an evident need to improve the outcome of patients suffering cardiac arrest on the wards. An important step is to reduce the time interval to defibrillation. Therefore, we emphasize that all medical and nursing staff should have CPR training with periodic renewal, and essential equipment should be readily available at all sites.

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Ambulatory blood pressure monitoring for 24 hours in children with type-1 diabetes mellitus

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Hypertension accompanying type-I diabetes is a major risk factor for the development of cardiovascular, renal, and atherosclerotic vascular disease. While the prevalence of hypertension in the normal population is 4.4%, it can reach up to 14.7% in diabetic patients.[1] The value of blood pressure (BP) measurement in the outpatient clinic setting is limited by the effects of white coat, stress, and exercise on arterial BP. Therefore, the 5th Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure recommends 24-hour BP monitorization, especially in diabetic patients.[2] With ambulatory BP monitorization (ABPM), it is possible to follow BP for 24 hours and detect diurnal variation. At least a 10% reduction in BP during sleep compared to daytime values is known as diurnal variation (dipper). Disturbances in diurnal variation are known as "non-dipping," which is an early indication of diabetic hypertension.[3] In this study, we aimed to assess ABPM and investigate the effects of metabolic control and duration of diabetes on BP in children with type-I diabetes.

This study took place at Ankara Diskapı Children Hospital, Ankara, Turkey between January 2003 and January 2004. Eighty-five cases (49 females, 36 males) with type-I diabetes mellitus and 40 healthy children (15 males, 25 females) with matching age and gender were included in the study. The mean age was 12.4±2.8 (6-18) years in the type-I diabetes group and 11.7±3.3 years in the control group. Inclusion criteria was normotensive, and the absence of clinical evidence of heart, renal, systemic disease, and of normo-albuminuria defined as albumin excretion rate (AER) <20 µg/min. We determined the relative body mass index (RBMI ([weight/height (m2)] / [age-and gender-matched weight / height (m2)] * 100)) of the cases to screen for obesity, which is likely to affect BP. Cases with an RBMI of >120 were considered obese. Lipid profile (cholesterol, triglyceride, HDL, LDL, VLDL), which is likely to affect BP measurement is determined in serum samples obtained at least 8 hours post-prandially. To determine the metabolic control of diabetics, glycosylated hemoglobin (HbA1c) levels were measured using the spectrophotometric method by Roche-Hitachi autoanalyzer. An HbA1c level of <7.7% was considered as indicative of good metabolic control, 9% of moderate, and >9% of poor control. For all the cases, systolic and diastolic BPs were measured in the outpatient clinic and 24-hour ambulatory BP was monitored. The BP values were evaluated according to Tumer standards.[4] Cases with systolic and diastolic values above 95% were considered hypertensive. A Nissei DS-250 instrument was used for 24-hour monitorization of BP. The BP was recorded with 20-minute intervals (07 am - 10 pm) during daily activities and with 30-minute intervals (10 pm - 07 am) during night, totaling 24 hours. Cases with a nocturnal drop of 10% or more in the mean systolic and diastolic BP were classified as "dippers," while others were called "nondippers". Independent samples t-test was used to compare between the diabetics and the control groups, and Mann-Whitney-U test was used for subgroup comparisons. One-way analysis of variance (ANOVA) test was used to analyze metabolic control of diabetics and Chi-Square was used to compare between dippers and nondippers. A $p<0.05$ was considered significant.

While age, gender, length deviation, and body mass index were similar between the diabetic and the control groups, systolic and diastolic BPs measured in the outpatient clinic were significantly higher than the mean 24-hour and daytime BPs measured by ABPM, for both diabetic and healthy children ($p<0.05$). There were no significant differences between the diabetic and the healthy children for BPs measured in the outpatient clinic. However, the mean 24-hour systolic BP measured by ABPM, daytime diastolic BP, and nocturnal systolic and diastolic BP were significantly higher in the diabetic patients (**Table 1**). At 24-hour monitorization, while 40 (47.1%) of the diabetic patients had normal diurnal rhythm of BP, 45 (52.9%) patients had disturbed diurnal rhythm. In the control group, these ratios were 60% and 40%. Although the ratio of disturbed diurnal BP rhythm (nondipper) was higher in the diabetic group than the control group, this difference was not statistically significant ($p>0.05$). When we analyzed the diabetic patients in relation to the degree of metabolic control, cases with good metabolic control had significantly lower mean 24-hour diastolic BP than cases with moderate metabolic control. Other parameters did not differ among cases with good, moderate, or poor metabolic control. When we analyzed the patients in relation to the duration of diabetes, cases with more than 5 years of disease had significantly higher levels diastolic BP measured both in the outpatient clinic and for 24 hours than cases with less than 5 years of disease. When factors likely to affect BP were analyzed separately, only one of the 5 patients with an RBMI >120 had the

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Table 1 - Outpatient and ambulatory blood pressure monitorization values of the diabetic patients and the control group.

