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PCSOFT PROGRAM FOR THE ACQUISITION AND ANALYSIS OF VOLTAGE-DEPENDENT PROPERTIES OF MACROSCOPIC CALCIUM CHANNEL CURRENTS IN RAT VENTRICULAR MYOCYTES

Ömer Hotomaroğlu* • Belma Turan**

SUMMARY

Computers that exist with improving priority in physiology laboratories facilitate the automation of experiments as well as the storage and analyses of physiological signals by the performance of real-time and on/off-line dedicated custom softwares. Physiological signals converted to electrical signals by transducers can be transferred into computers via analog to digital converters (ADC) or inversely, digital electrical signals generated by computers can be applied to physiological systems via digital to analog converters (DAC). In the above view of computers role in electrophysiology, it has been developed a homemade software named PCSoft for both on-line recording and real-time and off-line analysis of L-type Ca-channel currents by patch-clamp technique. PCSoft can also be able to measure the activation time and inactivation time constants as well as voltage-dependent properties of this type channels. Besides the above properties of this software, the ease of use of it, in contrast to many commercial softwares, was discussed under the light of literature.

Key Words: Data analysis, Isolated ventricular myocytes, L-type Ca-current, Patch-clamp technique, Software program

The membranes of all cells are permeable to ions and small molecules, whose passage through the membrane is usually by means of specific transport proteins, termed "channels". Channel proteins are thought to form continuous pores through the membranes. When channels are open, they allow ions to flow passively through them, down an electrochemical gradient.

Ionic channels are undoubtedly found in the membrane of all cells. Their known functions include establishing a resting membrane potential, shaping electrical signs, gating the flow of messenger Ca²⁺ ions, controlling cell volume and regulating the net flow of ions across epithelial cells of secretory and re-uptive tissues. Ca²⁺-channels are present in a large variety of excitable and non-excitable cells. In heart muscle, Ca²⁺-channels give rise to the slow inward Ca²⁺ current (I_{Ca}) and participate in many aspects of cardiac function (1,2). For example, I_{Ca} is involved in the development of the cardiac action potential (3,4) in the generation of electrical activity in nodal tissue

(5), in the initiation and development of myocardial contraction (6,7) and in the neurohormonal control of these functions (8,9). Our understanding of how Ca²⁺-channels function has been greatly facilitated by the use of the patch-clamp technique (10), which, in its various configurations, has permitted the study of Ca²⁺-channels at both the macroscopic (i.e. whole-cell I_{Ca}) and microscopic (i.e. single Ca²⁺-channel) levels. This technique has demonstrated that multiple forms of Ca²⁺-channels exist. In heart, two different types of Ca²⁺-channels have been found in mammalian atrial and ventricular cells (11-13).

Despite the considerable advances in understanding Ca²⁺-channel function, it has been difficult to explain all the properties of the macroscopic I_{Ca} in terms of the properties of the single Ca²⁺ channels. For example, the inactivation of Ca²⁺-channels induced by a depolarization of the membrane and the recovery from inactivation (reactivation) seen upon repolarization remain poorly understood. It has been proposed that Ca²⁺-channels may be inactivated both by a vol-

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** Department of Neurology, Ankara University Faculty of Medicine

*** Department of Pathophysiology, Ankara University Faculty of Medicine

tage-dependent mechanism and by a Ca^{2+} -dependent mechanism which depends upon the influx of Ca^{2+} into the cell during the Ca^{2+} current. Reactivation of whole-cell I_{Ca} has also been shown to be affected by Ca^{2+} ions entering the cell during a pre-pulse in cardiac preparations (14,15).

A sensitive PC and signal acquisition interface programming is absolutely necessary in order to monitor, save and analyze slow inactivating L-type Ca^{2+} current which is the single type Ca^{2+} current in isolated (rat) ventricle cells, by whole-cell patch-clamp method.

In this study, development of a budget-priced (unextravagant) software for our patch-clamp experiment system is aimed for both on-line recording and real-time and off-line analysis of L-type Ca-channel current of isolated ventricle cells. The developed software (PCSoft) is featured with measurement of activation and inactivation time constants of Ca^{2+} current and voltage-dependent properties of Ca-channels such as I-V relation, inactivation and reactivation. Functionality and touch-key, ease of use of this developed software in contrast to extravagant commercial software is discussed under the light of literature.

METHODS & MATERIALS

Materials:

The methods used for cell dissociation, whole-cell patch-clamp recording, superfusion and internal perfusion of the cells have been extensively described in our previous paper (16) and were used with no major modification in this present study.

Briefly, myocytes were isolated according to the method of Puceat et. al. (17). At the end of the collagenase perfusion, a piece of left ventricle was cut off and stirred gently to obtain cells. The cells were suspended in tissue culture petri dishes at 37°C . These cells were used within 6-8 hours of their isolation.

The cells in a tissue culture dish were suspended by gravity with a solution containing (mM) CsCl 20, NaCl 117, CaCl_2 1.8, MgCl_2 1.7, Na_2HPO_4 1.5, NaHCO_3 4.4, glucose 10 and HEPES 10, pH 7.4 at 20°C and bubbled with 100% O_2 . To measure accurately I_{Ca} with no contamination of other ionic currents, the cells were bathed in K^+ -free (above) solution and added $50\ \mu\text{M}$ tetrodotoxin in the capillary to block the sodium current.

The internal solution in the patch electrode (where patch electrode pipette resistance R_p , $R_p=1-3\ \text{M}\Omega$)

contained (mM) CsCl 120, MgCl_2 4, Na_2ATP 3, Na_2 -creatine phosphate 5, Na_2GTP 0.4, K_2EGTA 5, CaCl_2 0.062, and HEPES 10, adjusted to pH 7.1 with KOH.

By recording current passing through pipette by application of voltage pulses on pipette with 1 mV of amplitude yielded the patch-electrode pipette (R_p) resistance which has been monitored continuously by the developed software (PCSoft). The extremely low pipette capacitance (3-5 pF) has been omitted in this study like in most of whole-cell patch-clamp studies.

Experimental Arrangements and Data Analysis

The methods used for whole-cell patch-clamp experiments and for the analyses of data were derived from those developed on single cells (10, 18, 19).

Recording and analyses software of patch-clamp named PCSoft was totally developed in our laboratory with Turbo Pascal programming language and run on a 386-DX 40 MHz IBM compatible PC with a programmable signal acquisition card (Advantech, PCL-818PG) featured with analog-digital conversion, digital-analog conversion and digital output modules. Signals from cell and electrode filtered at 1 kHz by 5-pole Tchebicheff filter of patch-amplifier (Bio-Logic RK300) were sampled at 5 kHz. Conversions were handled by interrupt transfer method and were saved on harddisk on-line. Voltages applied on electrode and cell via headstage of patch-amplifier were generated by PCSoft programming the digital-analog conversion module.

PCSoft facilitates user to set the cell holding potential, duration and amplitude of the test pulse to obtain any cell current, labeling of capillary tubes those apply various solutions to the cell in order to record application solution and time course. Also, it allows the user to record the amplification gain by one key touch and warns the user if the amplifier was left on AC mode at current recording mode, if the amplification gain was not good enough or excessive and detects if there are any cable disconnection. PCSoft, continuously monitors each Ca-current ($I_{\text{Ca}}(t)$) along with I_{peak} and I_{200} values variation during the whole experiment and displays quality of patch. Channel kinetics (I-V curve, Inactivation and Reactivation) are also displayed real-time and saved on-line, as well as every recorded current. Each current is saved with including the information of recording gain, solution and quality of patch during recording period.

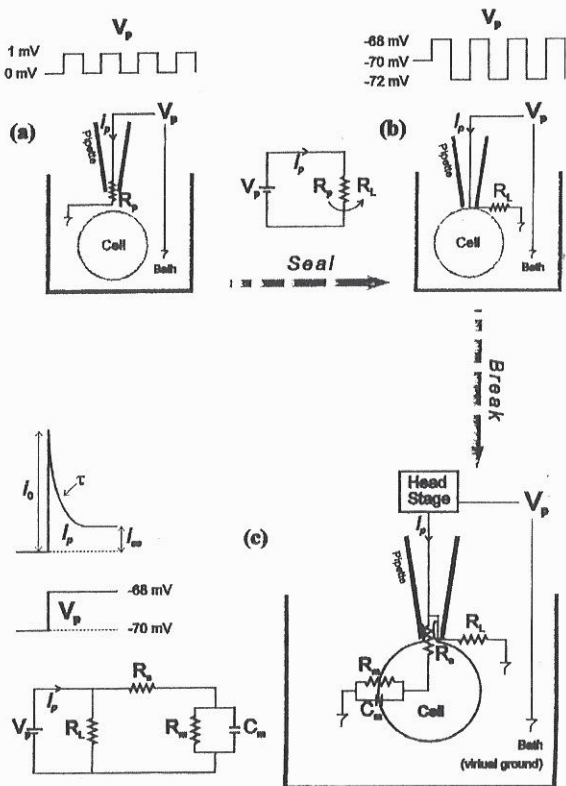


Fig. 1. Whole-Cell Patch-Clamp Configuration and Its Electrical Equivalent Circuit

Electrode resistance (R_p) (a), as seal progresses becomes negligible besides leak resistance (R_L). (b). To consider the seal to be completed the R_L should be in the order of G Ohms. When the cell membrane breaks open the whole-cell configuration is established (c) and this configuration can be represented by the equivalent electric circuit which is shown in the figure. R_m and C_m values can be calculated by using currents induced by application of 2 mV square pulses.

Determination of Serial Resistance (R_s) and Cell Capacitance (C_m) and Resistance (R_m)

An electrical equivalent circuit was designed to simulate patch-clamp electronics (shown in Figure 1) have been used to identify C_m and R_m (20). Cell and electrode features have been considered to establish the simulation circuit and cell parameters R_m and C_m are included at the incidence of the rupture of the membrane (in the whole-cell configuration of patch-clamp method). Serial resistance (R_s) corresponds to the sum of pipette tip resistance which dominates mainly, pipette solution resistance, bath resistance and any minimal serial resistance occurs between the reference electrode and bath junction. Leakage resistance (R_L) represents the resistance which occurs between

the wall of pipette tip and the membrane pieces adheres on the pipette wall after the rupture of cell membrane. R_L increases rapidly and remarkably upto gigohms during a perfect seal.

The current passing through the cell generated by application of -68 mV pulse from holding potential of -70 mV which does not evoke any voltage-dependent channels has been evaluated to calculate membrane resistance and capacitance. 10 separate, totally membrane resistance and capacitance depended currents have been averaged in time domain. Time constant (t) of the exponential decay has been determined by measuring the time passing for the current decreasing to the value of i_0 / e from $t=0$ to calculate the membrane capacitance (C_m). Calculation of membrane capacitance (C_m), serial resistance (R_s) and membrane resistance (R_m) has shown below:

Mesh and node analyses of above patch-clamp equivalent circuit yields below initial equations:

$$i_s = i_p - \frac{V_p}{R_L} \quad (1) \quad V_m = V_p - i_s R_s \quad (2) \quad i_s = \frac{V_m}{R_m} + C_m \frac{dV_m}{dt} \quad (3)$$

Derivation of first equation which yields $di_p/dt = di_p/dt$ at voltage-clamp condition $dV_p/dt = 0$ and above equations reveals the differential equation of current passing through pipette (i_p),

$$a = \frac{R_m R_s C_m}{R_m + R_s} \quad a = \frac{R_m + R_s + R_L}{R_L + (R_m + R_s)} \Rightarrow a = \frac{di_p}{dt} + i_p - bV_p = 0$$

with solution of $i_p(t) = bV_p(t) + ke^{-t/a}$ where a corresponds to the time constant (τ) of exponential decay in i_p : $a = \tau = R_m R_s C_m / (R_m + R_s)$ and where $R_m \gg R_s$, $\tau \approx R_s C_m$ and experimentally, $R_m > 200 \text{ M}\Omega$, $R_s < 10 \text{ M}\Omega$ in general as well.

At $t=0$; all of the current flows through uncharged C_m and $i_0 \approx V_p / R_s$ since $R_L \gg R_s$. At $t=200 \text{ ms}$; C_m gets charged and steady-state current (i_{ss}) flows only through the resistors. Finally, $i_{ss} \approx V_p / (R_s + R_m)$ can be obtained under the condition of $R_L \gg R_s + R_m$.

Validity of all of the above solutions depends on the high resistivity attenuation of R_L which also shows the success of the seal, so called *gigaseal*.

Recording of Ca-Channel Current

The depolarization of a cell was developed by clamping the cell to 0 mV, from holding potential of -

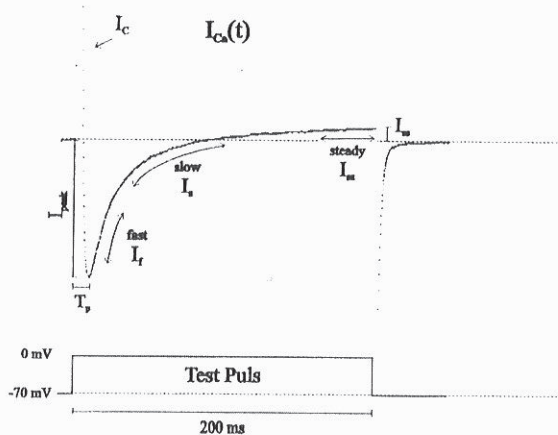


Fig. 2. Time Course of the Calcium Currents I_{Ca} recorded from a cell clamped at 0 mV for a duration of 200 ms. I_f represents the fast, I_s represents the slow, I_{ss} represents the steady state components and T_p represents the activation time of the current.

70 mV for 200 ms to obtain Ca-channel currents (I_{Ca}). This square pulses was used to record I_{Ca} and was called testpulse (TP) (Fig 2). I_{Ca} , being much slower with respect to cell capacitive current (I_C), Na^+ and K^+ channels being blocked with channels antagonists, is recorded without any disturbance (Fig 2). I_{Ca} recordings are obtained by depolarization of cell once in 4 s with testpulse. The amplitude of elicited I_{Ca} is determined by calculation of the difference between the peak value of the inward current (I_{peak}) and totally inactivated (steady-state) value of current after 200 ms (I_{200} or I_{ss}). The period between the onset of application of testpulse and development of I_{peak} is measured as activation time (T_p), where inactivation of I_{Ca} is defined according to the following equation (Scamps et al. 1990).

$$I_{Ca} = A_f e^{-t/\tau_f} + A_s e^{-t/\tau_s} + A_{ss}$$

fast slow steady

Inactivation of I_{Ca} has 3 phases which can be expressed as a total of 2 exponential (one fast and one slow) and 1 DC current. A_f , A_s and A_{ss} coefficients corresponds to amplitudes of fast, slow and steady components, respectively. At initial state ($t=0$), amplitude of initial current (I_0) has simply been determined only by serial resistance (R_s) since the membrane has not been charged yet ($R_s = \Delta V / I_0$). As the membrane gets charged, the current passing through the membrane

exponentially decreases until it forms into a steady-state current (I_{ss}) which depends on R_s and membrane resistance (R_m).

Determination of Current-Voltage Relation, Inactivation and Reactivation of Ca-Channels:

Currents (I) developed by applied potentials (V) are obtained to reveal the macro characteristic conductance of Ca-channels. Cells at holding potential of -70 mV are depolarized to different potentials ranging from -60 mV to +60 mV by 10 mV stepwise increments and the magnitude of I_{Ca} at each depolarization has been determined to form current-voltage (I - V curve) (Fig 3a).

To identify the inactivation of Ca-channels, first cells are depolarized by a prepulse (PP) of 200 ms ranging between -60 mV to +60 mV, then rested at holding potential of -70 mV for 3 ms and then depolarized by testpulse (TP) again (Fig 3b). Each I_{Ca} obtained by TP after corresponding PP is proportioned to I_{Ca} obtained by TP at the absence of PP and plotted according to PP potentials. Proportions of I_{Ca} developed by TP 3 ms after PP to a control I_{Ca} , reveals the percentage of inactivated Ca-channels.

The reactivation of Ca-channels is determined by applying two consequent testpulses (TP1, TP2) while resting the cell at holding potential for varying duration (ΔT) in between the testpulses (as shown in Fig 3c). Ratios of I_{Ca} obtained from TP1 to TP2 is plotted according to application intervals (ΔT , in seconds). Reconstruction of I_{Ca} amplitude obtained from TP2 compared to I_{Ca} amplitude obtained from TP1, demonstrates the reactivation of Ca-channels.

RESULTS

The mean membrane resistance (R_m) and capacitance (C_m) values with the standard deviations of enzymatically isolated rat ventricle myocytes recorded by developed PCSoft patch-clamp software utilizing the whole-cell patch-clamp method are given in Table 1.

Membrane parameters (capacitance and resistance) are measured after averaging 10 separate capacitive currents in time domain and calculated as described in methods. High standard deviations in both measured parameters are representing the variation between the rats in terms of their weights. The mean serial resistance (R_s) during our all current recordings were $8.54 \pm 1.99 \text{ M}\Omega$.

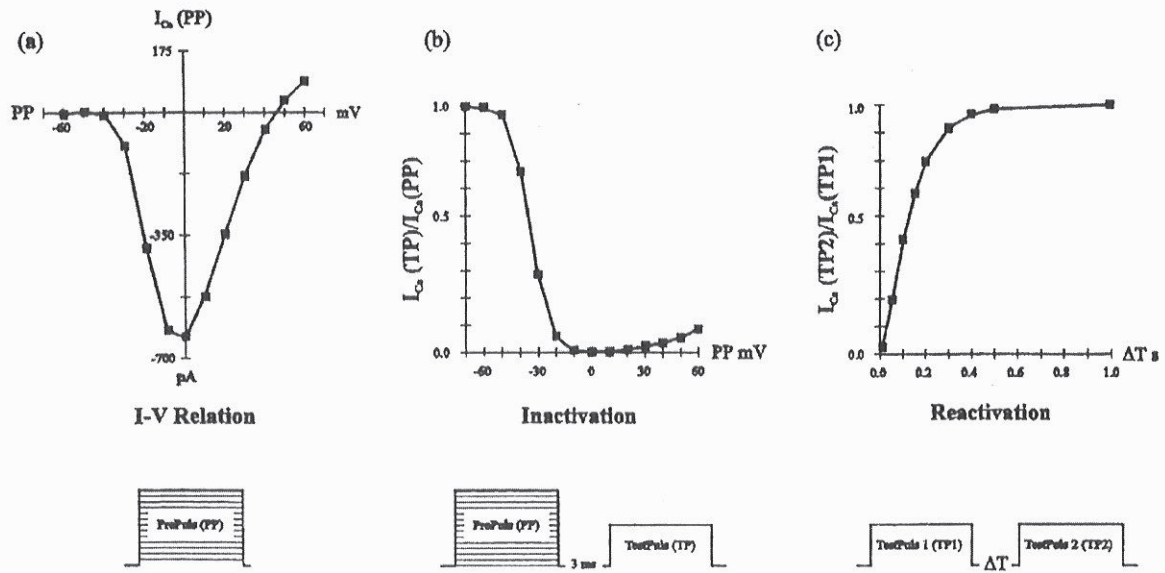


Fig. 3. Kinetics of L-type Ca-currents.

(a) Current-voltage relation, (b) inactivation and (c) reactivation curves and related voltage pulse paradigms for L-type Calcium currents.

Table 1: The Measured Membrane Resistance and Capacitance of Rat Ventricle Myocytes

Parameters	C_m (pF)	R_m (MΩ)
n=17	221±54.14	308.48±84.94

L-Type Ca-Channel Currents

Current passing through L-type Ca-channels (I_{Ca}) of rat ventricle myocytes has been generated and recorded by PCSoft. Voltage-dependent L-type Ca-channels which can open as a response to membrane depolarization are activated by clamping the membrane to 0 mV from holding potential of -70 mV for 200 ms. In order to reveal the I_{Ca} , the voltage-dependent K^+ -channels are blocked both from inside and outside by replacing the KCl with CsCl in electrode solution and external perfusion solution, while Na^+ -channels are blocked by addition of TTX in perfusion solution only.

Although a steady I_{Ca} amplitude is reached within first 1-2 min. of onset of recording, sometimes, almost a linear decline in amplitude of I_{Ca} (rundown) is seen, as shown in Fig 4, which is, mostly due to the leakage between the pipette wall and membrane. Since this rundown causes miscalculations when the variation of I_{Ca} amplitude is considered, the slope of decline has been deducted (by fitting to regression) from rundown before any calculations are done.

