Using computer based data acquisition and analysis system for nasal potential difference measurement in cystic fibrosis

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SUMMARY: Ergönül Z, Balkancı ZD, Yılmaz G, Kiper N, Yalçın E, Doğru D, Özçelik U, Göçmen A. Using computer based data acquisition and analysis system for nasal potential difference measurement in cystic fibrosis. Turk J Pediatr 2004; 46: 339-343.

Nasal potential difference (PD) measurement has been used as a diagnostic test for cystic fibrosis (CF). It has been shown that large differences in reproducibility of nasal PD measurement can exist between different study sites. These differences reduce the validity of studies. In our study we tried to measure nasal PD values for a group of Turkish CF patients by using a computer based data acquisition system, which could eliminate the bias due to using different voltmeters.

The CF group (n=40, mean age 9.3 years) value was -39.21±1.74 mV, and the control group (n=36, mean age 17.08 years) value was -18.24±1.48 mV (mean±SEM). Using the electronic data acquisition and analysis systems gave reliable results with high specificity (92%), sensitivity (79%), positive predictive value (95%) and negative predictive value (72%). Computer based data acquisition and analysis system provides suitable monitoring and continuous recording during measurements and facilitates repeat readings at the same distances along the nasal floor. Using electronic data acquisition may help to minimize the subjectivity in voltmeter measurements and hand analysis.

Key words: cystic fibrosis, nasal potential difference measurement.

The diagnosis of cystic fibrosis (CF) is made by the presence of one or more characteristic phenotypic features, a history of CF in a sibling, or a positive newborn screening test results plus laboratory evidence of an abnormality as documented by elevated sweat chloride concentration, identification of mutations in each of transmembrane conductance regulator (CFTR) gene known to cause CF, or in vivo demonstration of characteristic abnormalities in ion transport across the nasal epithelium[1]. According to the consensus statement by Rosenstein ad Cutting[1], genetic analysis and nasal potential difference (PD) measurement may be helpful in the diagnosis of CF, but these new tests have some limitations. Mutation testing for CFTR is limited by the fact that commercial laboratories currently test for only up to 87 of the almost 1,000 identified CFTR mutations[1], Nasal PD measurement requires a meticulous technique carried out by experienced individuals in accordance with standardized protocols and is not generally available outside a limited number of research centers[2]. In order to eliminate standardization problems of nasal PD measurement techniques, electronic data acquisition and analysis have been suggested[3].

Cystic fibrosis patients demonstrate a markedly more negative PD across respiratory epithelia than normal controls[4]. In normal epithelia, chloride ions enter the cell across the basolateral membrane and exit the apical surface down an electrical gradient via chloride channels[5]. The CF gene product, CFTR, functions as a chloride channel that also acts as a regulator of heterologous ionic channels[6]. Regulation of these channels is by phosphorylation in response to an increased intracellular cyclic adenosine monophosphate (cAMP) concentration. Sodium ions enter the

apical surface of the cell from the lumen via amiloride-sensitive sodium channels, thus creating a voltage or PD across the apical surface of the cell[5]. The airway epithelia of CF patients have impaired cAMP mediated chloride secretion and increased transcellular sodium absorption. This results in increased viscosity of airway secretions, probably in association with other mechanisms, such as abnormal transport of nonionic solutes and oversulfation of respiratory mucins[7]. Accelerated sodium absorption across a relatively chloride-impermeable barrier generates an increased negative transepithelial PD that can be measured in the nasal epithelium with a method developed by Knowles and coworkers in 1981[8].

Nasal PD measurement techniques commonly vary between centers. These differences reduce the validity of studies that use nasal PD as study outcomes. In order to minimize variance, standardization of the voltmeter input impedance and electronic data acquisition have been suggested[3]. In our study we measured nasal PD using a computer based data acquisition and analysis system, which could eliminate the bias due to different voltmeters and hand analysis.