Blood pressure (mm Hg)	Diabetic patient group	Control group	*P*-value
OP-SBP	106.41 ± 10.01	102.77 ± 10.46	>0.05
OP-DBP	68.94 ± 7.68	71.25 ± 13.99	>0.05
Mean 24-hour SBP	101.74 + 7.81	98.25 ± 8.59	<0.05
Mean 24-hour DBP	65.51 ± 6.53	64.62 ± 6.02	>0.05
Mean daytime SBP	105.69 ± 8.92	103.77 ± 11.50	>0.05
Mean daytime DBP	69.25 ± 6.24	66.90 ± 6.76	<0.05
Mean nocturnal SBP	96.31 ± 9.60	91.15 ± 10.55	<0.05
Mean nocturnal DBP	62.30 ± 8.73	58.57 ± 4.69	<0.05

OP-SBP - systolic blood pressure measured in outpatient clinic,
OP-DBP - diastolic blood pressure measured in outpatient clinic

mean daytime systolic BP above the 95th percentile. The same patient had the mean daytime diastolic BP between the 90th-95th percentiles. In this study, the mean 24-hour systolic BP, daytime and nighttime systolic and diastolic BP measured by 24-hour ABPM were found to be significantly higher in type-I diabetic patients compared to the control group. As type-I diabetic children are known to have higher arterial BP than healthy children or adolescents, early diagnosis of hypertension is highly important, especially in diabetic children in order to prevent ensuing complications.[5] For this reason, 24-hour ABPM is recommended, especially in diabetic patients, both to avoid measurement errors and to facilitate early diagnosis. White coat hypertension is defined as higher BP measured at the outpatient clinic than at home or by ABPM. White coat hypertension is especially common during the adolescent period. In a study, 70% of hypertensive children between 14-18 years of age had normal BP profiles measured by ABPM.[5] For this reason, measurement by ABPM is recommended for hypertensive patients before starting treatment. In our study, both diabetic and healthy children had significantly higher systolic and diastolic BPs measured at the outpatient clinic than measured for 24 hours with ABPM. In cases of high BP measurement during a random check at the outpatient clinic, ABPM should be applied both to confirm and to start, if required, antihypertensive treatment as early as possible. The ABPM allows detection of early changes in BP of adolescents and young adults. The ABPM utilization is emphasized for detection of subclinical increases in BP of diabetic children, which can be interpreted as the beginning of hypertension and as a warning sign for the development of diabetic nephropathy.[5] Several studies have shown elevations in systolic, diastolic, or both, BPs of diabetic children. In a multicentric study conducted by Holl et al,[5] while diabetic patients had higher daytime systolic BPs than healthy controls, there was no difference for daytime diastolic BPs. In our study, diabetic children and adolescents were found to have significantly higher mean daytime diastolic BPs as well as nocturnal systolic and diastolic BPs. A reduction of 10% or more in nocturnal BP during sleep compared to daytime BP is defined as diurnal variation of BP. If changes in daytime BP regulation are together with nocturnal increase in BP, it is called the "nondipping" pattern. The nondipping pattern is one of the poor prognostic factors and is associated with the development of renal disease, increased end-organ damage, and increased cardiovascular morbidity.[3] Although the nondipping rate was higher in the diabetic patients in our study, this difference was not significant when compared to the control group. This situation might be due to the inclusion of normoalbuminuric patients in our study. Obesity is a risk factor for hypertension in all age groups, diabetic or non-diabetic. In our study, the obesity rate was 5.8% (n=5). However, only one of those had hypertension in daytime systolic BP. Holl et al,[5] reported an association between obesity and systolic hypertension, and emphasized that this association is stronger for adolescents and young adults than for adult diabetics In the same study, duration, and metabolic control of diabetes were found to affect diastolic BP in young diabetic patients. Additionally, a significant association between metabolic control and diastolic BP was reported in this study. In our study, only the group with moderate metabolic control had significantly higher mean 24-hour diastolic BP than the group with good metabolic control. This result might have been influenced by the fact that the majority of

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our patients were in groups with good or moderate metabolic control. As a result of this study, we conclude that 24-hour ABPM is much more sensitive than the BP measurements at the outpatient clinic, especially for type-I diabetics with more than 5 years of disease duration and thus, ABPM is strongly recommended for prevention of diabetic complications.

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