Activation duration time (T_p) of I_{Ca} is measured as time takes I_{Ca} reaching upto its peak value (I_{peak}). I_{peak} and I_{200} are measured on-line as described in methods. Inactivation of I_{Ca} is fit in 2 exponential by Mar-

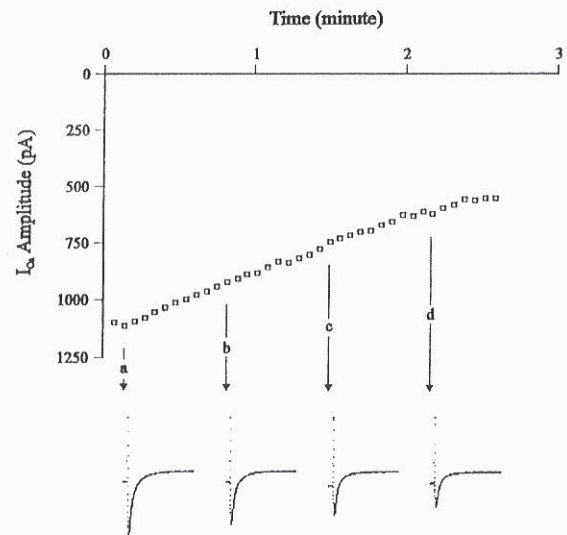


Fig. 4. Time course of run down in Ca-current traces. Time course of I_{Ca} peak values recorded from a cell for a duration of approximately 3 minutes. I_{Ca} amplitude gets smaller (rundown) with time because of the leak caused by an imperfect seal, but as seen, in a faster time scale, for time points a, b, c and d, shapes of the currents are not effected.

Table 2: I_{Ca} amplitude, activation time and inactivation time constants.

Parameters	T_p (ms)	τ_f (ms)	τ_s (ms)	I_{Ca} (nA)
n=17	19.93±3.01	13.29±6.64	34.80±10.84	1.43±0.62

Values in this table are calculated as mean±standard deviation. n represents the number of myocytes obtained from different rats. T_p represents the activation time of Ca-current τ_f and τ_s are representing the fast and slow components of inactivation of Ca-current.

quardt numerical method and slow (τ_s) and fast (τ_f) time constants of inactivation are derived. All above mentioned parameters of I_{Ca} are summarized in Table 2.

Values in table 2 shows I_{Ca} activating slower than I_{Na} ($T_p > 5$ ms) as well as inactivation of I_{Ca} progress in 2 phases (fast, slow). Above results are in correspondence with other published data for L-type Ca-channel findings.

Current-Voltage Relation, Inactivation and Reactivation of L-Type Ca-Channels

Current-voltage relation of Ca-channels is studied to reveal voltage dependency of activation of Ca-channels. Figure 5 shows averaged I-V relation of Ca-channels obtained from 14 different ventricle myocytes in control conditions. The expected bell-shaped I-V relation of macroscopic L-type Ca-channels is likely to be found in most cardiac tissues.

However, high standard deviation was exposed in measured I_{Ca} amplitudes as shown in Figure 5. Although rats were chosen with similar weight, still the cellular differences in size and hence C_m yielded in high standard deviation and couldn't have been corrected by calculating current density instead of amplitude due to lack of cellular size measurement system. Nevertheless, it is noticeable in Figure 5 that the activation of Ca-channels becomes obvious at -40 mV, reaches to a peak at 0 mV while the reversal potential is at 60 mV and these findings are in harmony with previously reported characteristics of L-type Ca-channels.

Today, it is accepted that the inactivation of Ca-channels occurs by both voltage and intracellular free Ca^{2+} level depended mechanisms (8, 21, 22). To investigate channel kinetics, inactivation of Ca-channels has been studied and an averaged inactivation curve of Ca-channels obtained from 10 different ventricle myocytes in control conditions is given in Figure 6 and as illustrated, the inactivation of Ca-channels is in U-shape. Although the Ca-channels inactivate totally at 0 mV, an activation regenerates at positive pulses.

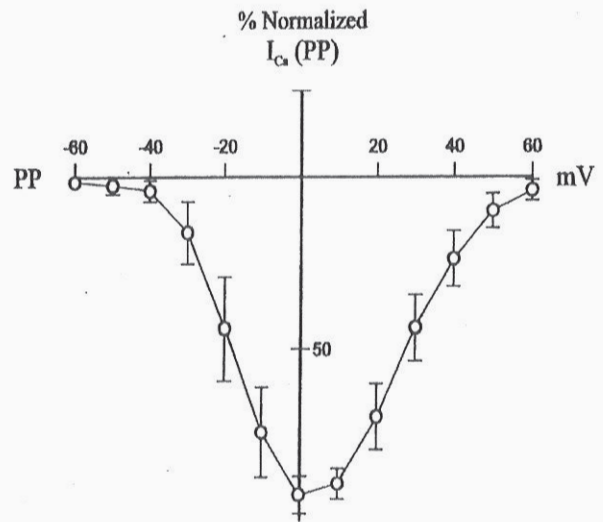


Fig. 5. Averaged current-voltage relation for the Ca-current. Effect of different concentrations of Selenite on the voltage-current relation of Calcium currents (I-V). I-V relation is obtained as mentioned in the methods section. Delta I_{Ca} values are measured as the difference between the maximum value and the value at the end of the 200 ms. pulse. I-V curves are normalized to their maximum value.

Furthermore in channel kinetics, the reactivation of Ca-channels has been studied and an averaged reactivation curve of Ca-channels obtained from 5 different ventricle myocytes in control conditions is plotted in Figure 7. The 90% reactivation of Ca-channels, approximately 1 s after inactivation is also found in parallel with the previous reports.

DISCUSSION

Computers are becoming more and more important for the automation of the physiological experiments as well as for the storage and analysis of experimental data via real-time and on/off-line software. By using transducers physiological responses can be translated into electrical signals which in turn can be sampled and stored in computers as digital data using

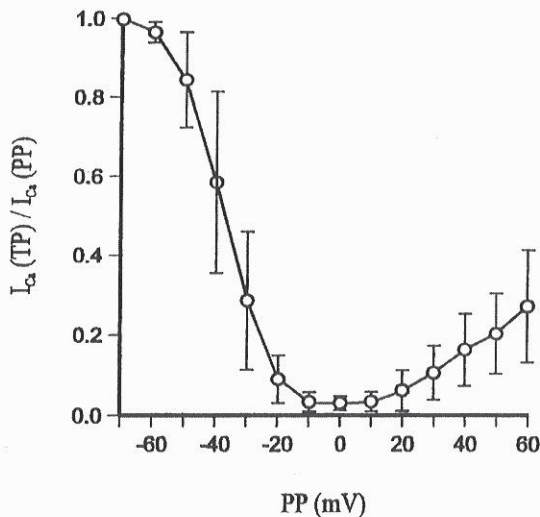


Fig. 6. Averaged inactivation curve for the Ca-current. Inactivation of the Calcium currents in normal ventricular myocytes. Inactivation curves are obtained by the double pulse protocol as mentioned in the methods section. In the figure the ratios of the delta I_{Ca} obtained by the test-pulse after the application of the pre-pulse to the delta I_{Ca} obtained in the absence of the pre-pulse are shown.

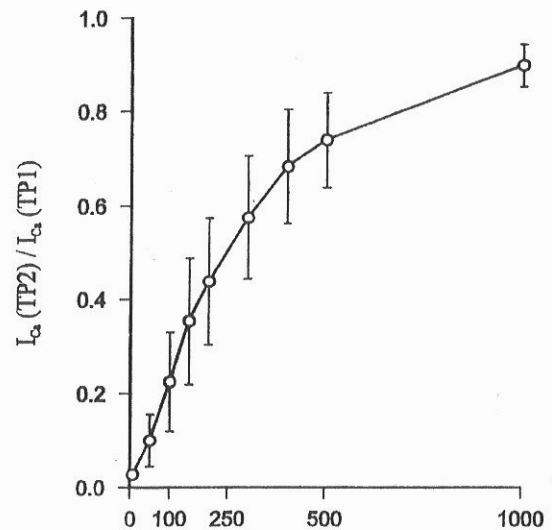


Fig. 7. Averaged reactivation curve for the Ca-current. Reactivation of the Ca-currents in normal ventricular myocytes. Reactivation curves are obtained by the double test-pulse protocol with a variable interval as mentioned in the methods section. In the figure the ratios of the delta I_{Ca} obtained by the second test-pulse to the delta I_{Ca} obtained with the first test-pulse are shown.

analog/digital converters. Alternatively any desired stimulus can easily be applied to a biological system using the same set-up. The most critical component required for these systems to function effectively is the software that controls and runs the system.

In this study we developed a software (PCSoft) which is suitable to our experimental set-up and budget requirements, to record and to analyse (real time or off-line) inward Ca-Currents in isolated cardiac myocytes. This program is comparable with similar commercial software currently available on the market today (10, 14, 18, 19, 22, 23).

Today patch-clamp is a popular method with a wide range of applications. This technique makes it possible to perform voltage clamp experiments on single-cells with dimensions measured in microns, in addition to this it enables researchers to measure and analyse currents passing from a single ionic-channel which was impossible before.

The connection between the computer and the patch-clamp amplifier was established by using an interface unit with an A/D and D/A converter.

PCSoft monitors the series resistance (R_s) real-time by applying a square voltage pulse to the patch pipette. Series resistance is called "series" because it is

in series to the voltage source and always includes pipette resistance as a series component. Different than many other patch-clamp software PCSoft displays the value of the series resistance so the user can monitor seal development by observing the numeric value of the resistance as well (18, 21, 22, 24-27). After the seal is formed further application of this square pulse may cause break down of the membrane so the pipette potential should be changed to the resting potential of the cell (cardiac cells resting membrane potential is around -60 mV, -80 mV). PCSoft by monitoring the series resistance can detect the formation of the seal (The necessary condition is $R_s > 100 \text{ MW}$) and, even if the user does not act in time, it brings the membrane to more negative potentials and continues the R_s monitoring by applying pulses between -68 and -70 mV.

After the whole-cell configuration is established all the membrane and seal parameters are recalculated and displayed. Membrane resistance (R_m) and capacitance (C_m) values calculated by using PCSoft are in agreement with the values reported previously in the literature (19). Value of the series resistance (R_s) is recalculated alongside other membrane parameters and a specific feature of PCSoft, which differs from ot-

her patch-clamp softwares, displays the value of R_s and if this value is too high warns the user.

In addition to all these features, PCSOFT warns the user if the gain setting on the amplifier is too low or if the connection to the amplifier is lost. Even if the user forgets, when the current recording is started PCSOFT automatically calculates and stores the values of R_s , R_m and C_m . If the current recording has to be stopped due to reasons like changing the perfusion tube, when the recording is restarted PCSOFT does all the above mentioned calculations again and confirms that the membrane and seal parameters still have acceptable values.

PCSOFT shows the time course of each recorded calcium current and also allows the user to follow the

quality of the seal throughout the experiment by monitoring the base-line, maximum (I_{max}), and steady state (I_{ss}) currents on-line. Additionally it displays the inactivation and reactivation curves on-line.

All these online analysis functions and the array of warnings of PCSOFT allow an efficient and less error prone use of the program and make it possible for the user to make quick and appropriate decisions and change the course of the experiment as it progresses.

ACKNOWLEDGEMENT

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CYTOLOGIC DIAGNOSIS OF MEDULLARY CARCINOMA OF THE THYROID GLAND*

Şafak Atahan** • Işın Soyuer** • Cemil Ekinci***

SUMMARY

Fine needle aspiration cytology (FNAC) has been evaluated as a diagnostic method for medullary carcinoma of the thyroid gland (MCT) in 22 patients with verified hereditary or sporadic MCT. Out of all cases, there was one case of multiple endocrine adenomatosis type II with MCT and pheochromocytoma. An adequate bioptic yield was obtained in 18 patients. In eight cases, immunocytochemical techniques for calcitonin, thyroglobulin and carcinoembryonic antigen were used. The typical MCT cell was asymmetrical, sometimes triangular and often with a characteristic red granulation in May-Grünwald-Giemsa staining. The presence of spindle cells in addition to these polygonal cells is a common feature and significant clue to the diagnosis. Eccentrically positioned, bi/multiple nuclei were common. Amyloid, when present, can be seen both intra- and extracellularly.

Key Words: *Fine needle aspiration cytology, Immunocytochemistry, Medullary carcinoma of the thyroid gland*

Medullary carcinoma of the thyroid gland (MCT) was defined in 1959 by Hazard et al. (1) as a distinct clinical and pathologic entity and the authors emphasized the diagnostic significance of amyloid in the tumor and its metastases. It accounts for up to 10% of all thyroid malignancies. It is considered mainly to be derived from parafollicular or C cells, known to be the site of storage and secretion of the polypeptide hormones including calcitonin. There are two types of MCT, the sporadic and the familial. The familial form is inherited in an autosomal dominant pattern, and expressed clinically as multiple endocrine neoplasia (MEN), types IIA and IIB, or as familial MCT alone (2,3).

The role of fine needle aspiration cytology (FNAC) is particularly important in the initial assessment of patients with the sporadic form of MCT, the diagnosis of which is not suspected clinically (4,5). Despite its importance, however, the literature on the cytologic recognition of MCT is scanty, perhaps because of its relative rarity (6,7,8,9,10,11,12,13,14). A study was undertaken to analyze the morphologic changes seen in FNAC specimens from MCT and to

determine which features could be the most useful in establishing a definitive diagnosis.

MATERIALS AND METHODS

Of 278 malign epithelial tumors of the thyroid diagnosed at our department from 1988 through 1997, 22 cases of MCT occurred. Twenty-seven samples from these 22 cases including recurrences, were reevaluated.

FNACs were performed by clinicians or cytologists using 20-22-gauge needles attached to disposable 20-ml syringes. The samples were air dried and stained with May-Grünwald-Giemsa (MGG) stain. In eight cases immunocytochemistry using the avidin-biotin method was performed with commercially available antibodies for antithyroglobulin, carcinoembryonic antigen (CEA) and calcitonin (Zymed®).

In 18 patients, specimens from the thyroid tumors and from lymph node metastases were also examined histologically. Tissue sections were stained with hematoxylin-eosin, Crystal violet and alkaline Congo red.

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** Pathologist, Department of Clinical Cytology, Faculty of Medicine, Ankara University, Ankara, Türkiye

*** Professor and Chief, Department of Clinical Cytology, Faculty of Medicine, Ankara University, Ankara, Türkiye

Table 1: Cytologic features of MCT

Cytologic Criterias		No. of materials (%) (n=27)	
Cellularity	Low	3	(11)
	High	24	(89)
Cell Pattern	individually	20	(74)
	Chosive clumps	3	(11)
	Mix	4	(15)
Cell population	Uniform	8	(30)
	Pleomorphic	19	(30)
Shape of cells	Trianguler-poligonal	4	(15)
	Spindle	2	(7)
	Mix	21	(78)
Intracytoplasmic granules	Present	18	(67)
	Absent	9	(33)
Intracellular amyloid	Present	5	(19)
	Absent	22	(81)
Extracellular amyloid	Present	9	(33)
	Absent	18	(67)
Binuclear cels	Present	23	(85)
	Absent	4	(15)
Multinuclear cells	Present	14	(52)
	Absent	13	(48)

FNAC smears were evaluated with the following cytologic features; smear cellularity, architecture, cell shape, nuclear and cytoplasmic features, presence or absence of amyloid and bi/multinucleated cells. We also noted the features of background.

RESULTS

The patient population of this study ranged in age from 14 to 68, with average age of 40 years. There were 13 women and 9 men. Twenty-seven smears from each case shown a number of common features that summarized in Table 1.

Cells were dispersed singly without group formation in 20 samples (74 %); small cell groupings as well as abundant single cells were identified in 4 specimens (15 %). Three aspirates were characterized by a predominance of cohesive cell groupings, two of them obtained from local recurrences. In one case, follicular pattern was so predominant that a diagnosis of follicular carcinoma was made (Fig 1).

The nuclei were round or elongate and marked variation of nuclear size was only occasionally present. Cells showed a moderate degree of nuclear pleomorphism in 19 aspirates (70 %). Two basic cell types are represented. Although in each case polyhedral cells with round, eccentric nuclei and abundant cytoplasm were seen, a prominent feature was the presence of spindle-shaped cells. In two cases, there were only spindle cell clusters, and although a diagnosis of

MCT were made, a differential diagnosis of fibrosarcoma and carotid body tumor were also suggested (Fig 2).

Another feature of diagnostic importance was cytoplasmic granulation. The granules were usually rather coarse but sometimes fine and dustlike. In a few cells the granulation appeared as a diffuse red staining in part of the cytoplasm. Red-stained cytoplasmic granulation was occurred in 18 samples (67%) and prominent in 13 (Fig 3). Amyloid occurred as single, isolated clumps but sometimes also in the form of small, rounded deposits within or adjacent to clusters of tumor cells (Fig 4). Intracytoplasmic amyloid was seen in only five samples (19 %). In nine samples (33%) contained densely stained amorphous amyloid between the cells. Binuclear cells were found in 23 specimens (85 %). Also, the multinuclear cells were detected in 14 samples (52 %). Cytoplasmic vacuolation was seen in one case.

Intranuclear cytoplasmic inclusions were observed in five samples, one of them obtained from local recurrences. In two specimens with intranuclear cytoplasmic inclusions showed marked nuclear pleomorphism as well.

Immunocytochemical studies were performed in selected cases to confirm the diagnosis. In each of eight cases immunoreactivity for calcitonin and carcinoembryonic antigen could be demonstrated in both the polygonal and spindle types of tumor cell. Thyroglobulin was negative in these eight cases.

There were two samples from the patient with multiple endocrine adenomas syndrome Type IIA. In the first samples, which obtained before the operation there was typical features of MCT. But in the second

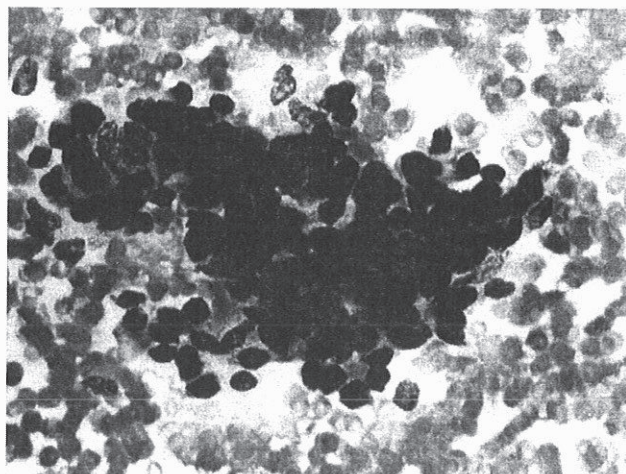


Fig. 1. Follicular arrangement of clusters of cells in an aspirate of MCT (MGG, x200).

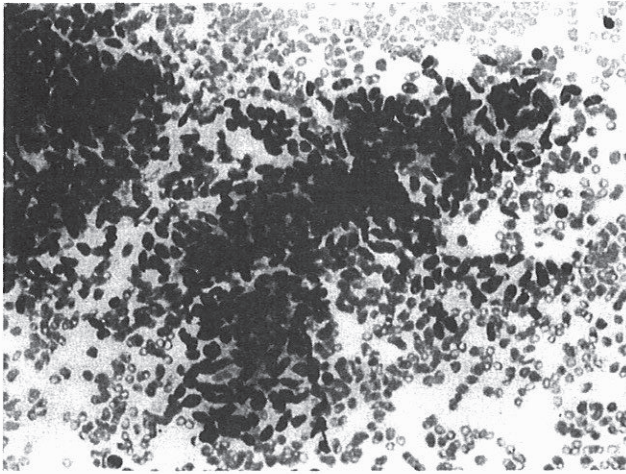


Fig. 1: Cellular groups of predominantly spindle cells (MGG, x200).

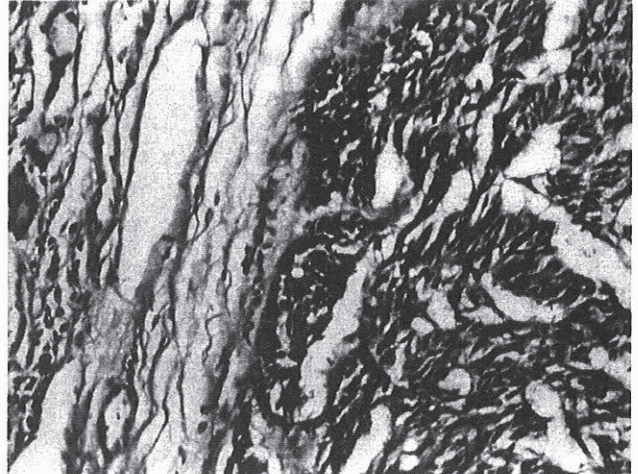


Fig. 2b: Tissue section showing spindle cells (H.E., x200).