Material and Methods

Subjects

Our study included 76 subjects in two different groups. The CF group comprised 40 patients (18 females and 22 males; mean age 9.3 years, range 2-20 years) with typical CF. The control group comprised 36 controls (17 females and 19 males; mean age 17.1 years, range 2-34 years). CF diagnosis was made by typical clinical findings, family history, elevated sweat chloride levels (>60 mmol/L) and/or by the presence of CFTR mutations. Genetic analyses of the eight most frequent mutations causing CF (ΔF508, N1303K, 621+1G-T, R1162X, R347P, 2789+5G-A, 2184delA, 1677Δ) were performed.

Nasal Potential Difference Measurement

Nasal PD was measured using an adaptation of the method described by Alton et al.[4] Briefly, an Ag/AgCl exploring electrode (SLE instruments, South Croydon, Surrey, UK) was positioned into a disposable Foley size 8 urinary catheter filled with an equal mixture of Ringer's lactate and electrode gel (Signa gel, Parker Laboratories Inc, Fairfield NJ, USA). The reference electrode was taped on the superficially abraded skin on the anterior surface of the forearm. Skin abrasion for superficial stratum corneum was performed with an abrasive pad. The computer based data acquisition system replaced a high impedance voltmeter for collecting, recording and evaluating nasal PD measurement data. Electrodes were connected to a DA100B differential amplifier module of MP100 computer based data acquisition and analysis system (Biopac System, Inc.). Before recordings, the offset of the electrodes was measured and appropriate corrections made. The electrodes were calibrated by placing the reference electrode in close contact with the exploring electrode in the gel. Values $>\pm 5$ mV were excluded and the electrodes replaced. The appropriateness of the equipment was ascertained by measuring the PD of the index finger and the values >-40 mV were defined as acceptable. The exploring electrode was passed along the floor of the nose and advanced until the maximal PD had been passed and then gradually withdrawn. The highest stable negative voltage was determined. Measurements were made from each nostril. The offset potential value was subtracted from the readings. The mean of the two side was used to give the nasal PD.

Informed written consent was obtained from all patients or from their parents. The Ethics Committee of Hacettepe University approved the study.

Statistical Analysis

The results of different groups were analyzed by the test performance criteria, namely sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). These were calculated using the following formulae: sensitivity a/(a+c), specificity d/(d+b), PPV a/(a+b), and NPV d/(d+c), where a is the test-positive CF group (n=38), b is the test-negative CF group (n=2), c is the test-positive control group (n=10), and d is the test-negative control group (n=26). According to test performance criteria, the optimal value helpful for the diagnosis was accepted as -25 mV. The negative values of greater than -25 mV were accepted as test-positive. All values were expressed as mean±SEM. Baseline nasal PD values were compared by the two-sample t test between groups. In all analyses p value <0.05 was regarded as statistically significant.

Results

The CF group nasal PD was -39.21±1.74 mV (range -21.7 to -61), and the control group nasal PD was -18.24±1.48 mV (range -1.6 to -37.5) (mean±SEM). Nasal PD values of the CF group were found to be significantly higher than control values ($p<0.00001$). Using the cut-off of ≥-25 mV for the nasal PD gave a sensitivity of 79% and a specificity of 92% with PPV of 95% and a NPV of 72%.

No significant difference was noted between left and right sides in the two groups. Neither the age nor sex of the subject influenced the measurements. There was no significant correlation between nasal PD and sweat chloride levels. Figure 1 shows the results of nasal PD obtained in CF patients and controls. Figure 2 shows a typical diagram of a healthy subject's nasal PD with the MP-100 system. Figure 3 is the nasal PD diagram of a CF patient. In two CF patients, readings were made at the time of an acute upper respiratory infection and presence of a nasal polyp. Both were retested after resolution of the acute infection or after nasal polyp surgery and showed typically more negative values.

