Tohoku J. Exp. Med., 2007, **212**, 159-167

Quantitative Correlation of *Helicobacter pylori* Stool Antigen (HpSA) Test with the Severity of *H. pylori*-Related Gastritis

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Adiloglu, A.K., Isler, M., Goren, I., Candir, O., Senol, A., Onal, S. and Karahan, N. *Quantitative Correlation of Helicobacter pylori Stool Antigen (HpSA) Test with the Severity of H. pylori-Related Gastritis*. Tohoku J. Exp. Med., 2007, **212** (2), 159-167 —— The *Helicobacter pylori* (*H. pylori)* load in both stomach and stool and the resulting severity of gastritis are important criteria in validating the status of *H. pylori* infection. We aimed to assess the reliability of the *H. pylori* stool antigen (HpSA) test for the primary diagnosis of *H. pylori* infection by calculating the best cut-off value to obtain the highest sensitivity and specificity in dyspeptic patients. We also investigated the correlation of HpSA test with the severity of gastritis and *H. pylori* load. The *H. pylori* statuses of 95 patients were evaluated by the positivity of both rapid urease test and microscopic detection of *H. pylori* in biopsy specimens, 88 subjects of whom were *H. pylori* positive. The sensitivity and specificity of the HpSA test were 51.1% (45/88) and 100% (7/7), respectively, according to the manufacturer's recommended cut-off value of 0.16. However, with the best cut-off value of 0.048, calculated by receiver operator characteristics analysis, the sensitivity of the test increased to 92.0% (81/88) with the same specificity. High values of the HpSA test were correlated with high scores of corpus *H. pylori* load and the severity of antrum and corpus inflammation ($p < 0.05$). With the best cut-off value of the HpSA test, the primary diagnosis of *H. pylori* infection can be made with higher sensitivity and specificity. The HpSA test is a helpful tool that evaluates the severity of *H. pylori* infection and the degree of gastric inflammatory activity and gastric *H. pylori* load. ———— *Helicobacter pylori*; stool antigen test; cut-off value; Sydney Grading System; gastritis

Helicobacter pylori (H. pylori) is one of the most common pathogenic microorganisms in human. Fifty percent of the population in developed countries and 80-90% of the population in developing countries are estimated to carry this pathogen (Rothenbacher et al. 1999). Besides gastritis, *H. pylori* infection has been involved in the pathogenesis of peptic ulcer, gastric adenocarcinoma and lymphoma (Hino et al. 2004). In recent years, noninvasive diagnostic tests used for

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H. pylori infection have gained significance. The Canadian and European consensus conference reports emphasize the importance of these noninvasive diagnostic tests both for diagnosis and post treatment control for children (Sherman et al. 1999; Drumm et al. 2000). Well established noninvasive tests are serology, urea breath test (UBT) and *H. pylori* stool antigen (HpSA) test. Although serological tests are useful in the diagnosis of current *H. pylori* infection, it is not useful for monitoring eradication therapy (De Oliverio et al. 1999; Koletzko and Feydt-Schmidt 2001).

In a meta-analysis, both the ^{13}C-UBT and the stool antigen test showed high sensitivity and specificity in adults (Vaira and Vakil 2001). The European *Helicobacter* Study Group recommended the stool antigen test and the ^{13}C-UBT for both diagnosis and assessment of eradication of *H. pylori* infection (Malfertheiner et al. 2002). United States Food and Drug Administration (FDA) approved the stool antigen test for both diagnosing *H. pylori* infection and monitoring eradication efficacy in adults (Vakil et al. 2000). The urea breath test can also be used for both pretreatment examination and post treatment follow-up. However, expensive instrumentation and a specialized technician are required. In addition, the performance of the test has been associated with some disadvantages in infants and very young children as well as in patients with certain neurological disorders. The HpSA test is an important alternative to the UBT (Gramley et al. 1999; Makristathis et al. 2000; Kato et al. 2004). The stool antigen test is recommended for several reasons; stool samples are easily available, and the test is noninvasive and easy to perform. In addition, the accuracy of the test has been confirmed in children. Moreover, this test may be the optimum test to screen and confirm the success of eradication therapy even as early as 2 weeks after the end of therapy (Ni et al. 2000; Oderda et al. 2000).