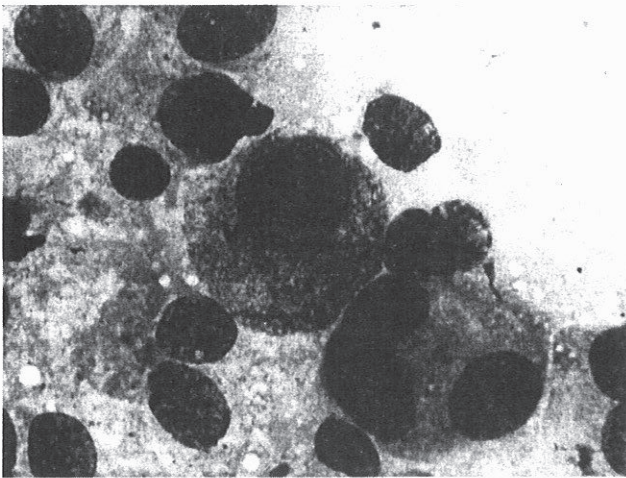


Fig. 3: Numerous granules are in the cytoplasm of the tumor cell. (MGG, x1000).

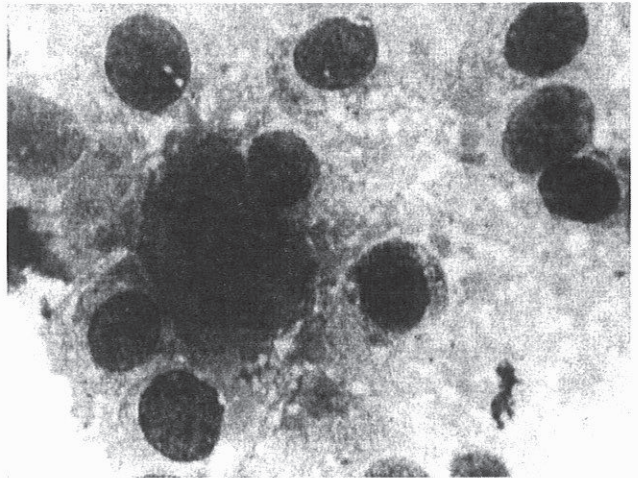


Fig. 4: Clumps of amyloid intermingled with groups of tumor cells (MGG, x400).

samples that obtained from local recurrence, the cells with scanty cytoplasm and round nuclei were arranged more commonly cohesive cell groups.

Among the 22 cases of MCT, the cytodiagnosis was medullary carcinoma in 21 and follicular carcinoma in one. There was no false-positive diagnosis of MCT on cytology. One case with cytodiagnosis of medullary carcinoma but an initial histologic diagnosis of follicular carcinoma were confirmed to be medullary carcinoma after positive staining with alkaline congo red staining.

DISCUSSION

An early and specific diagnosis of MCT is of considerable clinical consequence. The combination of

MCT with the other neuroendocrin tumors may cause unexpected cardiovascular emergency situations when general anesthesia and operation are attempted. This augments the need for an easy and reliable method of preoperative diagnosis of this tumor (7,13). In this paper the attention is drawn to the several cytological morphologies of MCT in FNAC.

Authors emphasized the difficulties of cytologically diagnosing of follicular lesions (4,5,9). The diagnostic problem as to MCT is different (7,10,13,14). This tumor yields a cytology with characteristic features, which usually permit the specific diagnosis: a somewhat asymmetrical tumor cell with one or more nuclei eccentrically positioned, many of them having a distinct bright red granulation. The amyloid deposits,

well visible in MGG, may finally secure the specific diagnosis (6,7,8,9,11,13). Although the cytologic picture is characteristic, there are many variations that may lead to incorrect diagnosis. Unusual varieties include a true papillary form, mucinous medullary carcinoma, clear cell variant and pigmented variant (12,13,15).

Intracytoplasmic granules were stained azurophilic with MGG and basophilic with Papanicolaou-staining method. But this type of granulation is not distinct in smears stained with hematoxylin-eosin, in which this eye-catching detail of the MCT cytology is lost (6,7,8,9,11). The granules are of great diagnostic importance. These granules are thought to correspond to neurosecretory granules identified in the cytoplasm by electron microscopy (13). However, it is of practical and theoretical interest to note that FNAC of other neuroendocrine neoplasms also exhibit red granulated cells that are akin to those of MCT: pheochromocytomas, chemodectomas and carcinoid tumors. This resemblance may evoke differential diagnostic problems when the biopsy specimen is taken from a mediastinal or neck tumor (7).

The presence of amyloid is not a frequent finding in cytologic samples of MCT. However, its presence is a good diagnostic feature. Extracellular amyloid was found in only 33 % of our specimens. There were cloudy, amorphous lumps of a substance staining gray-blue or violet with MGG. In a few reports, samples were stained with alkaline Congo red after destaining of MGG smears or papanicolaou smears (7,8,10). Since amyloid can be seen in only some cases of MCT sampled by FNAC, its absence is not diagnostically significant, but when present, it can provide useful confirmatory evidence for the diagnosis (13).

The immunocytochemical studies are used widely in MCT because of tumor cells are reactive for calcitonin, and nonreactive for thyroglobulin (8). Calcitonin is considered to be the antibody of first choice, while both chromogranin A and CEA can also provide valuable confirmatory evidence for the diagnosis. It can be seen in 80 % of MCT, with some of the positive cases showing only small and focal areas of positivity. Therefore, the absence of positive staining should not exclude the diagnosis of MCT, especially if the cytomorphology strongly suggests this diagnosis (13).

In this study, we have examined histopathologic materials of 18 patients retrospectively. In one patient, operation material had diagnosed as follicular carcinoma. The tissue sections from this case were stained with alkaline Congo red and positive result were seen. Also, prominent amyloid globules were present intra and extracellularly in smears of this case.

FNAC is a technic of proven value in the preoperative diagnosis of thyroid neoplasms. Of these tumors, MCT occupies a unique place in its implication for investigation and treatment of the patient and his or her family. In this study, the cytomorphologic features have been documented by reexamination of our own series of 27 samples of 22 cases. If the optimal and sufficient materials obtained MCT can be adequately diagnosed by FNAC despite the frequent cytomorphologic variations.

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TREATMENT OF HIRSUTISM WITH THE 5 α REDUCTASE INHIBITOR FINASTERIDE

A. Nuri Kamel* • Sevim Güllü* • Vedia Tonyukuk* • Demet Çorapçoğlu* • Nilgün Başkal*
Ali Rıza Uysal* • Gürbüz Erdoğan*

SUMMARY

We evaluated the clinical efficacy, safety and hormonal effects of the 5 α -reductase inhibitor finasteride in the treatment of hirsutism in women. Eleven patients with polycystic ovarian disease and nineteen patients with idiopathic hirsutism received finasteride 5 mg daily via oral route for 6 months. Hirsutism scores were monitored at third and sixth months during, and six months after the discontinuation of the therapy. Serum free and total testosterone, dehydroepiandrosterone sulfate, FSH, LH, prolactin, cortisol, 17 OH progesterone, estradiol levels, biochemical and hematological parameters were measured at the first visit and after 3 and 6 months. Serum dihydrotestosterone levels and bone mineral densities were evaluated before and after six months. Serum gonadotropin, dehydroepiandrosterone sulfate and dihydrotestosterone levels decreased significantly with the therapy. Other hormonal, biochemical and hematological parameters did not show any change. Bone mineral densities remained stable during the treatment. Hirsutism scores of the patients declined and found to be lower than initial values even 6 months after the discontinuation of finasteride. These results suggest that finasteride is an effective and safe drug in the treatment of both idiopathic hirsutism and polycystic ovarian disease.

Key Words: Finasteride, Hirsutism, 5 α -reductase, Polycystic ovarian disease

Hirsutism is characterized by the excessive growth of terminal hair, in male pattern, in women and is a common clinical condition (1-4). Increased conversion of vellus hair can occur as a result of excessive androgen production, increased concentration of free testosterone, increased 5 α -reductase activity or increased sensitivity of hair follicles to normal androgen levels (1). Testosterone is the major circulating androgen and is converted to its active form dihydrotestosterone (DHT) by 5 α -reductase enzyme in the hair follicle. DHT appears to be the androgen that promotes androgen dependent hair growth since the men with 5 α -reductase deficiency show reduced androgen-dependent hair growth (5,6).

Hirsute women have increased 5 α -reductase activity in skin and 5 α -reductase activity appears to be the main modulator of skin sensitivity to androgens (7-9). Finasteride is a 4-azasteroid inhibitor of type 2 isoenzyme of 5 α -reductase (10-12). It seems that the drug also has some inhibitory effects on type 1 isoenzyme, which is the main isoenzyme in skin, since it was found to be effective in the treatment of hirsutism by some investigators (13-16).

The aim of this study was to examine the efficacy of finasteride on hirsutism in women either with idiopathic hirsutism or polycystic ovarian disease. Its effects on hormonal and non-hormonal parameters and bone mineral density (BMD) were also evaluated.

PATIENTS AND METHODS

Subjects:

Eleven patients with polycystic ovarian disease and nineteen patients with idiopathic hirsutism were included in the study. The ages of the women were between 16-33 and the mean was 24 \pm 5 yrs. One fourth of the patients were obese. Written consent was obtained from all patients participating in the study and all of them were warned to use non-hormonal contraception methods during finasteride treatment because of the adverse effect of the drug on male fetus. The study protocol was approved by the Ethics Committee of the Ankara University Medical School.

Hirsutism was assessed by Ferriman-Gallwey score (17). The women who had a score \geq 10 were included. Other causes of the hirsutism such as Cushing's

* Ankara University Medical School, Endocrinology and Metabolic Disease Department, Ankara, Turkey

disease, congenital adrenal hyperplasia, ovarian or adrenal neoplasms, prolactinoma, were ruled out by clinical and laboratory examinations.

None of the patients had any other disease or drug history and none had received any hormonal treatment for hirsutism at least 6 months prior to the study.

The diagnosis of polycystic ovarian disease (PCOD) was based on clinical features of hyperandrogenism, oligo-amenore, elevated androgen levels, an LH/FSH ratio of greater than 2 and demonstration of polycystic ovaries by ultrasonographic examination. The idiopathic hirsutism was defined as hirsutism occurring in an androgen-dependent pattern without any known underlying disease, and these women all had regular menstrual cycles.

Protocol:

Finasteride was given 5 mg daily via oral route to all patients for six months. Clinical and biochemical evaluations were done basally, at third and sixth months of the treatment.

At each visit Ferriman-Gallwey scores were assigned by the same observer. Patients were requested not to depilate for at least two months before each evaluation. Clinical outcome of the treatment was also evaluated by the patients themselves.

Venous blood was collected for hormonal and non-hormonal parameters basally, at third and sixth months of the therapy. The blood samples of the women with idiopathic hirsutism were obtained at the early follicular phase of the menstrual cycle (3-6 days after beginning of the bleeding). Random sampling was performed in patients with PCOD since they had irregular menses.

Bone mineral densities of the women were evaluated before and after six months of the treatment.

All patients were also evaluated six months after discontinuation of finasteride treatment for the Ferriman-Gallwey scores.

Biochemical Evaluations:

Serum gonadotropins (FSH, LH), free testosterone (FT), total testosterone (TT), dehydroepiandrosterone sulfate (DHEA-S), estradiol (E2), 17 OH progesterone (17OHP), cortisol (F), prolactin (PRL) levels were determined at 0, 3 and 6 months. Serum DHT measurements were done at 0 and 6 months.

Body weights, blood pressures, hematological parameters (hemoglobin, hematocrite, leukocyte and platelet counts), fasting and postprandial two hour plasma glucose levels, renal and hepatic function tests and serum lipid profiles (total-low density lipoprotein-high density lipoprotein- cholesterol and triglyceride levels) were all measured at each visit.

Bone Mineral Density:

BMD (grams per cm²) was assessed by dual energy X-ray absorptiometry. Spinal BMD was measured at L₂₋₄ and hip BMD was measured at neck and trochanter basally and at the sixth month of the treatment. All measurements were performed on the same machine.

Assays:

After collection, blood samples were centrifuged and stored at -20 C ∞ until assayed. Samples were analyzed in duplicate in the same assay to avoid inter-assay variations. Serum FSH and LH (Medgenix, Belgium) and Prolactin (Orion Diagnostica, Finland) were measured by IRMA kits. RIA kits were used for DHT (Amersham, UK), TT and FT (Diagnostic Products Corp., USA), 17 OH P (Diagnostic System Lab, USA), Cortisol (Amersham, UK), Progesterone (Amersham, UK), Estradiol (Amersham, UK) determinations.

Statistical Analysis:

Student's test for paired samples, two tailed significance for comparisons and variance analysis tests were used. p values below 0.05 were accepted to be significant.

All values were reported as the mean \pm SD.

RESULTS

Hormonal Effects:

Table 1 shows the hormonal profiles of the women at baseline and during the finasteride treatment.

Serum FSH levels decreased from 6.6 ± 3.5 mlu/ml to 4.6 ± 2.2 mlu/ml at third month and to 4.9 ± 2.1 mlu/ml at sixth month ($p < 0.05$). LH concentrations also decreased significantly from 8.2 ± 4.4 mlu/ml to 5.6 ± 3.8 mlu/ml at third and to 3.9 ± 2.4 mlu/ml at sixth months (< 0.05).

Serum free and total testosterone levels remained unchanged during the therapy. Serum DHT concentrations significantly reduced during finasteride treatment (0.5 ± 0.08 nmol/L to 0.3 ± 0.04 nmol / L at sixth month, $p < 0.001$). Serum DHEA-S levels also decre-

Table 1: Serum hormone concentrations of the women before and during finasteride treatment

Hormone	Pretreatment	3rd month	6th month	p	Normal*
FSH (mlu/ml)	6.6±3.5	4.6±2.2	4.9±2.1	<0.05	(1.37-9.9)
LH (mlu/ml)	8.2±4.4	5.6±3.8	3.9±2.4	<0.05	(1.68-15)
FT (pmol/L)	12.1±6.5	10.7±5.5	10±6.5	>0.05	(3.5-29.5)
TT (nmol/L)	2.26±1.16	2.01±1	1.97±1.08	>0.05	(0.52-2.43)
DHT (nmol/L)	0.5±0.08	-	0.3±0.04	<0.001	(0.14-0.76)
E2 (pmol/L)	203±157	172±76	178±40	>0.05	(73-551)
DHEAS (Cmol/L)	10.6±8.2	7.9±5.2	4.7±2.5	<0.05	(0.8-21.1)
F (nmol/L)	485±176	499±253	430±174	>0.05	(138-635)
PRL (C g/L)	18±5	16±3	17±5	>0.05	(3.8-23.2)
17 OH P (nmol/L)	1.2±0.6	1.4±0.5	1.3±0.4	>0.05	(0.4-2.1)

Values are the mean±SD, E2; Estradiol, FT; Free testosterone, TT; Total testosterone, DHT; Dihydrotestosterone, DHEAS; Dehydroepiandrosterone sulfate, F; Cortisol, 17 OH P; 17 OH progesterone

* Follicular phase normal ranges

ased with the therapy (10.58 ± 8.18 mmol/L to 4.7 ± 2.5 mmol/L at sixth month, $p < 0.05$).

Serum 17 OH progesterone, estradiol, cortisol and prolactin levels also remained unchanged throughout the treatment.

Clinical effects:

Ferriman-Gallwey scores significantly improved with finasteride treatment (Figure 1). Basal hirsutism score of 17.6 ± 4.2 reduced to 13.5 ± 4.3 at third month and to 11.5 ± 4.5 at sixth month of therapy ($p < 0.05$). All of the patients satisfied with the treatment (self-evaluation).

Six months after the discontinuation of the therapy Ferriman- Gallwey scores were found to be lower than baseline scores (13.8 ± 3.9).

Side Effects:

No serious side effects were reported by the women. One of the patients complained about head-ache at the beginning of the therapy which was resolved spontaneously.

Body weights and blood pressures remained stable. Hematological tests, fasting and postprandial two hour plasma glucose levels, liver and kidney function tests and lipid levels did not change during the study period.

Bone Mineral Densities:

L2-4, femoral neck and trochanter bone mineral densities showed no alteration with the finasteride treatment (Table 2).

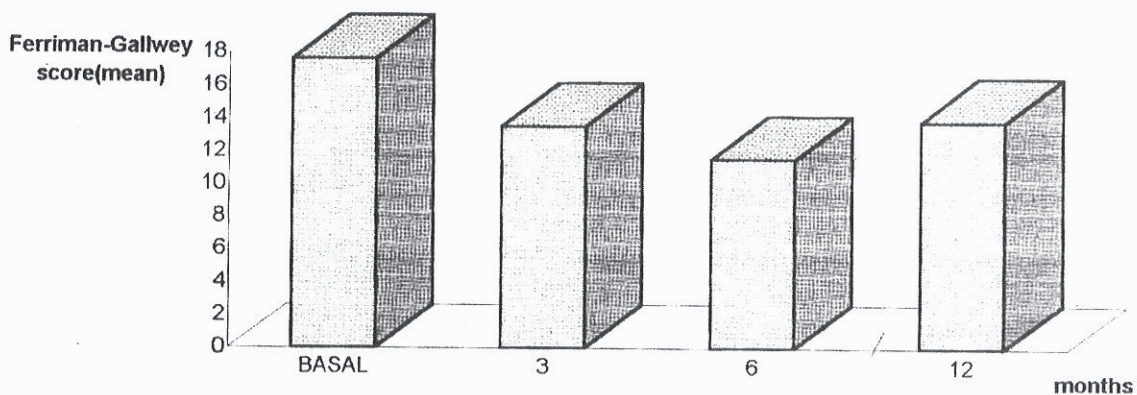


Fig. 1. Mean Ferriman-Gallwey scores in patients treated with finasteride. Evaluations were performed before, at 3rd and 6th months during therapy, and 6 months after the completion of treatment.

Table 2: Bone mineral densities (g/cm²) of the women before and after finasteride treatment

Hormone	Pretreatment	6th month	p
L 2-4	1.089 \pm 0.098	1.046 \pm 0.110	>0.05
Femoral Neck	0.962 \pm 0.101	1.012 \pm 0.123	> 0.05
Trochanter	0.805 \pm 0.108	0.792 \pm 0.100	> 0.05

DISCUSSION

This study was planned to evaluate the effects of finasteride on hirsutism score, hormonal and non-hormonal parameters in hirsute women. Finasteride is a potent inhibitor of 5 α -reductase enzyme and was developed for the treatment of benign prostate hyperplasia in men (11,12).

As the male patients with 5 α -reductase deficiency show a decreased body hair pattern (5,6) and as the women with this defect show no clinical abnormalities, it can be assumed that inhibition of 5 α -reductase activity with finasteride should have no harmful effect on female patients. As the increased activity of the enzyme in skin was proposed to play a role in the development of hirsutism, it can also be suggested that inhibition of its activity by finasteride must have some beneficial effects in the treatment of excessive hair growth in women.

The results of the present study showed that the 5 mg daily administration of finasteride to hirsute women is effective in 5- α reductase inhibition. The baseline DHT levels of the women, which were all within normal limits, decreased significantly with the treatment. In addition, hirsutism scores showed marked improvement. The change in hirsutism scores were clearly detectable at the third month of the therapy (24%) and reached to 34 % at the sixth month. Previous studies with finasteride also showed similar results. Fruzzetti et al.(13) reported a 27% decrease at third month and Moghetti et al.(14) found a 50 % reduction at sixth month in hirsutism scores. Wong et al.(15) also observed significant reduction in hirsutism score with finasteride. In the present study the effect of finasteride continued for at least six months after the discontinuation of the treatment as the Ferriman-Gallwey scores of the patients were found to be under the pretreatment levels (13.8 vs. 17.6, $p < 0.05$). The mean hirsutism score was 22% lower than the baseline level. Castello et al(16) also reported an ongoing improvement in hirsutism score at one-year after the finasteride treatment. These observations of them and ours are

very important because complete reversal of clinical effects of antiandrogen drugs in a short period after the discontinuation of treatment (18) is a major problem in the treatment of patients with hirsutism.

Interestingly serum gonadotropin levels of our patients decreased with finasteride treatment. No change in gonadotropin levels were reported in studies done with male volunteers (19,20). Reports on finasteride therapy in hirsute women however showed discrepancies. Increased or unchanged gonadotropin levels were reported (13,14,16). As serum total and free testosterone concentrations of the women did not change in our study; it is hard to explain the decrease in gonadotropin levels.

An unexpected decrease in DHEAS levels was observed in this study. Wong et al.(15) also reported a similar fall in DHEAS levels. Finasteride has no effect on the synthesis of this hormone but an increased metabolism of the hormone can play a role in this decrease.