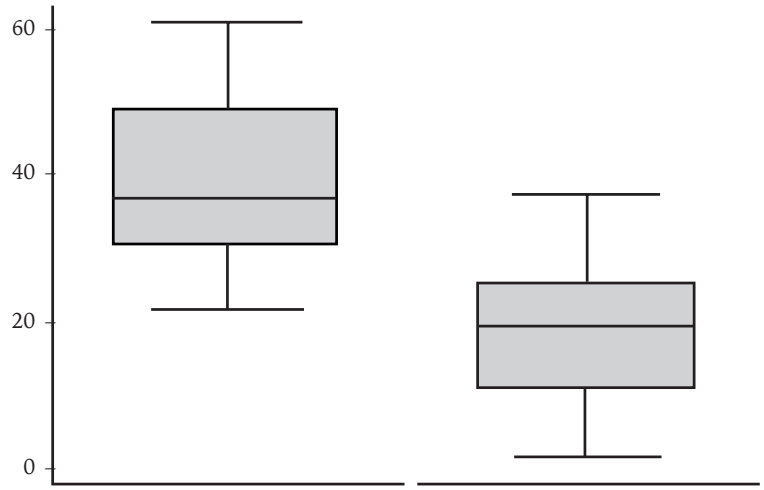

Fig. 1. Distribution of nasal potential difference (PD) of cystic fibrosis (CF) patients (n=40) and controls (n=36) ($p<0.00001$).

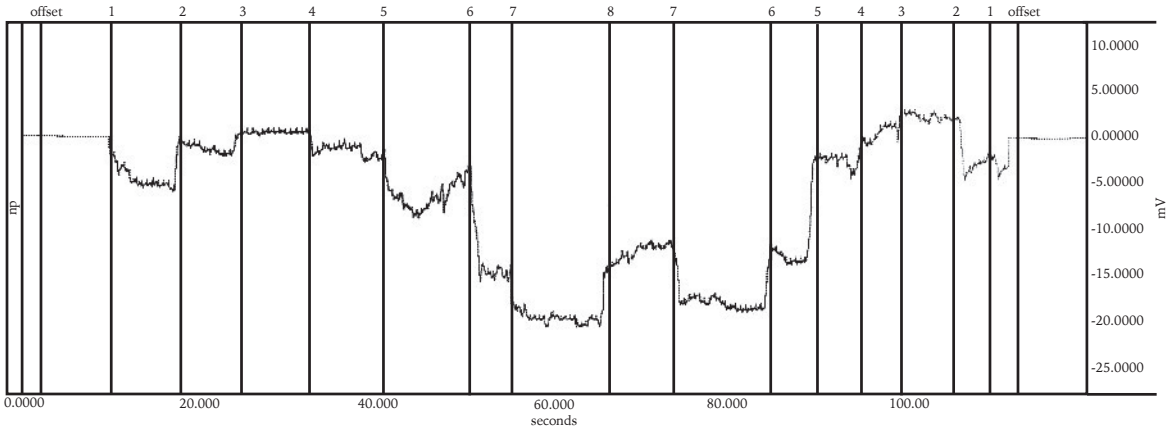

Fig. 2. The nasal potential difference measurement diagram of a control subject by MP-100 system. Recordings were taken from the floor of the nasal cavity at 1 cm intervals. The electrode was advanced until the most negative point had been passed and then slowly withdrawn. The most negative potential was accepted as the nasal PD value.