The HpSA test was also found to be more sensitive and specific than the polymerase chain reaction methods using stool samples (Li et al. 1996; Casswall et al. 1999; McNamara et al. 1999; Makristatis et al. 2000). Polymerase chain reaction of fecal samples can easily yield false negative results because a variety of chemicals in the stool can inhibit the reaction (Wilde et al. 1990; Mapstone and Quirke 1992).

In contrary to the above mentioned studies in favor of HpSA test, commercially available HpSA kits sometimes provide different diagnostic accuracies in different populations and geographical regions (Ohkura et al. 2000). To overcome this problem, in different studies, it was reported that by calculating the cut-off values of HpSA test by receiver operator characteristics (ROC) curve analysis, the sensitivity and specificity of HpSA test increases (Leodolter et al. 2001; Kim et al. 2002; Kato et al. 2003; Syam et al. 2005). Makristatis et al. (2000) also stressed the importance of local test validation especially in post treatment follow-up.

There are only a few reports which emphasize the correlation between gastric inflammation degree and *H. pylori* load and HpSA optical density (OD) levels (Labenz et al. 1996; Chou et al. 1997; Chang et al. 2002). In regard to the diagnostic tests other that HpSA; C-UBT or urease test either significantly correlated (Labenz et al. 1996; Chou et al. 1997; Chang et al. 2002) or did not correlate (Graham et al. 1987; Logan et al. 1991) with gastric inflammation degree and gastric *H. pylori* load.

The aim of this study was to determine the cut-off value of the HpSA test to obtain the highest sensitivity and specificity for the diagnosis of *H. pylori* infection in our dyspeptic patients and evaluate the correlation of HpSA OD titers with gastric *H. pylori* load and histological inflammatory activity levels according to updated Sydney System (Dixon et al. 1996).

Materials and Methods

Patients

One hundred and thirty-two consecutive patients referred to endoscopy for dyspeptic symptoms lasting longer than 3 months were investigated in the study. Patients who have used proton pump inhibitors, histamine-2 receptor antagonists or bismuth containing compounds in the preceding month, and non-steroidal anti-inflammatory drugs in the preceding 15 days before

endoscopy, patients with abnormal bleeding tests or allergic to anesthetics, pregnant patients, and patients having had gastro-duodenal surgery were excluded. Out of 132 patients, 22 either refused to participate in the study or refused to give feces. Eight patients accepted to participate in the study but were not eligible for the study because of the above mentioned exclusion criteria. Finally a total of 102 patients were included in the study.

Informed consent was obtained from the eligible patients after the nature of the procedure was explained. The study was performed in compliance with human-studies guidelines and the study has been approved by the Suleyman Demirel University Faculty of Medicine Dean's Office Local Ethical Committee.

During the esophago-gastro-duodenoscopy, biopsy specimens were sampled from both gastric antrum and corpus. Rapid urease test was performed from antrum biopsy samples and microscopic histopathological examinations were performed from both antrum and gastric corpus biopsy samples. *H. pylori* infection was accepted as positive if both microscopic *H. pylori* detection and urease test of the biopsy specimens were positive and as negative only if both of these tests remained negative and these results were accepted as standard test results. Seven patients were excluded from the study because microscopic *H. pylori* detection and urease results were not both positive or both negative, thus the results of 95 patients were chosen for statistical analysis.

Laboratory analysis

H. pylori-related histological changes were evaluated by updated Sydney System. Briefly, polymorphonuclear leukocyte (PMNL) density, chronic inflammation (stained by H&E) and *H. pylori* load (stained by toluidine blue O), were scored from 0 to 3 (Dixon et al. 1996; Deveci and Deveci 2004). All specimens were examined by the same experienced pathologist. Patients were asked to collect a specimen from their first stool after endoscopy. Stool samples were divided into aliquots, frozen, and stored (−70°C) until analysis. Stool samples were analyzed for *H. pylori* antigen using the Premier Platinum HpSA Enzyme Immunoassay (Premier Platinum HpSA, Meridian Diagnostics, Cincinnati, OH, USA). In regard to the manufacturer's cut-off, values greater than or equal to OD = 0.16 were considered positive, while those less than 0.14 were considered negative. A value between 0.14 and 0.159 was equivocal. There was no equivocal OD value in our study.