Serum TT and FT levels were found to be unchanged as mentioned above. Although an increase can be expected, some studies on males also demonstrated no change in testosterone levels (19, 21-23). So the results of the present study is not a disparity.

Finasteride was well tolerated by the patients and neither clinical nor biochemical adverse effects were observed. Although we did not evaluate the ovulatory functions of the women, Moghetti et al.(14) showed the continuity of ovulation during finasteride treatment. As the external genitalia abnormalities in male fetuses, exposed to finasteride in utero in rats, was reported (24), an effective contraception method should be used in women taking finasteride. In the present study we warned the patients to use non-hormonal methods for contraception in order not to affect the results of the study.

Normal serum DHT levels of our patients before the finasteride treatment indicate that the serum levels of this hormone do not correlate well with the presence of excessive hair growth. Elevated local levels of DHT as a result of its production in the skin seems to be the major factor in the development of hirsutism at least in some women. Although finasteride has a greater affinity for the type 2 5 α -reductase, which is the dominant form of the enzyme in genital tissue including prostate (25), our results indicate that it can also effectively inhibit type 1 isoenzyme, which is present in all skin tissue throughout the body, in vivo with chronic treatment.

Menstrual irregularities and decreases in bone mineral density in women treated with GnRH agonists or spironolactone for hirsutism were reported by some investigators (26-28). Estrogen replacement was recommended to prevent the bone loss in patients treated with spironolactone. Finasteride neither changed the BMD nor caused menstrual irregularities, in any women, in the present study. No change in bone turnover was also reported in men taking finasteride for benign prostatic hyperplasia (29). Although a six month period for the evaluation of BMD is short, it seems to be safe also from this point of view, but further

studies must be conducted to observe its long-term effects on bone turnover in hirsute women. In this way it can be taken into consideration especially for hirsute women in whom estrogens are contraindicated or not tolerated.

In conclusion, depending on the results of this study, finasteride can be proposed as an effective and safe drug in the treatment of both idiopathic hirsutism and polycystic ovarian disease. To clear the discrepancies in the hormonal parameters further studies should be conducted.

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INCOMPLETE ANDROGEN INSENSITIVITY SYNDROME: A FAMILY PRESENTING WITH SMALL PENIS, GYNECOMASTIA AND UNDERVIRILIZATION

Gürbüz Erdoğan* • Sevim Güllü** • Ramazan İdilman*** • Alim Uzunalımoğlu* • Ali Rıza Uysal*

SUMMARY

A family with incomplete androgen insensitivity syndrome is presented. The phenotypic features of the two postpubertal patients were small penis, gynecomastia and undervirilization, whereas one prepubertal child showed only small penis. The genotypes were found to be 46 XY. Studies of the basal and stimulated plasma testosterone levels indicated a defect in androgen action. Mesterolone, an orally active androgen, were given to postpubertal patients. A partial response in body hair development was observed with the treatment course.

Key Words: Androgen Resistance, Therapy, Mesterolone

Hereditary resistance to androgen action in men results in a broad spectrum phenotypic abnormalities. Androgen resistance can occur as either complete or incomplete forms in subjects with 46 XY genotypes. While the complete androgen insensitivity syndrome presents with a normal external female appearance (testicular feminization), partial androgen insensitivity syndrome shows a wide phenotypic spectrum ranging from perineoscrotal hypospadias, bifid scrotum and cryptorchidism to minimal virilization defects including gynecomastia and/ or infertility or even to fertile but undervirilized men (1-9).

The endocrine profile of androgen resistance consists of elevated serum testosterone (T) and luteinizing hormone (LH) concentrations and also increased serum estradiol (E₂) levels in postpubertal patients (1,2,4). These disorders are inherited in a X-linked pattern and accepted to be the consequence of mutations in the androgen receptor gene, which was located on the X chromosome (1-3, 10-15).

As a result of abnormal androgen receptor, these patients were believed to be resistant to the action of both endogenous and exogenous androgens, but some benefits by giving high dose testosterone were reported (5,15,16). Worsening of existing gynecomastia in postpubertal individuals, as a result of peripheral con-

version of testosterone to estradiol, especially when given in supraphysiological doses, is a problem.

We here report a family of incomplete androgen insensitivity syndrome, three affected brothers, including two postpubertal boys with gynecomastia and small penis who were treated with mesterolone, an androgen which is not converted to estradiol peripherally, and a prepubertal boy with just small penis.

SUBJECTS AND METHODS

Clinical findings of the patients are given in table

Prenatal, neonatal and childhood histories were unremarkable. Family history was also unremarkable, demonstrating no any other affected family member. None of the subjects had any coexisting systemic disease. Karyotypes were found to be 46 XY. Breast ultrasonographies (US) of subjects 1 and 2 revealed an increased glandular tissue echogenicity. Pelvic and scrotal US demonstrated normal sized testes, normal epididymis and absent Mullerian duct formations. Testes biopsy of case 1 showed hypospermatogenesis and disorganization of the spermatogenetic series. No biopsy performed to subjects 2 and 3. Basal FSH, LH, free and total testosterone and estradiol levels were measured and hCG (human chorionic gonadotropin) stimulation

* Professor of Endocrinology and Metabolic Diseases

** Teaching Professor in Endocrinology and Metabolic Diseases

*** Resident of Internal Medicine

Table 1: Clinical characteristics of the patients

Case	Age	External Genitalia	Testes	Gynecomastia	Body Hair	Sperm Count
1	17	Small penis Normal scrotum	Normal sized	Yes	Sparse	Oligo spermia
2	15	Small penis Normal scrotum	Normal sized	Yes	Sparse	Normal
3	12	Small penis Normal scrotum	Normal sized	No	-	-

tests were performed. For hCG stimulation tests were performed. For hCG stimulation test 5000 units of hCG was given intramuscularly for 4 days and blood samples for the measurement of serum total and free testosterone levels were taken on days four and five. Mesterolone 60 mg daily were given to subjects 1 and 2 via oral route for 6 months, case 3 had no treatment. Informed consent of the family was taken for the therapy. Basal and hCG stimulated and posttreatment hormonal values are given in table 2. Other hormonal and biochemical tests of the patients showed no abnormalities.

Amerlex (UK) RIA kit for estradiol, DPC (USA) RIA kit for free and total testosterone and Medgenix (Belgium) IRMA kits for FSH and LH were used for hormonal measurements.

RESULTS

The subjects showed the clinical and laboratory features of the incomplete androgen insensitivity

Table 2: Hormonal profiles of the patients

Hormone	Subject 1*	Subject 2	Subject 3*
FSH (miu/ml)	6.4 (0.9-8.9)	5.2	1.8 (1-3)
LH (mlu/ml)	29.8 (2.5-9.4)	32.2	7.1 (1-4)
TT (ng/dL)	1069 (280-880)	1228	1.8 (3-10)
FT (pg/ml)	59 (9-47)	57	1.7 (0.15-0.6)
E2 (pmol/L)	103 (0-87)	114	11 (0-40)
sTT	3272	3058	351
sFT	73	68	13.2
mLH	24	36.9	-
mTT	3200	2000	-
mFT	72	57	-
mE2	172	150	-

*: Normal ranges for ages are given in parenthesis

TT: Total testosterone, FT: free testosterone, E2: estradiol, sTT: total testosterone after hCG stimulation, sFT: free testosterone after hCG stimulation, mTT, mFT, mLH, mE2: levels under mesterolone treatment

syndrome. The most characteristic phenotype in this family consisted of a male with small penis, postpubertal gynecomastia and diminished virilization as evidenced by decreased chest, body and facial hair and female pattern of pubic hair. Elevated basal total and free testosterone levels with high LH levels indicated a normal androgen synthesis but a defect in hormone action. hCG stimulation tests which revealed normal increases in testosterone levels confirmed the diagnosis. The defect in virilization in this family appears to be transmitted as an X-linked trait.

As the undervirilization and small penis size disturbed both the patients and family, mesterolone treatment was started to subjects 1 and 2. After six months of treatment no improvement in virilization, sperm count or penile size could be detected in subject 1 but pubic hair increased in subject 2. Testosterone and estradiol levels were found to be increased according to baseline levels, where LH levels remained unchanged. Gynecomastia in subject 1 worsened during the treatment and eventually he went under a reconstructive mastoplasty operation. All biochemical tests including liver functions, were remained within normal ranges during mesterolone therapy.

DISCUSSION

We describe a family exhibiting some phenotypic features- gynecomastia, undervirilization and small penis- of incomplete androgen resistance. Infertile man syndrome and undervirilized male syndrome are the mildest forms of the partial androgen insensitivity syndrome. Our patients all showed normal external genitalia apart from small penis. Although the pattern of inheritance of the defect in this family was compatible with X-linkage, an autosome mode of inheritance could not be excluded.

Mutations in the androgen receptor gene which is located on the X- chromosome, are believed to be the

cause of androgen receptor abnormalities (10-15). More than hundred different mutations were reported in patients with androgen resistance (2). No correlation was found between the degree of mutation and clinical features.

Although we could not able to perform a genetic study in our patients, we believe that they had the same gene mutation as they showed identical phenotypic findings. Clinical variations within the families who had similar degree of dysfunction at androgen receptor were reported (3,7). These observations can explain the different ejaculate volume and sperm count in two postpubertal cases.

Patients with androgen insensitivity are believed to be resistant to exogenous as well as endogenous androgens. Partial response can be seen with high dose testosterone treatment (5,15,16), but peripheral conversion of this exogenous testosterone to estradiol can worsen the existing gynecomastia in these patients. Mesterolone is an orally effective androgen which can not be aromatized to estrogens in peripheral tissues (1), so we preferred this drug for the treat-

ment of our two postpubertal patients. Virilization of subject 1 did not show any improvement, although his testosterone levels increased prominently. His gynecomastia worsened unexpectedly. As mesterolone has no effective negative feed back regulation of gonadotropin secretion, this can, in part, be explained with the ongoing high LH levels and endogenous testosterone to estrogens. Subject 2 showed minimal increase in body hair, but his penile size did not improve. His gynecomastia which was minimal before treatment remained unchanged. Although the basal and posttreatment estradiol values of the patients were similar, sensitivity of the breast tissue to estrogens must be different, so the gynecomastia of this subjects showed no progression.

In conclusion, patients with androgen insensitivity are usually resistant to androgen therapy. Although it is hard to make a conclusion with only two patients, mesterolone can be taken into consideration for the treatment of androgen resistance since a partial response can be gotten with the drug, it is safe and oral administration can be preferred by some patients.

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USE OF PAPAVERINE FOR THE TREATMENT OF SUDDEN DEAFNESS WITH DIABETES MELLITUS

Serdar Ensari* • M. Cem Özbek* • Serdar Çelikkanat* • Sevim Güllü** • Cafer Özdem*

SUMMARY

Diabetes mellitus is a well-known cause of progressive sensorineural hearing loss but there is only a few reports about sudden deafness in diabetic patients. In the present paper three patients with non-insulin dependent diabetes mellitus (NIDDM), presenting with idiopathic sudden hearing loss (ISHL), is reported. Papaverine hydrochloride, a selective cerebral vasodilator agent, was given by intravenous route to the patients. Two of the patients had complete and one had partial recovery with the therapy. No side-effect of the drug was observed and patients remained metabolically stable during treatment. We conclude that papaverine is a safe and effective agent in the treatment of diabetic patients with ISHL.

Key Words: Sudden hearing loss, papaverine hydrochloride

Sudden hearing loss is defined as a 30 dB sensorineural hearing loss occurring in at least three contiguous frequencies in less than three days (1).

There are many suggested causes for idiopathic sudden hearing loss (ISHL). These include vascular lesions, trauma, labyrinthine membrane rupture, viral and bacterial infections, immune complex diseases and acoustic neuroma. Even diabetes and other metabolic disorders are also well-known causes of sensorineural hearing loss, there has been only a few reports about sudden deafness related to diabetes (2). Although the efficacy of papaverine hydrochloride which is a selective cerebral vasodilator agent for the treatment of ISHL is discussed, this is the first report which evaluates the effect of papaverine in diabetic patients with sudden hearing loss. In this paper three patients who received papaverine hydrochloride for the treatment of sudden hearing loss are presented.

CASE REPORTS

In all of the three patients general physical examination revealed no abnormalities. Otolaryngologic examination was normal apart from the hearing loss in the related ear. Haematological investigations including full blood count, erythrocyte sedimentation rate,

urea and electrolyte, blood sugar, lipid profile, thyroid function tests, autoimmune profile, serology for syphilis and high resolution computed tomography of temporal bone were done.

All of the three patients were hospitalized and received intravenous drips of papaverine hydrochloride 5mg/kg/1¹/₂-2 hours for three consecutive days diluted in 250cc 0.9 % saline starting from 20 drops/min and gradually increasing the dose up to the level of 70 drops/min. After IV treatment for 3 days, therapy was continued orally up to 30 days again with papaverine (5mg/kg).

To assess the efficacy of papaverine treatment criteria of Siegel (3) were used.

CASE 1

A 64-year-old female presented to the Department of Otolaryngology out-patient clinics with a five-day history of tinnitus, ear fullness and sudden hearing loss. Diabetes mellitus had been diagnosed 10 years ago, upon which she was placed on glibornuride 25mg two times and metformin 500mg three times a day. She had hypertension for five years and was taking indapamid 2.5mg daily. On admission her plasma glucose was 221mg/dl (12.2mmol/L) and her

* Department of Urology, Ankara Numune Hospital, TURKEY

** University of Ankara, Faculty of Medicine Department of Endocrinology

HbA₁C level was 8.7% (n=4.5-6.5%). Urinary albumin excretion was 10mg/mL. High resolution computed tomography of temporal bone was also normal.

An audiogram revealed 50 dB sensorineural hearing loss in the right ear. There was a 30 dB sensorineural hearing loss with speech reception threshold(SRT). in left ear. After treatment with intravenous drips of papaverine hydrochloride, her hearing improved an average of 15 dB and this was classified in group 3 (partial recovery) according to the classification of Siegel (3). Serial audiograms are shown in Figure 1.

CASE 2

A 52-year-old male, with diabetes mellitus for one year, was admitted with a-two-day history of tin-

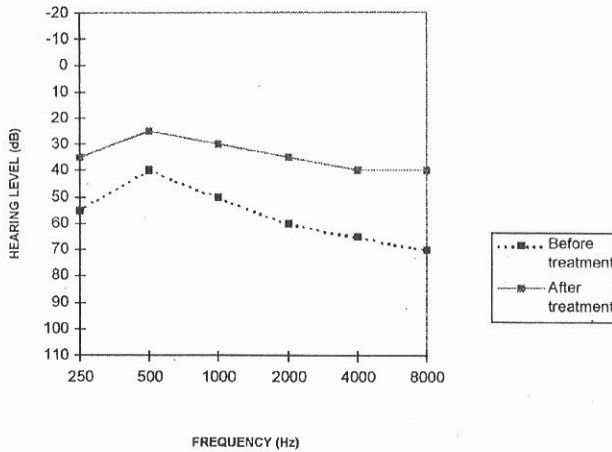


Fig. 1: Serial audiograms of the patient 1.

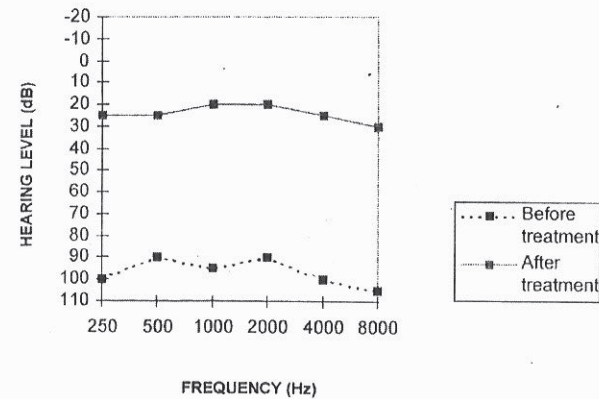


Fig. 2: Serial audiograms of the patient 2.

nitus and sudden hearing loss. He had no drug, smoking, alcohol, infection, stress or allergy or any notable past medical history.

His physical examination revealed no abnormalities, including neurological and opthalmological evaluations, except sensorineural hearing loss. His blood glucose level was 285 mg/dL (15.8mmol/L) and his HbA₁C level was 11.2%(N=4.5-6.5%). His urinary albumin excretion was (8.2mg/ml).

His audiogram revealed a total sensorineural hearing loss in the right ear. There was a 40 dB hearing loss at 4000Hz in the left ear possibly by occupational.

After treatment he had complete recovery. The serial audiograms of the patient, one week and one month after papaverine therapy are shown in Figure 2.

CASE 3

A 42-year-old diabetic male was admitted with no vertigo. He had diabetes mellitus for three years and was on gliclazide 80mg two times a day for the last 18 months. He had a history of alcohol intake and smoking for 14 years. He had also an emotional stress history for the last few weeks.

His plasma glucose was 142 mg/dL (7.8mmol/L) and HbA₁C was 6.8%. His audiogram revealed a 70 dB sensorineural hearing loss and speech discrimination was 72%, and SRT was 75 dB at the right ear. There was a 30 dB sensorineural hearing loss in his left ear. After treatment he had complete recovery. The serial audiograms of the patient are shown in Figure 3. His blood glucose level was decreased to 142mg/dL with diabetic diet in three days.

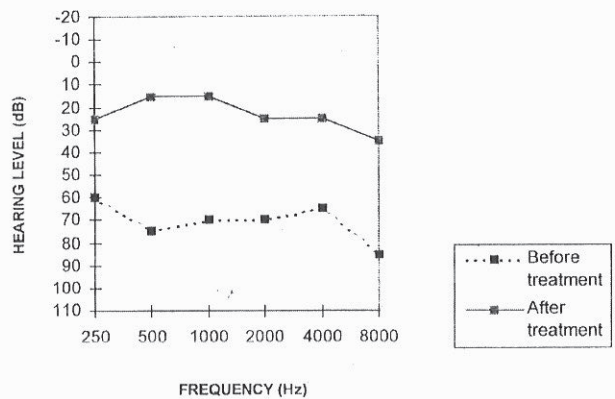


Fig. 3: Serial audiograms of the patient 3.

DISCUSSION

There is disagreement among authors regarding the existence of or incidence of hearing loss secondary to diabetes. While studies have not demonstrated an increase in hearing loss in the young diabetic population, there is some evidence that elderly diabetic patients suffer a bilateral, progressive, high frequency hearing loss at an earlier age than the non-diabetic population (4).

Most of the complications of diabetes mellitus are consequence of the metabolic derangements, mainly hyperglycemia(2). From evidence derived from temporal bone studies, it would seem logical that the diabetic patient might be at an increased risk of ischemic injury to the inner ear (5).

Sudden hearing loss occurring in all age ranges, which may or may not recover, has been described in diabetic patients (3).

Wilson et al(5) found no difference in the audiological pattern of ISHL in diabetic patients and controls with ISHL with steroid therapy.

Medical treatment for sudden sensorineural hearing loss is still controversial although many treatment regimens have been proposed. The Otolitic Medical Group has been using the vasodilator regimen for over 30 years with only minor changes (6). Belal (7) advised to give vasodilator therapy and/or heparin until a viral disease and membrane breaks were eliminated. In our opinion it is not necessary to eliminate a viral cause since it has been suggested that a viral etiology may cause certain vascular changes that result in a reduced blood flow to the cochlea.

By using papaverine in guinea pigs, Suga and Snow(8) showed the increase of blood flow to the cochlea to a rate of $208 \pm 71\%$. Although papaverine hydrochloride is a selective cerebral vasodilator agent

it has not been used frequently for the treatment of ISHL. Papaverine increase cerebral blood flow and decreases cerebral vascular resistance without altering oxygen consumption (9).

Umemura et al. (10), reported in their experimental study that papaverine caused an increase in inner ear blood flow in a dose dependent manner. No significant differences in the response to papaverine were observed between the diabetic and control rats.

Shaia and Sheehy (6) stated that favorable prognostic factors were absence of vertigo and early treatment. Our cases had no vertigo and were treated early. This may explain our successful results. However, in these early treated and vertigo free three patients, the possibility of having the same results with the treatment of placebo or at least other therapeutic agents can not be eliminated.

Ravi and Henderson (2), in their case report of a diabetic patient with sudden deafness, published the recovery of hearing only with the regulation of glucose level.

Wilson et al. (5) found no significant difference statistically with 15 diabetic and 15 non-diabetic patients who received steroid and who did not.

Shaia and Sheehy (6), reported diabetes, vertigo and late treatment as bad prognostic factors in their series of 1220 patients with ISHL who were treated with vasodilator regimens (histamine, niacin).