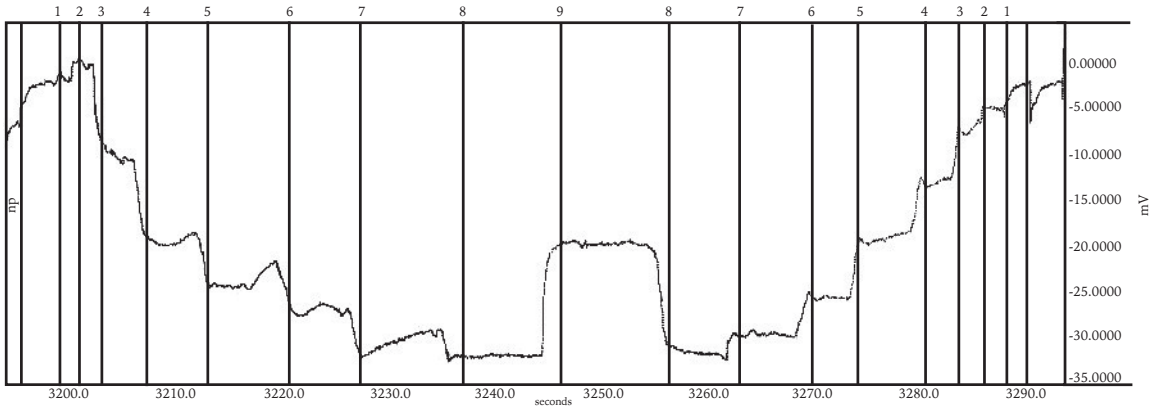

Fig. 3. The nasal potential difference measurement diagram of a CF patient by MP-100 system. Recordings were taken from the floor of the nasal cavity at 1 cm intervals. The electrode was advanced until the most negative point had been passed and then slowly withdrawn. The most negative potential was accepted as the nasal PD value.

Discussion

Nasal PD measurement has been used as a diagnostic test for assessing the abnormal transepithelial ion transport in CF[9,10]. In the Cystic Fibrosis Foundation Consensus Panel, in vivo demonstration of characteristic abnormalities in ion transport across the nasal epithelium has been accepted as a criterion for the diagnosis of CF[1]. Despite its proven diagnostic value, nasal PD measurement methods have been considered. It has been shown that there are large differences in reproducibility of measurements between different study sites[3].

In the conventional perfusion protocol for nasal PD measurement, subcutaneous placement of the reference electrode carries a risk of local infection and problems of cooperation in children[7]. It has been reported that the investigation was technically very difficult and uncomfortable even for the most cooperative adult volunteers[4]. This type of testing requires special training and meticulous attention[2]. A simplified method for measurement of nasal PD has been described by Alton[4]. The handling of this simplified device does not require extensive physiologic and bioelectric knowledge. The use of an epicutaneous reference electrode not only reduces the risk of infection but also improves cooperation and allows repeated nasal PD measurements[7].

One of the main problems in standardization of measurement techniques is to provide the appropriate clinical electrical engineering standards[1]. It has been reported that voltmeter input impedance standardizations may help to minimize differences in measurements. Electronic data acquisition and analysis systems were found desirable for alleviating subjectivity in nasal PD recordings[3].

For these reasons we selected Alton's protocol in our study with a computer based data acquisition and analysis system. The MP100 computer based data acquisition and analysis system is commonly used in medical schools and research centers for recording bioelectrical potentials. It provides suitable monitoring and continuous recording during measurements with 50/sec sample rate, and helps to detect the most negative potential points and to repeat readings at the same distances along the nasal floor. It is very useful for saving and printing data in graphs. It eliminates the subjectivity of the person who performs the measurement.

It has been reported that a raised basal nasal PD is strong evidence for diagnosis of CF. However, the absence of a raised PD does not rule out CF because a false-negative results occurs in the presence of an inflamed nasal epithelium[1,12]. In our study the measurements were repeated for the patients with upper respiratory tract infections when no nasal pathology was present. We observed more negative nasal PD values in CF patients after treatment of nasal pathology.

The nasal PD measurement test with electronic data acquisition and analysis system gave reliable results with high specificity, sensitivity, PPV and NPV. Our study was the first on nasal

PD measurement in a Turkish CF group. Our reference values may help other laboratories that plan to set up nasal PD measurement as a clinical diagnostic tool. We suggest that using electronic data acquisition may help to minimize the subjectivity in voltmeter measurements and hand analysis for nasal PD in CF patients.

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