Statistical analysis

Sensitivity, specificity, positive and negative predictive values of HpSA test were calculated by standard methods and evaluated according to the cut-off values recommended by the manufacturer as well as by the best cut-off value calculated by the ROC curve analysis (Swets 1988). The ROC curve analysis was performed by testing different OD values to obtain the highest sensitivity and specificity (Fig. 1). Kendall's tau-b correlation analysis was used to detect the correlations between HpSA OD values, gastric inflammation and *H. pylori* load scores. The graphics were designed as simple scatter graphics with line fit option according to correlation degrees. The statistical analyses were performed using the SPSS for Windows statistical package, version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

Of the 102 eligible patients, 88 were urease and histology positive, 7 were urease and histology negative, and 7 were histology negative and urease positive or vice versa. The last group was omitted from the statistical evaluation because of conflicting diagnosis of *H. pylori* infection. Finally, a total of 95 patients were eligible for statistical analysis. The mean of ages of the patients was 43.6 ± 14.2 ranging from 19 to 73 years. The endoscopic findings of the patients are listed in Table 1. The HpSA test results, obtained using two different cut-off values, recommended by the manufacturer, and calculated by ROC curve analysis, are listed in Table 2. By applying the manufacturer's cut-off value for HpSA test, the sensitivity, specificity, positive and negative predictive values were 51.1%, 100%, 100% and 14.0%, respectively. The best HpSA cut-off value calculated by the ROC curve analysis was 0.048 (area under the curve: 0.972, asymptotic confidence interval: 0.939-1.004) (Fig. 1). With the calculated cut-off value of 0.048, the sensitivity of the test increased without decreasing the specificity, which was still 100%. Using this cut-off value, 81 of the 88 *H. pylori* positive patients were also HpSA positive (92.0% sensitivity), and all of the 7 *H. pylori* negative patients were also HpSA negative (100% specificity). The positive and negative predictive values of the HpSA test were 100% and 50.0%, respectively.

TABLE 1. Endoscopic findings of the patients included.

Endoscopic findings	Patients (n = 95) (%)	Hp positive (%)	Hp negative (%)
Antral or corpus gastritis	86 (90.5)	79 (91.9)	7 (8.1)
Duodenitis	21 (22.1)	19 (90.5)	2 (9.5)
Gastric ulcer	2 (2.1)	2 (100)	0 (0)
Duodenal ulcer	8 (8.4)	8 (100)	0 (0)
Gastric and duodenal ulcer	1 (1.1)	1 (100)	0 (0)
Esophagitis	21 (22.1)	17 (81.0)	4 (19.0)

Hp, *Helicobacter pylori*.

TABLE 2. HpSA test results according to the cut-off value recommended by the manufacturer as well as by the best cut-off value calculated by the receiver operator characteristics (ROC) curve analysis.

	By using manufacturer's cut-off: 0.16		By using the best cut-off: 0.048	
	HpSA (+)	HpSA (–)	HpSA (+)	HpSA (–)
H. pylori (+) by standard tests	45	43	81	7
H. pylori (–) by standard tests	0	7	0	7

OD, optical density

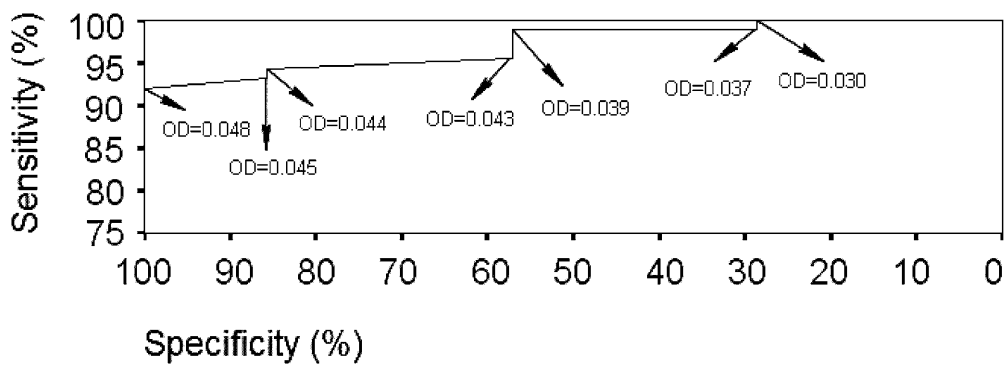

Fig. 1. The receiver operator characteristics (ROC) curve for the detection of best cut-off values of HpSA test. The optimal cut-off value was found to be optical density (OD), 0.048.

In patients with *H. pylori* infection, the significant correlations of the HpSA OD values with antral inflammation and corpus PMNL density are demonstrated in Figs. 2 and 3, respectively. In addition, gastric antrum PMNL density significantly correlated with HpSA OD values (r = 0.223, p = 0.005). Gastric corpus, but not antrum *H. pylori* load significantly correlated with HpSA OD values (Fig. 4).