Despite the well-known side effects of histamine and steroids, papaverine hydrochloride is a reliable and safe drug with positive effect on the recovery, so we concluded to advise the use of rapid infusion therapy of papaverine, a selective cerebral vasodilator, for the treatment of ISHL in diabetic patients especially with no vertigo and in whom fewer days (0-7) have elapsed from the onset of hearing loss.

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THE OPINIONS OF PHYSICIANS ABOUT THE ACTIVITIES OF THE CONSULTATION LIASION UNIT*

Hakan Kumbasar** • Elmas İnce*** • Nurşen Nur Atakan*** • Duru Kuzugüdenlioğlu****

SUMMARY

An inquiry of 13 questions was sent to 900 physicians working in the Ankara University, Faculty of Medicine, to learn the physicians' opinions about and suggestions for the Psychiatry Consultation Liasion Unit. Results of the questionnaires were evaluated. Most of physicians were familiar with the unit, and belived the unit was useful.

Key Words: Consultation, Consultation-Liasion Pschiatry Unit

In today's psychiatry, as the interaction of the biological, psychological and psychosocial variables is getting clearer, the psychiatry team began to have its own place in the general hospital and consequently the development of the psychiatry consultation-liasion unit is brought forward. According to the definition of Lipowski, the relation and the collabration of the general medicine and psychiatry is the principle target of the consultation liasion discipline (1).

In order to be able to response the increasing demand in our hospital in a systematic and scientific manner, a separate Colsultation Liasion Unit within the Psychiatry Department was established with the approval of the Academic Committe in 1994. The foundation of the unit was also approved by the Faculty Administrative Committee in 1995. The unit offers psychiatric and psychosocial services in the entire hospital. Our aims are to diagnose, treat and prevent the psychiatric and psychosocial morbidity accompanying the primary disease of the patients in the outpatient and the inpatient clinics; to perform research projects with the cooperation of different clinics; to present educational activities to the non-psychiatric staff (residents, nurses and other health workers) (2,3).

The consultation liasion practices were being carried out much before the establishment of the real unit. These activites were presented in many national and international psychiatry congresses (4,5,6,7).

In order to collect information about the physicians' opinions and suggestions for the consultation-liasion unit we sent questionnaires to physicians working in our hospital.

MATERIAL AND METHOD

Nine hundred questionnaires were sent to physicians (Figure 1). An information concerning the unit such as its foundation, its aim and activities was given as an introduction of the questionnaire. Following this brief explanation, questions were asked and applicants were requested to return the forms in a certain time specified clearly in the form and hand then to the secretary of department.

RESULTS

Nine hundred questionnaires were sent to physicians, and only 84(9,3%) of these 900 forms were returned.

* Poster presentation, XI th World Congress of Pschiatry, Geneva, Switzerlant, 1997

** Associate Professor, Ankara University Faculty of Medicine, Department of Psychiatry, Consultation-Liasion Psychiatry Unit

*** Psychologist, Departments of Psychiatry, Consultation-Liasion Unit, Ankara University

**** Resident, Department of Psychiatry, Ankara University Faculty of Medicine

1. When did you graduate from the medical Faculty?

2. What is your specialization?

3. What is your academic degree? Resident Fellow
 Assistant Professor Associate Prof. Prof.

4. Do you know Psychiatry Consultation Liasion Unit?
 Yes No

5. Have you ever asked for a consultation from the unit?
 Yes No

6. If you have, what are your impressions?
 I think it is useful.
 I don't believe in its benefit.
 The number of the staff member is inadequate.
 I think it is adequate
 I couldn't follow the results.

7. How do you behave when you want a consultation?
 I tell the patient to go to the unit by him(her)self.
 I don't give any information to the patient before the consultation.
 I fill the consultation form and I inform the patient.
 I send the patient with a brief note on any kind of paper.
 I don't ask for a consultation.

8. What are your opinions about asking a consultation?
 My patients do not want to go.
 I don't believe it will be useful.
 It takes a long time to fill the consultation form.
 It is easier to tell the patient to go to unit by him(her)self
 I do not know how to explain the situation to the patient.
 I believe it is useful to refer the patient to the unit.

9. What is your opinion about to be informed with the results of consultation?
 It is enough for me to see the prescription.
 The result is not important for me.
 A reply and an assessment form should be sent.
 A form should be written, and the doctor should be informed.

10. Do you want to cooperate with the unit in terms of liasion?
 Yes No

11. If you say "yes" to the 10th question, what kind of study do you suggest?

12. What do you suggest for a more efficient liasion service?

13. Which topics do you want to discuss in an "interdisciplinary organizaiton of psychiatry consultation liasion" symposium?

Figure 1. Questionnaire

Twenty eight (33,33%) professors responded the questionnaire; other responders were 20 (23,80%) residents, 18 (21,42%) associate professors, 14 (16,66%) fellows and 4 (4,76%) assistant professors.

Fifty seven (67,85%) of the physicians responded the questionnaire knew the unit, but the remaining 27 (32,15%) didn't know.

Thirty five (41,67%) of the physicians have asked for a consultation from the unit at least once, but the others (58,33%) have not.

Answers of the physicians for the 6 th question of the questionnaire were as follows:

Impression	Number	%
I think it is useful	29	82,88
I don't believe in its benefit	2	5,71
The number of the staff members in inadequate	2	5,71
I think it is adequate	1	2,85
I couldn't follow the result	1	2,85

Answers for the 7th question of questionnaire were:

	Number	%
I tell the patient to to to The unit by him(her)self	3	3,57
I don't give any information to the patient before the consultation	0	0
I fill the consultation form and I inform the patient	32	38,09
I send the patient with a brief note on any kind of paper	16	19,04
I don't ask for a consultation	33	39,28

Questions of physicians about asking a consultation were as follows:

	Number	%
My patients do not want to go	0	0
I don't believe it will be useful	5	8,73
It takes a long time to fill the form	1	1,72
It is easier to tell the patient to go to unit by him(her)self	6	10,64
I don't know how to explain the situation to the patient	0	0
I believe it is useful to refer the patient to the unit	46	79,31

Answers for the 9th question were:

Impression	Number	%
It is enough for me to see prescription	0	0
The result is not important for me	0	0
A reply and an assessment form should be sent	26	36,62
A form should be written, and the doctor should be informed	45	63,38

Sixty two (73,80%) of the physicians responded the questionnaire wanted to cooperate with the unit.

Some suggestions of physicians for a more efficient liaison service were close relationship and frequent meetings; specialized staff; to increase the number of the staff; to give more importance to the presentation of the unit; to develop sub-groups for psychiatric problems of specific patients; to educate a specialist for the unit; simple consultation forms instead of detailed ones.

Study topics suggested by physicians were disabled and amputated patients; patients with sexual problems in Urology Department; psychosocial support and management; patients with organ loss; mental disorders in endocrinologic diseases; long term cooperation with psychiatry in malignancies; cancer patients; psychosomatic problems in asthmatic patients; menopause; claustrophobic patients resisting MRI; chronic visual problems; cardiological problems; regular meetings with Plastic Surgery; doctor-patient relationships.

DISCUSSION

The purposes of this study were to present Consultation Liaison Unit founded in 1994 in Ankara University, Faculty of Medicine and to learn other departments' point of view; to learn other physicians ideas and suggestions; to structure the activity program regarding the needs of the clinics.

Only 84 (9,33%) of 900 questionnaires were turned back. Eventhough our activities have started in 1970s and consultation-liaison lectures have being given to medical students since 1986, the participation to the inquiry is very low. This result is similar with that of a previous study in which 39 inquires from a total of 210 (18,5%) were turned back (8).

In another study for determining the physicians' thoughts and feelings about child psychiatry; 61,7% of questionnaires were received (2). Higher participation rate in this study may be due to the fact that the forms were given and collected back by the investigators.

Most of the participants (67,85%) knew the unit; but only (41,67%) of the participants have asked for a consultation at least once. A great majority of the physicians responded the questionnaire (82,88%) thought the unit was useful. According to this study when physicians wanted consultation, most of them filled

the consultation form and gave information to the patients, and most of them want to be informed after consultation.

At the end of the questionnaire, physicians suggested important points for both research projects and

daily practices.

We believe that we have to tell more about consultation liaison unit to physicians; to perform research protocols with other departments; and to provide closer relationships with other clinics.

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SPONTANEOUS REMISSION OF CANCER: A TURKISH SAMPLE

Gülören Ünlüoğlu* • Ülgen Okyayuz* • Hakan Kumbasar* • Timur Oğuz*

SUMMARY

During recent years we started to hear about spontaneous remission/regression of cancer. If this is true, it carries the message of more hope for Ca treatment. Therefore time has come to determine the existence of such cases.

Since Ankara is one of the cities where cancer is treated in Turkey, our study team decided to work with cancer treating physicians in Ankara, trying to find out an answer to the question of "How many cases who had spontaneous remission in cancer could be detected" according to those doctors who could be reached. A questionnaire was prepared and given to 350 cancer treating doctors working in 3 university and 3 ministry hospitals.

A total of 117 forms were collected by the 1st of April, 1997 out of whom, 36 were female and 81 were male physicians. Twelve physicians witnessed 28 remissions and 14, physicians reported 125 regression cases, both concerning various types of cancer.

The results are given with tables and discussion made in the text.

Key Words: Cancer, Remission

During their training almost all physicians are exposed to the importance of stress and how it can effect the mind and body of any individual (1,2,6).

In 1968, I attended a medical conference in Ahmet Andiçen Cancer Hospital in Ankara, Turkey, where the relationship between stress and cancer was discussed by two of the most prominent physicians of the time, one of whom was a breast surgeon and the other a psychiatrist.

When the surgeon completed his lecture; he told us about one of his latest cases who was operated successfully, recently due to the cancer of breast. Since her cancer history was rather striking and demonstrative, he wanted to share with us the valuable information she gave to him. The story was as follows: This middle aged lady had breast cancer at the age of 18, when she was living with her parents in Rome, Italy, where her father was working as a diplomate. They took a biopsy from the lump in her breast and reported to her and her family that she had cancer of breast and unfortunately it was inoperable. Since she had only 3-6 months to live, all she could do would be to go and have all the fun she could. This was the doctors' commendation. She came back to Istanbul and she

swam, danced and tried to do every thing that she wanted within her reach. That year she had the best summer of her life. At the end of one year the lump got smaller and eventually vanished. Seeing that she did not die she finished her education, got married, had children who grew-up to be adults now. However some years ago she had cancer of the stomach and was operated successfully. Recently when she felt another lump in the breast she came to see what could be done. When her doctor suggested a biopsy she gave him the stated history of her illness. The breast surgeon contacted the hospital in Rome with the patient's written and signed consent asking for information or the pathology report of some 30 years ago. The reports were lost, but the biopsy material with her name tagged on it was found in the specimen archives of the hospital, and was sent to Ankara, Turkey, immediately. It was similar type of cancer which was found now. At the time of the breast examination of her new doctor, when she realized that he was suspicious of cancer and seemed quite worried, she said to him, "Don't worry too much doctor, I guess I am able to overcome cancer". After giving this information to us, he went to the backstage and brought the patient to

* Department of Psychiatry Medical School of Ankara, Turkey

stage with him, holding her shoulders with great tenderness and with admiration he introduced her to us. The whole crowd was breathless. I have never forgotten her face and her courage, after all she was the first person I had met who had won a fight against cancer. For many years she stayed so until I read doctor B.S. Siegel's book, "Love, Medicine and Miracles", where his wonderful work with *Exceptional Cancer Patients* (EcaP) is discussed from various aspects installing hope to professionals', minds (7). Another book of importance on the subject is "Getting Well Again" where the authors described their work with 159 terminal cancer patients all of whom had only one year life expectancy. At the end, 19 percent was completely cured and the disease regressed considerably in 22 percent of all patients. The rest could have better quality of life (8). To teach the patients and their families not to give up hope is very important. Visualization and usage of imagery techniques used by Greens (4) and Schülzenberger (5) have proven to be much effective within the last two decades.

A patient with great awareness not only fought his illness personally, but also inspired others to fight their illness with courage and dignity (3).

We as members of consultation and liaison team of Ankara medical school have been working with cancer patients on individual basis and their families in psychosocial support groups since 8 years. When we learned about the International Symposia on Spontaneous Remission of Cancer in Heidelberg between 17-19 April, 1997, we thought time had come to find out what was happening to cancer patients after the diagnosis of cancer in Turkey. To have a nationwide sample was impossible due to shortness time. Since Ankara, was the capital of Turkey and was one of the major cities where cancer was treated and also it was the city where all the members of the study team lived we decided to limit our study to Ankara's cancer treating physicians.

OBJECTIVES

The goals of our study were to find out:

- If the cancer treating specialist physicians had ever witnessed any remission/regression case/cases of cancer,
- The type of cancer case/cases which showed spontaneous remission or regression, that was more than expected,
- The number of such cases,

- What are the physicians' thoughts or views for reasons of such remissions/regressions.

METHOD

Procedure

An inquiry form was prepared by the study team investigating the details related to spontaneous remission/regression of cancer as observed by the treating physicians (see the inquiry form attached). We tried to determine the name of the hospitals where cancer patients were being treated by calling each hospital's superintendent and asking them if cancer treatment was available in their hospital. We found 8 hospitals treating various types of cancer. Since it was difficult to obtain permission for collecting information from military physicians we excluded the military hospital from our study which left us with seven Ankara hospitals 3 of which were university and the rest were ministry hospitals. The total number of beds in seven hospitals was 7136. However we could not calculate the number of cancer beds since cancer patients could be admitted to various clinics other than oncology, medical oncology or radiation oncology per se. The distribution of beds according to hospitals is: Medical School of Ankara University 2186, Hacettepe University Hospital 1200, Başkent University 210, Oncology Hospital 600, General City Hospital 1500, Hospital of Chest Diseases and Tuberculosis 1100, and Subspecialty Hospital 340.

The inquiry form was given personally by a team member to 250 cancer treating physicians whose specialties were Surgical Oncology, Medical Oncology, Radiation Oncology, Pediatric Oncology, Nuclear Medicine, Neurosurgery, General Surgery, Surgery of Thorax, Gastroenterological Surgery, Ear, Nose and Throat Surgery, Urology, Obstetrics and Gynecology, Internal Medicine, Gastroenterology, Chest Diseases, and Hematology. There were over 100 specialist in Oncology Hospital which was located at the outskirts of Ankara, therefore 100 forms were given to the superintendent of Oncology hospital to be distributed to the specialists of that hospital adding to a total of 350 forms. To collect the 250 individually distributed forms, each doctor was revisited by a team member and the completed forms were collected. Some doctors said that they did not have time to fill the forms, that they probably would complete them later. The completed inquiry forms of oncology hospital were fetched from the superintendent. We stopped collec-

ting the forms on the first day of April since time was running short.

A letter addressed to the respondent was attached to the form explaining the goals of the study team which was to make a pilot study in order to find out some information on the topic of remission and regression of cancer to present as a Turkish sample in the Spontaneous Remission Symposia which was going to take place in Heidelberg, Germany, at the date of April 17-19 of 1997. The letter ended with the expression of our gratitude to the physician for their contribution to our study by conveying their views and experiences to us. At the end of the letter the name and the application address of Heidelberg Symposia was given, in case anyone wanted to attend it.

Thirty six female and 81 male physicians adding up to 117 respondents completed the spontaneous remission/regression inquiry form out of 350 forms which were distributed to them.

The available data was processed by SPSS/PC. Since this is a descriptive study the statistical analysis of the data was limited to marginal tables indicating the number of respondents and frequencies in accordance to various dependent and independent variables.

In order to be able to clarify the general characteristics of the sample we tried to categorize the independent variables. For example since the ages were between 27 and 78 we divided the groups to decades. Year of graduation from medical school was between 1943 and 1993. The year of 1943 is by chance, but the year of 1993 is obligatory since specialty training in Turkey takes 4 years, 1993 is the last year the respondent could be accepted to the study since our study covers only the specialists. Therefore graduation years were divided into decades such as 1943-1953, 1954-1963, 1964-1973, 1974-1983, and 1984-1993. Years spent as a physician and as a specialist are divided to decades similarly. Duration of working with cancer patients are divided into decades again. However because of the importance of experiences, a subdivision was made to differentiate the beginners from the more experienced. This was done by sub-dividing the first decade in terms of less than 5 years and 6-10 years and later to decades as 11-20 and so on. The number of patients and the number of cancer patients examined were between, less than 150 to more than 5000. Therefore these had to be divided including 100 patients in every bracket starting from less than 150 until 1050 and from 1051 to 1500 and 1501-2500, and 2501-5000 respectively.

General Characteristics of the Sample

Of the 117 specialist physician respondents 69% (n=81) is male and 31% (n=36) is female. Their age range is between 27 and 78 having one physician at each age. Average age is 40, and the cumulation is between 31-40 with 54%, 41-50 age group follows with a ratio of 20.7% and 27-30 age group is third in row with a ratio of 11.3%.

The majority which is 56% is graduate of Ankara Medical School and second is Hacettepe University medical school with 30%. The rest is from various Schools of Medicine in Turkey, except the two physicians one of whom is from Azerbaijan and the other from Bagdad medical school of Iraq.

The year of graduation from medical schools varies between 1943 and 1993. The cumulation is on 1984-1993 range with 47%. It is followed by 1974-1983 group by 33%, and third in row is 1964-1973 group with a ratio of 13%.

In the duration of medical profession the first row is taken by 11-20 years group with 44.2% followed by the group who have been in the profession less than 10 years, with a ratio of 31.6%, and 14.5% is taken by 21-30 years group.

Distribution of the 16 specialty fields show a wide range, the highest percent being from general surgery which is 16.4% followed by radiological oncology with 12.9%, and the third is medical oncology with a percent of 12.1. Ear, nose and throat comes with a percentage of 6.9 and both hematology and gynecology follow with 6%. The rest is as follows: Internal Medicine, Diseases of Chest, Urology, Pediatric Oncology, Gastroenterological Surgery, Gastroenterology, Nuclear Medicine, Neurosurgery and Surgical Oncology within the percentage ranges of 5.2 to 1.7.

The distribution of year groups spent at the field of specialists show an accumulation of 44% for specialists who spent 5 and less years in their fields followed by 11-20 group with 25%. Six to ten years group takes 17% and 21-30 group 11.2%. The rest is insignificant for mentioning.

According to working in various hospitals the first row is taken by Ankara Medical School hospital with a ratio of 42%, followed by Oncology Hospital by 28.2%, then comes Hacettepe Medical School Hospital and Subspecialty hospital is the 4th in the row.

Data collected on the duration of work years in the same field is 39.3% for physicians working under 5 years, and the ones working 6-10 years have a ratio of 26.5%, 11-20 years 19.7%, 21-30 years 7.7% and 31-

40 years 5.9%. There is only 1 doctor working for over 40 years (0.9%).

The ratio of doctors working with cancer patients accumulate between 6-10 years (41%), followed by the group of less than 5 years with a ratio of 23%. Eleven to 20 years group is 20.5% and 21-30 group 12.5%, the ratio of the rest is less than 3%.

Number of patients examined in one year by each physician ranges from less than 150 up to 5000. Percentage of physicians covered by the study population according to the yearly number of patients shows that 15.4% of physicians have examined 1501-2500 patients in one year taking the highest percentage. 13.7% have examined 951-1050, 12.5% 1051-1500 respectively. The highest patient bracket is 1501-5000 and only 6% of the physicians have seen so many patients. The rest has between 5.1% to 2.6% which may not be considered too important.

The total number of cancer patients in treatment during one year seems to be pretty high in general. However the accumulation seems to have the highest ratio of 19.7% among the less than 150 cancer patients bracket. The second in line is the group of 151-250 treated cancer patients bracket with a ratio of 13.7%. The rest has the following row: 11.1% 251-350 cancer patients' group, 9.4% 451-550, 7.7% 1501-2500, 6.8% 1051-1500, and 5.1% 851-950 cancer patients group. The ratio of the rest is less than 3.4%.

Our sample of specialist physicians are diagnosing, following and treating cancer patients closely, therefore we tried to see if they had the chance of encountering remission/regression of cancer according to different variables. The findings concerning the depend variables such as remission and regression are reflected by 8 tables.

DISCUSSION

This descriptive study which aims to collect first hand information from cancer treating specialist physicians who work in various hospitals of Ankara on the subject of "Spontaneous Remission and Regression of Cancer" as observed and assessed by them. Since it is the first pilot study carried out on the subject in Turkey, we tried to reach to 500 physicians.

When we review our sample of $n = 117$ respondents, 30.8% of whom were female and 69.2%, male physicians. Their age range was quite wide and was between 27 and 78 with the mean age being 40. The years they spent in the profession as a physician differed from 4 to 54 years, cumulation being in the younger bracket. The number of years spent as a specialist is less. The number of patients examined/assessed in 1 year was between -150 and 5000+. As one can see some of our specialists could be considered overworked definitely which can answer the question why we could not collect all of the forms in three months time.

To the question of if they observed any spontaneous remission of cancer among patients they treated, 9 male and 3 female physicians answered affirmatively (Table 1). Nine male specialists reported 24 cases of remission whereas only 3 female specialists reported 4 remission cases. Since 11% of males and 8% of females encountered 28 cases, 10% of all doctors have seen remissions which can be considered an important finding. So spontaneous remission exists.

The situation changed in the item of regression (Table 2). Although equal number of male and female physicians reported regression one out of 9 males and 3 out of 9 females did not state the number of cases they had seen, the exact number of regression cases could not be calculated. Therefore the 4 doctors were excluded leaving 14 doctors who reported a total of 125 regression cases. Eight male specialists encounte-

Table 1: Distribution of 28 Spontaneous Remission Cases According to the Sex of the 12 Reporting Specialist Physicians

Spontaneous Remission Cases	Male Physicians		Female Physicians		Total	
	n=81	69.2%	n=36	30.2%	n=117	100.0%
n'	n	%	n	%	n	%
0	72	88.9	33	91.6	105	89.7
1	5	6.2	2	5.6	7	6.0
2	1	1.2	1	2.8	2	1.7
4	2	2.5	0	-	2	1.7
9	1	1.2	0	-	1	0.9
Total	81	100.0	36	100.0	117	100.0

Table 2: Distribution of 125 Regression Cases According to the Sex of the 14 Reporting Specialist Physicians

Regression Cases n' = 125 n'	Male Physicians n=81 69.2%		Female Physicians n=36 30.2%		Total n=117 100.0%	
	n	%	n	%	n	%
0	72	88.9	27	75.0	99	84.3
1	3	3.7	-	-	3	2.5
2	2	2.5	2	5.5	4	3.4
3	1	1.2	-	-	1	0.9
5	1	1.2	-	-	1	0.9
9	1	1.2	-	-	1	0.9
10	-	-	1	2.8	1	0.9
17	-	-	1	2.8	1	0.9
20	-	-	1	2.8	1	0.9
50	-	-	1	2.8	1	0.9
Not Known	1	1.2	3	8.3	4	3.4
Total	81	99.9	36	100.0	117	100.0

red only 24 cases whereas 6 females encountered 101 cases. It is interesting to find 10% of females reported 101 cases which means more than 4 times as much as the male doctors reported.

Table 3, shows the distribution of spontaneous remission cases according to the hospital of the respondent specialist. Only physicians of 3 hospitals have seen remissions. The first is Ankara Medical School from where 8 respondents saw (n'=20) cases. Oncology hospital's 3 physicians observed (n'=7) remission cases, and from Hacettepe Hospital 1 physician

witnessed only (n'=1) remission adding up to n'=28 cases of remission.

Table 4, reflects the number of doctors from 6 hospitals who encountered regression of cancer. Out of n=49 physicians of Ankara Medical School Hospital 8 respondents have seen cancer regression in n'=95 cases. Oncology Hospital from where we had 33 respondents, 5 specialist physicians saw n'=25 regression cases and from Hacettepe medical school hospital with 24 participating physicians only one subject had seen only one case of regression again. What the

Table 3: Distribution of Spontaneous Remission Cases According to the Hospitals the Reporting Specialist Physicians are Working

Spontaneous Remission Cases n' = 28 n'	Ankara University Hospital n=49 41.9%		Oncology Hospital n=33 28.2%		Hacettepe University Hospital n=24 20.5%		Subspecialty Hospital n=6 5.1%		Hospital of Chest and Tuberculosis n=3 2.6%		Başkent University Hospital n=2 1.7%		Total n=117 100.0%	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
0	41	83.7	30	90.1	23	95.8	6	100.0	3	100.0	2	100.0	105	89.8
1	5	10.3	1	3.3	1	4.2	-	-	-	-	-	-	7	5.9
2	1	2.0	1	3.3	-	-	-	-	-	-	-	-	2	1.7
4	1	2.0	1	3.3	-	-	-	-	-	-	-	-	2	1.7
9	1	2.0	-	-	-	-	-	-	-	-	-	-	1	0.9
Total	49	100.0	33	100.0	24	100.0	6	100.0	3	100.0	2	100.0	117	100.0

Table 4: Distribution of Regression Cases According to the Hospitals the Reporting Specialist Physicians are Working

Spontaneous Remission Cases n' = 125	Ankara University Hospital n=49 41.9%		Oncology Hospital n=33 28%		Hacettepe University Hospital n=24 20.5%		Subspecialty Hospital n=6 5.1%		Hospital of Chest and Tuberculosis n=3 2.6%		Başkent University Hospital n=2 1.7%		Total n=117 100.0%	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
0	40	81.6	25	75.8	24	100.0	5	83.3	3	100.0	2	100.0	99	84.6
1	2	4.1	1	3.0	-	-	-	-	-	-	-	-	3	2.5
2	3	6.1	1	3.0	-	-	-	-	-	-	-	-	4	3.3
3	-	-	1	3.0	-	-	-	-	-	-	-	-	1	0.9
5	-	-	-	-	-	-	1	-	-	-	-	-	1	0.9
9	-	-	1	3.0	-	-	-	-	16.6	-	-	-	1	0.9
10	-	-	1	3.0	-	-	-	-	-	-	-	-	1	0.9
17	1	2.0	-	-	-	-	-	-	-	-	-	-	1	0.9
20	1	2.0	-	-	-	-	-	-	-	-	-	-	1	0.9
50	1	2.0	-	-	-	-	-	-	-	-	-	-	1	0.9
Not Known	1	2.0	3	9.0	-	-	-	-	-	-	-	-	4	3.3
Total	49	99.8	33	99.8	0	-	6	99.9	0	-	0	-	117	100.0

reasons for such differences could be, needs further research.

Table 5, shows remission cases according to medical specialties. Although there were 16 specialties, affirmative responses came from 6 specialties for remission. One specialist from internal medicine saw 9 cases, one doctor from Hematology encountered one case, from Ear Nose and Throat specialty one doctor reported 4 cases, from general surgery 3 doctors reported 7 cases, from surgical oncology one doctor reported one case, from Medical Oncology one doctor and

one case, from Radiation Oncology 2 doctors and 3 cases from Pediatric Oncology 2 doctors and 2 cases with a general total of 12 physicians and 28 cases of remission. The highest number of remission cases (n = 9) was seen in internal medicine and only by one specialist. This is almost one third of all (n = 28) remission cases. Therefore this finding also must be investigated in more detail.

Table 6, repeats the issue for regressions, except the responding clinics differ. Two specialists from chest diseases saw n'=12 cases regressing, gynecolog-

Table 5: Distribution of the Number of Remission Cases of Cancer According to Medical Specialty (12 Physicians Reported 28 Cases of Remission)

Cases	Internal Med.	Hematolog	Ear, Nose, Throat	General Surgery	Surgical Oncology	Medical Oncology	Radiation Oncology	Pediatric Oncology	Total (Physician)	Total Cases
1	-	1	-	1	1	1	1	2	7	7x1=7
2	-	-	-	1	-	-	1	-	2	2x2=4
4	-	-	1	1	-	-	-	-	2	2x4=8
9	1	-	-	-	-	-	-	-	1	1x9=9
Total	1	1	1	3	1	1	2	2	12	28

yists 2 reported $n'=51$ cases, one urologist $n'=5$ cases, 5 general surgeons reported $n'=16$ cases, followed by one specialist only from surgical and medical oncologies with a total of $n'=20$ regression cases. Two specialists from radiation oncology responded with $n'=21$ cases. The total sum of regression cases was 125.

Table 7 shows the distribution of remission cases according to specialist physicians' number of years spent treating cancer patients. The highest number of responses came from -5 years group and 11-20 years group as $n=3$ responses with $n'=10$ cases of remis-

sion, 6-10 years group gave $n=4$ responses covering $n'=5$ remission cases. Twentyone -30, and 31-40 year groups saw one case each. These findings make us think the possibility of younger specialists having more hope than the older ones. It is a fact that people usually may see what they are looking for.

Table 8, reflects the regressions in terms of years spent as a specialist treating cancer. There are $n=20$ respondents in -5 year group with one regression case each. Among 6-10 years group $n=9$ respondents have seen $n'=42$ regression cases in comparison to only 5 remission cases of the same group. Regression is mo-

Table 6: Distribution of Cancer Regression According to Medical Specialty (14 physicians reported 125 cases of regression)

Cases	Chest Med.	Gynochology	Urology Throat	General Surgery	Surgical Oncology	Medical Oncology	Radiation Oncology	Total Physicians	Total (Cases)
1	-	1	-	1	1	-	-	3	$3 \times 1 = 3$
2	1	-	-	2	-	-	1	4	$4 \times 2 = 8$
3	-	-	-	1	-	-	-	1	$1 \times 1 = 1$
5	-	-	1	-	-	-	-	1	$1 \times 5 = 5$
9	-	-	-	1	-	-	-	1	$1 \times 9 = 9$
10	1	-	-	-	-	-	-	1	$1 \times 10 = 10$
17	-	-	-	-	-	1	-	1	$1 \times 17 = 17$
20	-	-	-	-	-	-	1	1	$1 \times 20 = 20$
50	-	1	-	-	-	-	-	1	$1 \times 50 = 50$
Totals	2	2	1	5	1	1	2	14	125

Table 7: Distribution of the 28 Remission Cases According to the Physicians' Number of Years Spent Treating Cancer Patients

Cases	Years					Physician	Cases
	-5	6-10	11-20	21-30	31-40		
n'	n	n	n	n	n	n	n
1	-	3	2	1	1	7	7
2	1	1	-	-	-	2	4
4	2	-	-	-	-	2	8
9	-	-	1	-	-	1	9
Total	3	4	3	1	1	12	28

Table 8: Distribution of the 125 Regression Cases According to the Physicians' Number of Years Spent Treating Cancer Patients

Cases	Years					Physician	Cases
	-5	6-10	11-20	21-30	31-40		
n	n	n	n	n	n	n	n
1	-	2	-	1	-	3	3
2	-	3	-	-	1	4	8
3	-	1	-	-	-	1	3
5	-	1	-	-	-	1	5
9	-	1	-	-	-	1	9
10	-	1	-	-	-	1	10
17	-	1	-	-	-	1	17
20	1	-	-	-	-	1	20
50	-	-	1	-	-	1	50
Total	1	10	1	1	1	14	125

re common. The third bracket of years treating cancer is 11-20 with $n=5$ responses and $n'=5$ where as they had seen $n'=10$ remission cases earlier.

When we come to what kinds of cancer were being treated by the members of our sample, the list of answers is very long, starting from visceral organs to squamous cell cancer, leukemia, neuroblastoma, non-hodgkin lymphoma and all kinds of metastasis, multiple myeloma etc.

For reasons of remission and regression 18 subjects responded. The answers could not be categorized. In several answer that they did not know. Five said that immunity or immune system played an important role. Some emphasized the importance of attachment to life and living, early diagnosis and the right therapies be it chemotherapy, radioteraphy or surgical intervention. Some wrote that biological behavior of the tumor was important. Active participation of the patient in treatment program, plus psychotherapy and to try to have high morals were sincerely recommended. One doctor stated that literature is available about spontaneous regression of lymphomas, but that she has not encountered any such case.

Now when we review our initial goals we see that 11% of male respondents and 8% of female specialists

had encountered cancer remissions while similar percent fo males and 25% of females had encountered regressions, but only 16% indicated how many.

The type of cancer cases showing remission and regression has a great variety and therefore could not be meaningfully classified.

The total number of remission cases reported by the subjects is 28 and for regression it is 125 which could be concidered more than our team expected.

The stated possible reasons for remission and regression of cancer by specialist physicians are quite parallel to what is reported by various researchers in our list of references.

We feel that even to talk about the possibilities of spontaneous remission/regression of cancer is important and it should be taken up more frequently and new questions should be raised and answers should be searched for since working doctors can report such cases giving us more hope.

However, the results make one realize how little we know, and how much more we need to know motivating us for working more.

Still the question exists: "Does a physician see remission/regression of cancer, because that is what he/she is hoping and looking for".

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PERSPECTIVES OF RELATIVES OF PSYCHIATRIC PATIENTS*

Engin Tuncer** • Hüseyin H. Özsan** • Aykut Özden*** • Işık Sayıl****

SUMMARY

The relatives of psychiatric patients can have a strong influence on the outcome of the treatment process. Modification of these perspectives can prevent a likely relapse or poor prognosis. We conducted the study among the relatives of depression and psychotic patients. 30 relatives of depressed and psychotic patients were included and a control group matching age, gender and education level were evaluated. Two questionnaires prepared by the authors were used to evaluation. Results showed that relatives attribute the causes of the disorders mainly to personal and biological causes than familial or esoteric causes. There were slight difference between the two groups regarding etiologic questions, but while relatives of depressed patients reported mostly personality issues for treatment, relatives of psychotic patients reported biological treatments. The results indicated that a regular and structured education programme is needed to cope with irrelevant attribution of relatives.

Key Words: *Relatives of psychiatric patients, Etiologic concepts*

Etiologic concepts of the relatives of psychiatric patients are known to have great impact on the course and outcome of their illness (1,2). Relatives who attribute their patients' illnesses to irrelevant causes may search for extra-psychiatric or extra-psychological treatments and induce non-compliance with the treatment. Moreover, relatives who have ascetic beliefs or blame their patients for their illness, may not search any treatment at all.

Attributing the cause of illness to irrelevant issues and searching treatment from extra-medical sources, para-professionals, religious or quasireligious persons are supposed to be highly prevalent in Turkey. While there is no reliable statistics on this subject, our experience suggests about a 50% of psychiatric patients visited an extra-medical person, almost always accompanied by their relatives. On the other hand, religion is a major phenomenon especially in the rural parts of Turkey, and we know that individuals' religious beliefs can sometimes interfere with their health behaviors (3).

The aim of this study is to determine the perspectives of the relatives of psychiatric patients on causes

and treatment of the illness. Findings of such studies would be useful in shaping the psychoeducation (PE) programs for the relatives, and in treatment difficult patients (4).

In Turkey, relatives usually continue their support to their members with mental illness, whatever the severity of the patient is, and as a consequence, live the burden of chronic mental illness to the end. Almost every psychiatrist and psychologist, who is working with chronic psychosis (CP) patients, tries to help the families, in a structured way or not. One of the major approaches to help the family is the provision of basic information about the patient's psychiatric disorder (5). Diagnosis, signs and symptoms, treatment approaches, side-effects of medication and course of illness are the main information given to the families, among others.

MATERIAL AND METHOD

This study was conducted in the Psychiatry Clinic of University of Ankara Turkey, in 1995. We randomly selected the relatives of 30 schizophrenics and 30

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** Associate Professor of Psychiatry, M.D. Department of Psychiatry, University of Ankara, Medical School.

*** Resident, M.D., Department of Psychiatry, University of Ankara, Medical School.

**** Professor of Psychiatry M.D., Department of Psychiatry, University of Ankara, Medical School.

depressives from our inpatient wards, and 30 "normal" persons as a control group for gender, age and education levels, the main variables that were likely to influence the results other than illnesses. These relatives were chosen if they had been living with the patients for the majority of the duration of the illness. As expected, they were mostly spouses or parents. Occasionally we included siblings or children, who live the patients.

Two questionnaires and one demographical data form were used in the study, all prepared by the researchers in the light of relevant literature and Turkish Culture. These two questionnaires consisted checklists about the causes and treatments of psychiatric disorders. The first one had 57 items on the etiologic issue, while the other had 15 items on treatment options.

In the first questionnaire, relatives pointed their opinions on whether that particular item could be a cause of a psychiatric disorder or not, on a 3-Point-Likert type answers sheet, as "I Agree", "neutral" or "I Disagree." The 57 items were divided into five major topics; "Biological causes", "Familial causes", "Psychosocial causes", "Personality causes" and "Esoteric causes", however, they are placed in the text randomly.

The other questionnaire was a rather short one (15 items), scanning for the beliefs of subjects on the treatment of psychiatric disorders. It was answered as mentioned above, but consisted four subgroups; "Medical treatments", "Esoteric treatments", "Personal growth" and "Environmental changes". In the demographical data form, relatives were also questioned for their religious beliefs and previous extra-medical treatment searches.

Scoring of the two questionnaires were as follows; "1" for Agree, "2" for Neutral and "3" for Disagree.

Thus, higher points indicate disbelief on the written cause or treatment. Total point for a subgroup was calculated and then divided to the number of items, which gave the weighed scores that show the inclination of relatives clearly. For instance, a score of 2.5 shows an inclination towards a disagreement with the items, compared with a score of 1.5 or less. The means of all subgroups for the three study groups are also calculated. Then, the means of the two relative and control groups are compared with variance analysis, and if a significance is found, students' t-test for independent samples is used, in order to find the origin of the significance. SPSS for Windows program is used in the statistical analysis.

RESULTS AND DISCUSSION

Table 1 shows the demographical features of the subjects. Statistical analysis was made for only age, educational years and gender (chi-square for gender, t-test for others) and no significance was found between the groups. Schizophrenic patients, as expected, seemed to have more hospitalizations and longer duration of illness.

In Table 2, relatives' religious and extra-medical searches are outlined. Most of the relatives in all groups practice religion moderately, but many of them had visited some sort of extra-medical source for help, even the "normal" group. There is a difference between the relatives of schizophrenics and the other two groups, since almost 25% of the latter two groups reported searching of no extra-medical help. This may be a result of the more devastating course of schizophrenia, which eventually led relatives to search for a "cure". Nevertheless, these findings prove the high affiliation of Turkish people with non-medical, spiritual issues, and we believe that they would be higher, if the study was conducted in a rural settlement.

Table 1: Demographical Features of the Subjects

n=30 for all	Relatives of Schizophrenia Patients	Relatives of Depression Patients	Controls
Gender (Male)	50.0%	43.3%	50%
Marital Status (Married)	80.0%	83.3%	76.7%
Occupation (Employed)	56.7%	50.0 %	63.3%
Age*	48.3	46.1	45.9
Education*	9.8	9.7	10.8
Duration of Illness*	6.5	4.6	-
Number of Hospitalization	2.9	2.2	-

* Mean years

Table 2: Religions and Extramedical Treatment Searches (%)

n=30 for all	Relatives of Schizophrenia Patients	Relatives of Depression Patients	Controls
Religious Practices			
Frequent	40.0	33.3	26.7
Moderate	30.0	43.3	26.7
Very few/None	33.3	36.7	30.0
Extra-Metical Help			
Healer, sacred place	60.0	30.0	0.0
Fortuneteller	40.0	16.7	53.3
Meditation	33.3	3.3	50.0
Psychology sources*	26.7	50.0	33.3
None	6.7	56.7	24.0

Another striking finding was the high percentage of relatives referring to relevant publications available to them. A similar result was found in another study conducted with psychiatry "patients" in the same clinic (6). These results are important for the PE programs. Educational materials for the patients and relatives have to be published, and the influence of the media should not be overlooked.

Table 3 outlines the questionnaire on causes, with statistical analysis between each group of subjects. We can see from this table that; both relative groups put personality factors as the leading causes of illness, followed by biological causes, while placing "family factors" and the infamous "esoteric" factors backwards, which is a contradictory finding with a similar study made with male psychotics' relatives (7). However, this result is somewhat expected, as the relatives would certainly externalize the cause of illness, but they might have chosen the esoteric factors more readily, so this is a comforting result in that sense. However, pointing the finger on the patients' personality as the cause of illness, must not be ignored. In our experience, blaming of the patients' personality is particularly seen in substance dependents' relatives, but it definitely has a counter-therapeutic effect in all patient groups, which makes it an important topic on PE sessions.

The control group reported all factors, but esoteric ones, as the likely causes of illness. There are even significant differences with the relative groups on family and psycho-social factor groups.

Özsan and colleagues (6) performed the same study with schizophrenic and depressive patients, and showed that patients were usually undecided on the cause of illness, nevertheless placing the psycho-social factors ahead. They did not rank esoteric causes high as well. These and our findings made us think

that, although our people give credit to various extra-medical issues, they generally do not believe in them as a cause of illness. However, this result may be a bias from subject selection, since our patients and relatives are mostly educated urban settlers, and their illness had a long duration, by which one can obtain correct information.

In Table-4, the results of relatives' opinions on the treatment issues are outlined. All three groups reported "personal growth" and "medical treatment" in the first place, but medical treatment is more evident in schizophrenia group ($p < .01$), and moreover depression group reported "external treatments" significantly more than the control group ($p < .05$).

These results reflect that relatives of schizophrenic patients believe in the efficacy of medications more than the others. Although we witness that outcome with anti-depressants in depression is more favorable than antipsychotics in schizophrenia, it is interesting to find the opposite in the relatives' report. This may be a result of different expectations. Relatives of schizophrenics might have lived the results of non-compliance with the drugs, so that they believe more to drugs even though they do not "cure" their patients completely. On the other hand, relatives of depression patients expect more than drugs could give. They want their patients either to change themselves (personal growth) or their environment (external factors).

These issues are important in PE sessions conducted with relatives. They have to learn that "personal growth" can not be achieved as it is supposed to be and environmental changes are not as easy as they seem to be. Nevertheless, it is comforting to find that neither group believe in esoteric treatments, while they practiced it frequently. Frankly, there is a chance that relatives might have hidden their real opinions on esoteric treatments. As a matter of fact, it may be more

Table 3: Attributions on causes of Psychiatric Disorders

n=30 for all	Relatives of Schizophrenia Patients	Relatives of Depression Patients	Controls	Statistics
Biological	1.7	1.9	1.6	Non-significant
Family	2.4	2.3	1.5	p<.001 (S/C, D/C)
Psychosocial	2.1	2.0	1.4	p<.001 (S/C, D/C)
Personality	1.5	1.3	1.7	Non-significant
Esoteric	2.3	2.4	2.6	Non-significant

S: Schizophrenia D: Depression C: Control

Table 4: Opinions on the Treatment of Psychiatric Disorders

	Schizophrenia	Depression	Controls	Statistics
Medical	1.3	1.7	1.8	p<.01 (S/D, S/C)
Personal	1.3	1.2	1.1	Non-significant
External	2.2	1.9	2.4	p<.05 (D/C)
Esoteric	2.2	2.4	2.8	Non-significant

S: Schizophrenia D: Depression C: Control

clever to be skeptical on this issue, and not overlook it in the PE sessions.

CONCLUSION

Patients' and sometimes their relatives' attributions to the causes of illness and their opinions on the treatment methods are usually overlooked by the clinicians. We suppose that; "they know many things and can always think rationally" and the worst of all; "they will accept -and obey- our suggestions and explanations readily". These myths can break the patient-clinician relationship and result in non-compliance, drop-outs and even symptom exacerbation. We have to give enough time to the thoughts of the patients and the-

ir relatives. This study was made in order to give systemic support to the presence of great diversity and unpredictability in relatives' attributions and opinions on causes and treatment of their patients' illnesses.

Contemporary Western psychiatry has already integrated PE programs into their practice and began to get their harvest (5, 8, 9, 10). Since patients and their relatives actively search for explanations for the occurrence of psychiatric disorders, clinicians are well advised to take the initiative and to discuss this issue. It would certainly affect their help-seeking behavior (1,2). In our clinic we do have various PE hours. With the help of studies like this one, we try to shape and revise it.

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A CLINICAL AND RADIOLOGICAL STUDY OF LUMBAR SPINE IN RHEUMATOID ARTHRITIS

Serpil Savaş* • Şehim Kutlay** • Suat Fıföz*** • Süreyya Ergin**** • Serdar Akyar*****

SUMMARY

Study Design: Thirty Rheumatoid Arthritis (RA) patients and age and sex matched 30 chronic back pain patients were studied by using a structured questionnaire, clinical examination of the back and radiology of the lumbar spine.

Objectives: To evaluate the clinical and the radiological characteristics of the lumbar spine in RA.

Summary of the Background Data: The characteristic radiological appearances of lumbar spine in RA include disc narrowing associated osteophytosis, spondylolisthesis, facet joint erosions and osteoporosis. Little is known about the relationship between back pain and these radiological patterns.

Methods: RA patients were asked about the symptom of chronic low back pain. Clinical examination included lumbar spine movements: flexion, extension and lateral flexions and radiology included lumbar spine X-rays and lumbar Computerized Tomography (CT).

Results: Nineteen (%63.3) of RA patients had chronic back pain. Clinically extension was more limited in RA group ($p < 0.05$). Radiologically facet joint erosions ($p < 0.05$), sacroiliac cysts ($p < 0.001$) and sacroiliac osteophytes ($p < 0.001$), compression fractures ($p < 0.05$) were significantly higher in RA group. When these radiological findings were correlated to back pain, we found poor correlation between the sacroiliac osteophytosis, compression fracture and back pain ($p < 0.05$ $r=0.36$). There was a moderate correlation between facet joint erosions and back pain ($p < 0.05$ $r=0.53$).

Conclusion: Our study supports the previous findings characterising lumbar involvement such as facet erosions and compression fractures. However we could not find the other typical radiological findings of RA such as disc narrowing without osteophytosis and spondylolisthesis. We found a correlation between facet involvement and back pain and also the characteristics of the pain in our patients resembled facet syndrome.

Key Words: Rheumatoid Arthritis, Low back pain

The radiological and the clinical appearances of the cervical spine in Rheumatoid Arthritis (RA) are well known however there are limited number of studies about the lumbar spine (1-5). RA is a widespread inflammatory synovitis. The facet joints are affected in much the same way as other diarthrodial joints in RA. Lawrence (3) had identified characteristic radiological patterns in RA including disc narrowing without osteophytosis, spondylolisthesis, facet joint erosions and osteoporosis. Ball's (2) findings also supported these findings. There was only one study about the correlation between back pain and radiology of the lumbar spine in RA in the literature (1). However Helliwell's results did not indicate a clear characteristic syn-

drome which would characterize inflammatory synovitis of the facet joints in RA.

Facet joints have always presented problems in radiological examination as their oblique angulation makes assesment by standart anteroposterior and lateral radiographs difficult. So as being the gold standart in examining the facet and the sacroiliac joints, CT was used in our study.

The aim of this study is to evaluate the clinical patterns of the back pain and also radiological changes of the lumbar spine in RA. The correlation between back pain and these radiological parameters were also evaluated.

* Research Fellow, Ankara University Medical School, Department of Physical Medicine and Rehabilitation

** Teaching Fellow, Ankara University Medical School, Department of Physical Medicine and Rehabilitation.

*** Research Fellow, Ankara University Medical School, Department of Radiology

**** Professor, Ankara University Medical School, Department of Physical Medicine and Rehabilitation

***** Professor, Ankara University Medical School, Department of Radiology

METHODS

Thirty patients with RA who attended to İbni Sina Hospital in Ankara were asked whether they had low back pain. The patients with RA with back pain were asked about the duration of the back pain, radiation of the pain on the body and the activities that relieve or aggravate the pain and also the duration of steroid therapy and dosage. Age and sex matched 30 chronic back pain patients (pain longer than 3 months) were selected as controls. Inflammatory, infectious, metabolic and neoplastic etiologies were excluded by history, examination, biochemical and haematological tests and radiology. In both groups lumbar spine movements were measured; flexion was measured by Modified Schobers method, lateral flexion was assessed by using the marks used in Modified Schobers method, lateral flexion was assessed by measuring the distance traveled by the outstretched arm down the ipsilateral leg. Functional disability was assessed by using Stanford Health Assessment Questionnaire. Number of the swollen and the tender joints, duration of the early morning stiffness, erythrocyte sedimentation rate (ESR) determined the clinical disease activity. Both RA and control groups were invited to undergo a radiological examination consisting of x-ray examination and computerized tomography (CT). X-ray examination included a pelvic view and routine anteroposterior and lateral views of lumbar spine and CT included the views of L3-L5 lumbar intervertebral distances, facet joints and sacroiliac joints. All graphics were read by two observers without knowledge of the diagnostic group. On X-rays disc

narrowing (number of discs, maximum score:5), corpus degeneration (0:Normal, 1:minimal, 2:moderate, 3:severe), vertebral crush fracture (scored for each vertebra, maximum score: 5)(1), disc calcification, spondylolisthesis, scoliosis were noted. Because of the difficulty in examining the facets and sacroiliac joints on routine X-rays, we preferred using CT. Facet erosions, facet osteophytes, facet cysts, facet air, facet sclerosis, sacroiliac erosions, sacroiliac osteophytes, sacroiliac cysts, sacroiliac air and sacroiliac stage were examined on CT. Sacroiliac joints were staged according to the New York Criteria (Grade 2 and above was recorded as positive) (1).

STATISTICAL ANALYSIS

All statistical analysis were performed on the SPSS/PC statistical package (SPSS/PC+3.1, SPSS Inc., Chicago, Illinois, USA). All data were presented as the means plus or minus the standard deviation (mean \pm SD). Correlation analyses were done by using Spearman test and differences among groups were evaluated by Mann Whitney - U test. Differences were considered significant when p was less than 0.05.

RESULTS

In the RA group the mean age was 53.5 ± 11.73 . All of them were women. The demographic and the clinical details of RA group is given in table 1. In the control group the mean age was 49.6 ± 6.03 . All of them were women like the RA group.

Nineteen patients (%63.3) with RA had back pain and the mean duration was 2 years. None of them had back pain before the start of the disease. The nature of the pain was sharp in %63.3 of these patients, dull in %36.8 of the patients. Pain was aggravated with extension (%46.7), exercise (%63.3) and flexion (%33). The pain radiated to hip (%40), leg (%20) and upper back (%33.3). There was no difference in flexion and lateral flexion measurements on clinical examination of lumbar spine however extension was more limited in RA patients ($p < 0.05$). The details of the lumbar spine measurements are given in table 2.

Table 1: Demographic and the clinical details

	Mean	Standart deviation	Range
Age	53.5	11.73	36-58
Duration of disease	11.02	10.49	1-46
Morning stiffness	2.30	2.40	0.4-2.5
Number of swollen joints	3.03	3.05	0-12
Number of tender joints	8.17	473	1-20
ESR	41.93	21.61	15-100

Table 2: The details of the lumbar spine measurements. *NS: Nonsignificant

	Rheumatoid Arthritis		Controls		p
	Mean (cm)	Standart deviation	Mean	Standart deviation	
Modified Schober	21.49	0.42	21.15	0.29	NS*
Right lateral flexion	13.73	0.76	14.10	0.66	NS
Left lateral flexion	14.08	0.75	15.02	0.66	NS
Extension	13.52	0.48	12.41	0.23	$p < 0.05$

Table 3: Comparison of radiographic findings in RA and chronic back pain groups

	Rheumatoid Arthritis		Controls		
	n	%	n	%	
Disc narrowing					
0					
1	11	36.7	12	40	
2	15	50	17	56.7	NS
3	3	10	1	3.3	
4	1	3.3	0		
5	0		0		
	0		0		
Corpus degeneration					
0	2	6.7	1	3.3	
1	9	30	11	36.7	
2	7	23.3	10	33.3	NS
3	12	40	8	26.7	
Compression fracture	6	20	1	3.3	p<0.05
Spondylolisthesis	4	13.3	3	10	NS
Scoliosis	2	6.7	6	20	NS
Face cyst	17	56.7	11	36.7	NS
Facet osteophyte	22	73.3	22	73.3	NS
Facet sclerosis	26	86.7	24	80	NS
Facet air	7	23.3	10	33.3	NS
Facet eorosin	9	30	2	6.7	p<0.05
Sacroiliac erosion	1	3.3	0	0	NS
Sacroiliac air	26	86.7	21	70	NS
Sacroiliac osteophyte	18	60	8	26.7	p<0.05
Sacroiliac cyst	10	33.3	1	3.3	p<0.05

Radiologically facet joint erosions ($p < 0.05$), sacroiliac cysts ($p < 0.001$) and sacroiliac osteophytes ($p < 0.001$), compression fractures ($p < 0.05$) were significantly higher in RA group. Details are given in Table 3.

When these radiological findings were correlated to back pain, we found poor correlation between the sacroiliac cysts, sacroiliac osteophytosis, compression fracture and back pain ($p < 0.05$ $r = 0.36$). There was a moderate correlation between facet joint erosions and back pain ($p < 0.05$ $r = 0.53$).

When the patients were divided into two groups as facet positive and facet negative, back pain was more frequent in the facet positive group ($p < 0.07$). Clinical activation parameters did not differ between facet positive and facet negative groups (Table 4).

DISCUSSION

We have found facet erosions more frequently in RA patients. Diarthrodial joints are the target tissues in RA. Because of the diarthrodial composition, facet joints must be involved in RA. This hypothesis was first supported by Lawrence in 1964 (3). They noted that radiographic changes in the apophyseal joints of the lumbar spine occurred in 3-5% of an RA population. They found an increased incidence of lumbar facet joint erosions in RA when compared to 50 controls. Sims-Jayson and Baddeley were able to demonstrate rheumatoid erosions in apophyseal joints on stereoscopic radiographs (4).

Sacroiliac cysts ($p < 0.005$) and osteophytes ($p < 0.005$) were significantly higher in our patients.

Table 4: Comparison of clinical activation parameters in facet positive and facet negative RA patients

	Facet Positive		Facet Negative		p
	Mean	SD	Mean	SD	
ESR	37.0	16.6	44.04	23.4	NS*
Duration of morning stiffness	2.55	2.39	2.19	2.45	NS
Number of swollen joints	3.33	3.0	2.90	3.12	NS
Number of tender joints	9.55	4.39	7.57	4.85	NS

Our findings support the findings of Frigo and Dixon (6,7) who suggested that the abnormal sacroiliac joints in Rheumatoid Arthritis patients may be caused by degenerative changes. Sacroileitis was found %26.7 in our RA patients. Wilkinson has found erosive sacroileitis in up to % 30 in his cases (8).

Compression fractures were more frequent in our patients with RA than the controls. There were no correlation between steroid usage and these compression fractures. The usage of steroids predicted vertebral crush fracture in Helliwell's study, but it was not significant statistically (1).

Loss of bone mass in RA is a common clinical problem. There are two types of osteoporosis seen in RA; periarticular and generalized osteoporosis. Several mediators (Pg E2, IL-1, TNF α) that are known to be associated with rheumatoid synovitis may be involved in the pathogenesis of periarticular osteoporosis. Periarticular bone loss occurs early in the disease course. Generalized osteoporosis occurs more gradually. Many studies have examined bone mass in patients with RA. Many studies have examined bone mass in patients with RA (9-13). Magaro et al. Found a significantly lower lumbar spine bone mineral density in patients in functional class III/IV than in functional class I/II. It has been suggested that longer disease duration and disease activity is correlated with loss of bone mass in patients with RA (11). However most studies have not found disease duration to be a significant predictor of bone mineral density (11, 14, 15). It is well known that glucocorticoid therapy is associated with development of osteoporosis and an increased risk for fracture. Controversy exists; however as to whether low dose corticosteroid therapy contributes to the development of osteoporosis in patients with RA or alternatively contributes to the maintenance of bone mass by decreasing disease activity and improving functional status.

Patients with RA appear to be at increased risk of fracture compared with subjects in general population. Factors intrinsic to the inflammatory process of rheumatoid disease and functional impairment accompanying the disease with its resulting limitation on physical activity both appear to contribute to an excessive decline in bone mineral density in patients with RA. The role of the treatment with low doses of glucocorticoids in the development of osteoporosis on the resulting increased fracture risk remains unclear and obviously further studies are necessary.

Anteroposterior instability were equal between the groups in our study. The pathogenesis of the insta-

bility was explained in three pathways in Heywood's study in 1986 (5). First one is the beginning of synovitis in the apophyseal joint which erodes the cartilage slowly and subchondral bone in exactly the same way as it does in peripheral joints. Second includes two theories a) rheumatoid discitis which probably starts at the discovertebral junction as an entesopathy (inflammatory theory of Shichikawa) b) Martel (16) suggested that the disc involvement in the servical spine is derived from microtrauma from the instability leads to major disruption. (Mechanistic Theory of Martel). Third pathway is direct spread into the disc space from involvement of the costovertebral joints as demonstrated at autopsy by Bywaters.

Back pain is a common symptom and its diagnosis is often difficult. The source of back pain in RA is not clear. In RA patients with back pain the history, physical examination and conventional radiology are often unhelpful in differentiating inflammatory joint disease from other causes of back pain. CT is the gold standart in examining facet joints and it showed the erosions of the facet joints which resembles rheumatoid erosions seen in other joints of our patients. The presence of osteophytes does not negate the diagnosis of rheumatoid involvement of the facets (5). Severe back pain with sciatica in RA may result from several mechanisms including inflammation of facet joints, steroid induced compression fractures, an intraspinal rheumatoid cyst arising from the synovial membrane of a facet joint (17), lumbar spinal stenosis due to the infiltration of capsules, ligamenta flava, facets, and lamina by rheumatoid granulation tissue (18). Gait abnormalities associated with lower limb joint involvement.

The type of the involvement of sacroiliac joints in RA is controversial. Our data suggests sacroiliac joints in RA are mostly degenerative nature. Dixon (7) found that abnormalities of sacroiliac joints in RA were purely age related changes.

Helliwell's results did not indicate a clear characteristic syndrome which would characterize inflammatory synovitis of the facet joints (1). We found a correlation between low back pain and facet erosions. Extension was more limited in RA patients than the controls and extension aggravated the back pain. Pain radiated to the hips and buttocks more frequently. These characteristics of the pain in our patients resembled facet joint syndrom. When RA patients were divided into two groups as facet positive and facet negative groups, back pain was more frequent in RA group.

Our study supports the previous findings characterising lumbar involvement such as facet erosions and compression fractures except disc narrowing without osteophytosis. However we could not find disc narrowing without osteophytosis and spondilolisthesis more frequently in RA. We found a correlation between facet involvement and back pain and also

the characteristics of the pain resembled facet syndrome.

In conclusion, although our data does not support characterising radiological of the lumbar spine in RA, when a patient with RA complaints of back pain; we have to keep the rheumatoid involvement of the facet joints in mind.

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IN VITRO SUSCEPTIBILITY OF MYCOBACTERIUM TUBERCULOSIS TO CLARITHROMYCIN*

Nezihe Saygun** • Buket Cicioğlu*** • Meral Saygun**** • Selçuk Esengür*****
Sedef Bengisun**

SUMMARY

In this study forty three clinical isolates of M.tuberculosis were investigated to determine their in vitro susceptibility to clarithromycin. All the cultures of M. tuberculosis were maintained on Lowenstein-Jensen medium for three weeks. The following concentrations (mg/l) of clarithromycin were used 0.25, 0.5, 1.0, 2.0, 4.0. Thirty-nine of these isolates were susceptible to this drug at 0.25 mg/l concentration. Three of 43 strains were resistant to rifampicin, that two of them were resistant also to clarithromycin at all concentrations. One strain to streptomycin and one strain to isoniazid were resistant.

Key Words: *Mycobacterium tuberculosis, Clarithromycin*

Clarithromycin, a new macrolide, has been to have improved pharmacokinetics and in vitro activity compared with those of erythromycin (6). Rapidly growing mycobacteria are responsible for numerous types of infections, including osteomyelitis, cellulitis, multiple nodular soft tissue abscess. Clarithromycin was found 10 to 50 times more active than erythromycin against mycobacterium chelonae (2). The efficacy of clarithromycin in the elimination of mycobacterium avium bacteremia in AIDS patients has been shown (4). Bacteriostatic and bactericidal effects of clarithromycin against M. avium were determined (2,7,10). The activity of clarithromycin against intracellular M. avium were done in the concentration of 4.0 mg/ml in blood (10). The in vitro activities of clarithromycin were determined by agar dilution method (6). The aims of the present study were to determine the in vitro susceptibility of fortythree mycobacterium tuberculosis strains against clarithromycin.

MATERIALS AND METHODS

This investigation was made between October-November 1993. The strains of M.tb were grown from

sputums of tuberculous patients which were hospitalized with clinical TB diagnosis in Chest and TB Clinic of Ankara Medical Faculty, Ankara University.

Antimicrobial agent: Clarithromycin was obtained from Abbott Laboratories imports exports and trade anonymous company. To make a stock solution, we dissolved 50 mg of the clarithromycin in 5 ml of methanol and then completed to volume of 25 ml with phosphate buffer (pH 6.5). This stock solution (2 mg/ml) was further diluted with phosphate buffer to make appropriate working solutions, which were kept in refrigerator for not more than two weeks (10).

Media: Löwenstein-Jensen (L-J) media with 0.25, 0.50, 1.0, 2.0 and 4.0 mg/l concentrations of clarithromycin were prepared.

M. tuberculosis: M. tb strains of forty three patients were grown on L-J media. Colonies were collected and the turbidity of the resulting suspensions were adjusted with distilled water to match that of a standard suspension of M. bovis BCG (1.0 mg/ml). For inoculation of L-J media, the suspensions were further diluted 10^{-3} and 10^{-5} and were inoculated with and without drugs L-J media (3).

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** Department of Chest Diseases and Tuberculosis, Faculty of Medicine, Ankara University

*** Department of Microbiology, Faculty of Medicine, Ankara University

**** Health, Culture and Sport Department, Faculty of Medicine, Ankara University

***** Dispensary of Tuberculosis Eskişehir

The cultures were evaluated after 3 to 4 weeks incubation at 37°C.

The susceptibilities of all strains against antituberculosis drugs (streptomycin 4.0 mg/l, isoniazid 0.2 mg/l, ethambutol 2.0 mg/l, thiacetazone 2.0 mg/l and rifampicin 40.0 mg/l) were studied at the same time.

RESULTS

Thirty-nine of *M. tuberculosis* strains were inhibited by the lowest concentration of clarithromycin (0.25 mg/l, 90.0 %). Forty strains were inhibited from on 0.5 mg/l (93 %) concentration (Table 1).

Three of isolates against to rifampicin and two of these isolates were also showed resistance to clarithromycin at all concentrations. One strain to isoniazid and one strain to streptomycin were resistant (Table 2).

DISCUSSION

New erythromycin derivative which is stable under acid conditions was synthesized by modifying functional group namely, the hydroxyl group at C-6 (Clarithromycin). This macrolid has been recently tested against both *M.tb* and *M.avium* complex organisms (12).

The pharmacological profile of clarithromycin is a favourable one. It is rapidly absorbed from the gastrointestinal tract. The steady state peak serum concentrations of clarithromycin are 1-1.5mg/l after a

250mg dose and 2-3mg/l after a 500mg dose. After doses of 250mg, levels in lung removed at operation have been 8.8mg/l compared with 1.7mg/l in serum. Clarithromycin has had an intracellular to extracellular ratio of 9 in human neutrophils, and it is concentrated in alveolar macrophages. These concentrations would far exceed the MICs of *M. tuberculosis* (11). We have found that clarithromycin is active against *M.tb* at 0.25 and 0.50 mg/l concentrations at the rate of 90% and 93% respectively.

Clarithromycin is active against common pathogens. Most respiratory pathogens are inhibited by £0.25mg/l, except *H. influenzae*. Clarithromycin has also been used to treat *Mycobacterium avium* infection, but it is too early to decide on its efficacy.

The adverse reactions to clarithromycin in clinical studies have been minor and have not included serious neurological, cardiac, or renal reactions. Adverse effects were shown to be neither age nor dose dependent (11). Very recent publication showed that clarithromycin produces also postantibiotic effect against the bacterial species (14).

The in vitro activities of the newer macrolides azithromycin, clarithromycin, roxithromycin and erythromycin against isolates of *M. fortuitum*, *M. chelonae* and *M. chelonae*-like organisms were studied (2, 15). Clarithromycin and azithromycin are new macrolides which are well absorbed after oral administrations than erythromycin. These compounds have high activity against the nontuberculous mycobacteria (2, 8, 9, 15).

The in vitro activity of the erythromycin against 34 clinical isolates of *M. tb* were studied by standard proportion method on L-J medium. The minimal concentration (MIC) of erythromycin for 50% and 90% of the 34 clinical isolates were 16 and 111.5mg/l respectively. Because of high MIC values obtained, erythromycin wouldn't be effective on *M. tb* (1).

In our investigation; in vitro susceptibilities of 102 clinical isolates of mycobacteria (100 *M. tb*, one *M.*

Table 1: In vitro susceptibility of the 43 *M.tuberculosis* isolates against to clarithromycin.

Concentration of Clarithromycin (mg/l)	Susceptibility of strains to Clarithromycin Number (n)	Rate (%)
0.25	39	90.0
0.50	40	93.0
1.0	40	93.0
2.0	40	93.0
4.0	40	93.0

Table 2: Numbers of the resistant strains against to clarithromycin and antituberculous drugs

Antituberculous drugs and number of resistant strains Drugs	Number (n)	Resistance to concentrations of clarithromycin (mg/l)				
		0.25	0.50	1.0	2.0	4.0
Streptomycin	1	-	-	-	-	-
Isoniazid	1	-	-	-	-	-
Rifampicin	3	2	2	2	2	2
Ethambutol	-	-	-	-	-	-
Thiacetazone	-	-	-	-	-	-

bovis, one M. non tb Runyon Group 3) to roxithromycin were studied by standard proportion method on L-J medium. The MICs of roxithromycin for 50% and 90% of the strains were 1 and 5 mg/l respectively (13).

Forsgren, has been studied susceptibility of the 20 clinical isolates of M. marinum to various antibiotics by radiometric respinometric technique (BACTEC). M.marinum strain has been found susceptible against

clarithromycin at 2.0 mg/l concentration, at the rate 95% (5).

Clarithromycin has definite activity against M.avium in patients with AIDS (4).

Peak serum and lung tissue levels reached with 250 and 500 mg P.O. are higher than the MICs of clarithromycin. This results exhibited growth inhibition in mycobacteria at the rate of 93%.

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FERTILITY POTENTIAL IN THE PATIENTS WHO HAD UNDERGONE ORCHIDOPEXY FOR RETANSIO TESTES

Bülent Öztürk* • Ercüment Ulusoy* • Ekrem Güner* • Mesut Çetinkaya* •
Ali Memiş* • Öztuğ Adsan**

SUMMARY

We investigated fertility potentials of former cryptorchidic patients in adulthood, who had undergone orchidopexy at the age of 5 or later in this study. Patients were separately grouped as unilaterally/bilaterally operated cases and cases operated before/after 15 years of age. Unilaterally operated 38 cases were divided into 3 groups according to their respective ages and their seminal parameters and testicular volumes were statistically compared.

Normal sperm densities were found in 58 % of unilaterally operated cases and 38 % of bilaterally operated cases, disregarding to operation age and primary localization. Moreover, a statistically significant difference was found between two groups in sperm counts ($p=0.003$). Normal sperm concentrations were found in 61 % of the patients operated before the age of 15, while only 20 % had normal sperm concentrations in the group operated after the age of 15, disregarding to the side laterality and primary localization of the testis. There was a statistically significant difference in sperm concentrations between two groups ($p=0.003$). In 3 groups of 38 unilaterally operated cases divided according to their ages, both sperm concentrations and testicular volumes decreased with increasing age, and this finding had statistical significance ($p<0.05$).

Better testicular volumes and sperm concentration determined in cases with lower operation ages form the basis for the thought that, germinal loss and testicular atrophy parallel to the duration of cryptorchidic period could be prevented or at least stopped with orchidopexy at an early age.

Key Words: Cryptorchidism, Fertility, Orchidopeksi, Semen analysis.

Cryptorchidism is among the most common abnormalities of childhood. In many studies undescended testis incidences are given as 3 to 5 % in newborns (1). Similarity of incidences in the 3rd and 12th months of life shows that most of the spontaneous descents happens in the first 3 months (1). In 9 months old infants undescended testis incidence was found similar to postpubertal age, namely 0.8 % (2). Prematureness, low birth weight and multifetation are the factors increasing the incidence. Cryptorchism is seen in 25 % of premature newborns. In 15 % of these cases the condition is bilateral (3).

Orchidopexy is currently the most widely used elective operation for cryptorchidism. The microscopic abnormalities beginning in the first months of life in undescended testis cases become significant in the age of 2 (4) and increase in the following years (5). Accordingly recommended age of treatment for cryptorchidism has lowered significantly. However, there

is no evidence supporting the probability that earlier interventions might affect fertility potential, and fertility after real cryptorchidism has not been explained adequately.

Postpubertal fertility potentials of cryptorchidic cases are investigated by seminal analysis, hormonal studies, testicular biopsy and paternity investigation. We studied fertility potentials of individuals who had undergone orchidopexy at and over 5 years of age by seminal analyses done in puberty or postpubertal period.

MATERIALS AND METHODS

In this retrospective study two hundred and fifty patients who had undergone single stage orchidopexy in the Urology Clinic of Ankara Numune Hospital between the years 1984 and 1993 were recalled by post. Ninety-five of them came to our clinic for cont-

* Department of Urology, Ankara Numune Hospital, TURKEY

rol, and 75 individuals who had undergone surgery at or over the age of 5 and who were currently over the age of 15 accepted examination of their fertility potentials.

After genital and systemic examinations, individuals with absent testes or who had undergone orchiectomy were excluded from the study. Similarly individuals who had other pathologies that could affect fertility (varicocele), who had encountered inguinal surgery or orchitis were excluded, as well. Seminal specimens were taken from the 51 cases included in the study, and better of the results obtained from two specimens taken with one month interval was taken into consideration. Bilateral testicular volumes of these 51 subjects were measured ultrasonographically.

Seminal specimens were collected after 3 days of sexual deprivation. All specimens were obtained by masturbation and put into glass jars that were dry, sterile and pre-heated to room temperature. Volume and sperm quality (density, morphology and motility) of the specimens were evaluated according to World Health Organization (WHO) criteria, within 15 minutes of their collection (6). All seminal analyses were done in the same laboratory and by the same person.

Seminal parameters were accepted as normal, when the volume was at least 1.5 ml, with at least 20 million per milliliter in number, of which at least 50 % had normal morphology and at least 50 % moved progressively, in accordance with WHO criteria (6).

While spermatic density of more than 20 million in milliliter of semen was accepted as normal, less than 20 million was recorded as oligospermia, and absence of sperm in semen was recorded as azospermia.

Morphologic examination of the sperms was performed microscopically in hematoxylin-dyed, dried seminal slides. In morphologic classification the cells are roughly classified as normal, abnormal and immature cells.

Ellipsoid shaped testis' volume was calculated by 'length x width x antero-posterior depth x 0.52' formula, in cubic centimeters (7). All measurements (in

longitudinal, transverse and anteroposterior axes) were performed by ultrasound scanner.

The 51 subjects included in this study were grouped as unilaterally versus bilaterally operated patients and patients who underwent surgery below 15 years of age versus over 15 years of age. Unilaterally operated 38 subjects were further divided into 3 groups according to their ages (5-10 years, 11-15 years, over 15 years), and their seminal parameters and testicular volumes were statistically compared. In statistical analysis one way variance analysis and independent t test were used.

RESULTS

Study consisted of 51 patients who had undergone orchidopexy operation for cryptorchidism between the years 1984 and 1993. The mean orchidopexy age of all patients was 12.3 (range 5-29), and mean age in examination was 21.1 (range 15-33). The mean age of 13 bilaterally operated patients was 12.1 (range 5-29), and 38 unilaterally operated patients was 13.7 (range 6-27) at the time of orchidopexy. Seminal analyses of unilaterally and bilaterally operated patients are given in Table 1.

Patients were divided into two groups according to the mean puberty age, which was accepted as 15 years. Ignoring laterality, 41 of 51 patients were operated before 15 years of age, and 10 were operated after 15 years of age. Seminal analyses of the patients who were operated before and after 15 years of age are given in Table 2.

Bilateral testicular volumes of 38 unilaterally operated patients were measured. While mean testicular volume was found $14,95 \pm 4,88$ cc. in non-operated testes, it was found $8,43 \pm 4,41$ cc. in the operated testes ($p=0,001$).

Unilaterally operated 38 patients were divided into 3 groups to further investigate the age of orchidopexy's influence over seminal parameters and testicular volume. Table 3 shows seminal analyses and testicular volumes of unilaterally operated 38 patients,

Table 1: Seminal analysis results of 51 patients (38 unilaterally-operated, 13 bilaterally operated)

	Unilateral	Bilateral	p value
Volume (ml)	2,54±1,82	2,46±2,62	0,925
Density (10 ⁶ /ml)	45,59±47,01	15,68±20,01	0,003
Mobility (normal%)	56,57±25,95	53,75±27,22	0,795
Morfology (normal%)	70,43±15,41	62,5±14,88	0,205

Table 2: Seminal analysis results of patients who were operated before and after the age of 15

	< 15 age (n=41)	≥ 15 age (n=10)	p value
Volume (ml)	2,22±1,83	3,75±2,43	0,087
Density (10 ⁶ /ml)	44,12±45,57	12,75±21,76	0,003
Mobility (normal%)	56,81±25,3	52,14±30,53	0,715
Morfology (normal%)	70,56±13,56	59,29±20,5	0,205

Table 3: Seminal analysis and testicular volumes of 38 unilaterally operated patients according to age groups.

	5-10 age	11-15 age	> 15 age	p value
Volume (ml)	1,86±0,74	2,81±2,42	2,81±0,88	0,359
Density (10 ⁶ /ml)	68,5±66,42	44,82±34,14	15,94±23,47	<0,05
Mobility (normal%)	64±23,19	54,17±26,19	52,14±30,53	0,568
Morfology (normal%)	69,5±17,71	75,28±9,15	59,29±20,5	0,600
Operated testes volume (cc)	10,37±5,94	8,58±3,6	5,39±1,72	<0,05
Nonoperated testes volume (cc)	14,55±4,98	14,87±4,33	15,7±6,44	0,879

grouped as 5 to 10 years of age, 11 to 15 years of age and over 15 years of age.

According to seminal analyses' results, 3 (7.9 %) of 38 unilaterally operated cases and 5 (38.5 %) of 13 bilaterally-operated cases had azoospermia. These 8 patients comprised 5 (12.2 %) of 41 patients operated before the age of 15 and 3 (30 %) of 10 patients operated after the age of 15.

DISCUSSION

Adulthood fertility problem of cryptorchidic patients, which is among the most frequent developmental problems of childhood, is known for centuries. One of the most important aspects of cryptorchidism is that it affects fertility even though it is corrected (8). This influence is seen in bilaterally undescended testis cases more often. Similarly it is more significant in cases with testes located higher than scrotum basis and with longer period of cryptorchidism.

As to its established effect on fertility, it is important to explain fertility in cryptorchidism. Numerous studies have been conducted up to now to explain the loss of fertility and understand treatment efficiency in cryptorchidism.

Seminal analysis is the most easily accessible and most often used technique in assessment of fertility potential. Very different sperm concentrations have been reported in the studies examining semen specimens after cryptorchidism. In the studies disregarding to treatment age, normal sperm concentrations have been reported in 50 % of the patients with unilateral cryptorchidism and 25 % of the patients with bilateral

cryptorchidism (8, 9). In our study normal sperm concentrations have been found in 58 % of the unilaterally operated patients and 38 % of the bilaterally-operated patients without regarding to operation age and primary localization, which is very similar to other studies with comparable features. Although no significant relationship was found between two groups in seminal volume, sperm motility and morphology, a statistically significant relationship was found in sperm density ($p=0.003$, Table 1).

Analysis of 21 studies conducted on this subject reveals that while subnormal sperm concentrations were reported in 28-82 % of the unilaterally cryptorchidic patients, in bilaterally cryptorchidic group this percentage increases to 44-100 %, with azoospermia in more than half of them (10). Chilvers has presented a review of 27 articles investigating fertility after orchidopexy in 1986. According to him oligospermia and azoospermia have been found respectively in 31 % and 41 % of treated unilateral cases, and 31 % and 42 % in bilateral cases (8). In this study oligospermia and azoospermia was found 34 % and 8 % respectively in unilaterally operated cases, and 23 % and 38 % respectively in bilaterally-operated cases, disregarding to operation age and primary localization. These rates are similar to the results of other studies given above.

There are a number of studies investigating fertility potential in cryptorchidic patients in view of treatment age. It is reported that patients treated before 6 years of age benefit early surgical intervention (11). Similarly Ludwig has reported normal seminal analy-

ses in 7 of 8 patients who had undergone orchidopexy before 2 years of age, and stressed the importance of an early surgical treatment (12). Chilvers has grouped unilateral and bilateral cases in his review as patients who had undergone surgery before and after the age of 9. In this review with no subjects in the age group currently accepted as ideal for treatment, no significant difference was found between two groups in sperm concentrations (8). Grasso has found that 83.5 % of the patients who underwent orchidopexy in postpubertal period had oligospermia or azospermia (13). We examined the subjects in our study with two separate classifications, as unilaterally or bilaterally operated patients and as patients operated before or after the age of 15. In our study where there are no patients who were operated before the age of 5, in the group of subjects operated before 15 years of age 61 % had normal sperm densities, 27 % had subnormal densities and 12 % showed azospermia. These rates were 20 %, 50 % and 30 % respectively in patients who had undergone surgery after 15 years of age. While there were no statistically significant differences between two groups in seminal volume, sperm motility and morphology, sperm counts showed significant differences ($p=0.003$, Table II). Results show that patients who were operated after puberty and in later ages have worse sperm concentrations, which is confirmed by the findings of Grasso's study consisting of similar subjects.

Mandat has reported no statistically significant differences in sperm concentrations between age groups, when the subjects are divided as ages of 2-6, 6-10 and over 10 (11). Okuyama has not found any significant difference in sperm concentrations investigating unilateral and bilateral cases treated in 2-5 and 9-12 age groups (14). Puri and O'Donnell have emphasized that operation age is not important in their study on sperm densities of patients who had undergone orchidopexy at 9-14 years of age (9). Kumar has performed the same study with paternity assessment and found better results in the patients who were operated before 12 years, but the findings has not been remarkable (15).

We examined unilaterally operated 38 patients in three age groups consisting of 5-10, 11-15 and over 15 years to further examine orchidopexy age's influence on fertility. While there were no statistically significant differences among three groups in seminal volume, sperm motility and morphology, sperm counts showed

significant differences ($p<0.05$, Table 3). Namely worse sperm concentrations were found when orchidopexy age was higher and therefore cryptorchidic duration was longer. In very few studies examining cryptorchidic patients according to orchidopexy ages it was shown that early orchidopexy conserved fertility (11, 12). On the other hand, in most of the studies it was reported that orchidopexy age had no positive influence on fertility in the future. In these studies without any subjects operated before the age of 2, significant differences were not observed in sperm concentrations between the age groups (8, 9, 11, 14, 16). To summarize, there is very few evidences supporting the necessity of an early operation and its success in improving fertility (16). Nevertheless the germinal loss and testicular atrophy increasing parallel to duration of cryptorchidic period should be prevented at an early age by orchidopexy. We obtained evidence supporting early orchidopexy in our study. This finding should be further elaborated and supported by other studies performed on early operated patients.

Most undescended testes are smaller than its contralateral descended pair and this volume loss becomes significant in 6 months (3). Puri and Sparron have reported that surgically treated cryptorchidic testes are significantly smaller than normal testes. We compared both testicular volumes of 38 unilaterally operated cases disregarding to operation age and primary localization. Results revealed a highly significant difference between operated and non-operated testicular volumes ($p=0.001$). Volume loss is highly pronounced in operated testes when mean volumes are compared. Operated testis volume was found to be almost half of non-operated testis; in other words almost half the volume was lost.

Pronounced volume loss in operated cryptorchidic testes has been reported in many studies (3, 13, 16, 17, 18, 19) but association between this loss and orchidopexy age has not been reported in any study. We examined bilateral testicular volumes in three groups of unilaterally operated patients to investigate the probable association between volume loss and orchidopexy age. Thus we investigated possible influence of orchidopexy age and therefore duration of cryptorchidic period, on testicular size in adulthood and spermatogenesis. In the comparison without primary localization classification, there was no difference between 3 operated age groups of 5-10, 11-15 and over 15 years in non-operated testicular volumes, but operated testicular volumes showed significant dif-

ferences ($p < 0.05$, Table III). Namely, volume decreased with later orchidopexy age as a result of increased testicular atrophy, and sperm count per milliliter decreased significantly as well (Table III). This comparison supported the view that testes with larger volume are more functional. Once more it was shown that delaying orchidopexy age and prolonging cryptorchidic period of the testes negatively affects testicular volume and consequently fertility.

Fertility potential of cryptorchidic patients, determined by prognostic factors such as testes localization, palpability, uni- or bilaterality, duration of cryptorchidism and orchidopexy age, might be influenced by some intrinsic defects as well. Numerous signs indicate that germinal epithelium damage and testicular atrophy in cryptorchidism are due to high temperature, and shows acquired, irreversible and progressive characteristics. However, predominantly it is not clear whether the pathogenesis is due to an acquired irreversible defect or caused by an intrinsic congenital defect. Similarly volume and spermatogenesis loss in

cryptorchidic testes might not develop due to environmental factors such as high temperature and pressure, but because of an intrinsic defect. It is not possible to make this differentiation definitely at the present time.

Better results of testicular volume and sperm concentrations obtained when orchidopexy age is lowered, gives birth to the thought that the germinal loss and testicular atrophy increasing parallel to cryptorchidic duration could be prevented, or at least stopped by orchidopexy at an early age. Therefore the operation should be performed as early as possible to permit testicular development and consequently protect the fertility. It is necessary to further investigate and support the influence of orchidopexy age to spermatogenesis and testicular development, by conducting more research especially on patients operated at the age of ideal treatment. Studies on this subject should be focused on bilateral cases who experience important fertility problems and show the highest rates of oligospermia-azoospermia.

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