DISCUSSION

In previous studies, the HpSA test was investigated for the diagnosis of *H. pylori* infection and the sensitivity and specificity were reported to be over 80% in symptomatic untreated patients (Makristathis et al. 1998; Puspok et al. 1999; Trevisani et al. 1999; Vaira et al. 1999). In our study, the sensitivity of the HpSA test was found

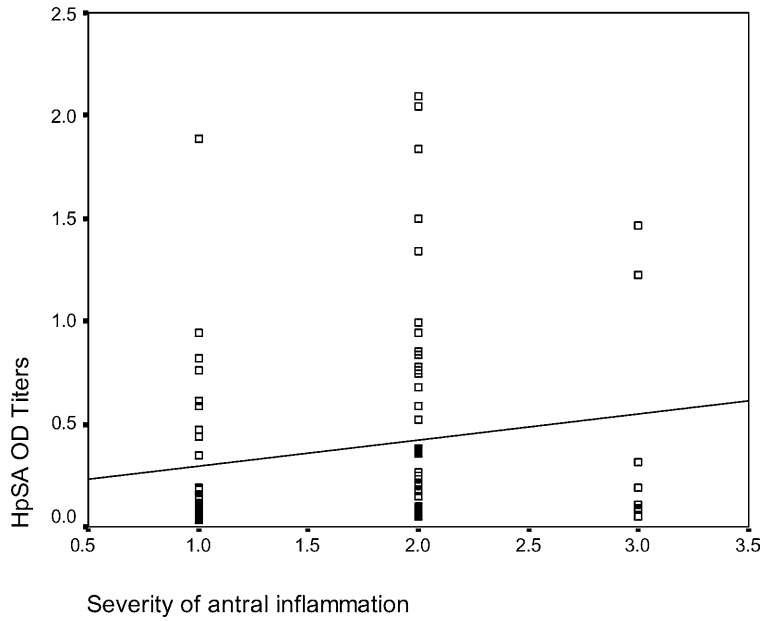

Fig. 2. The significant correlation of the HpSA test optical density (OD) titers with antral inflammation degree ($r = 0.217$, $p = 0.011$).

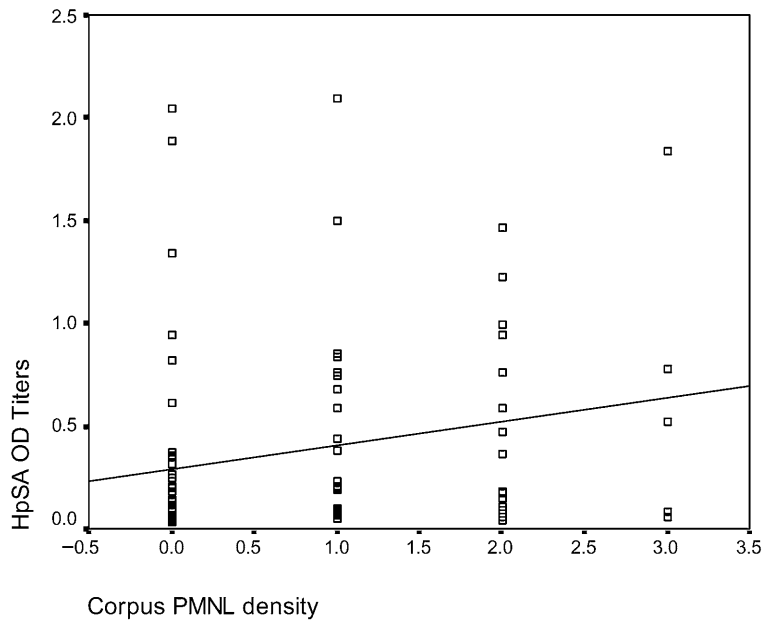

Fig. 3. The significant correlation of the HpSA test optical density (OD) titers with corpus polymorphonuclear leukocytes (PMNL) density ($r = 0.222$, $p = 0.007$).

to be quite low (51.1%) when determined on the basis of the manufacturer's cut-off value. However this does not mean that HpSA test does not have high diagnostic accuracy. In fact, according to several reports, HpSA test can be considered reliable in terms of diagnostic accuracy by adjusting the cut-off values for every population separately to obtain the highest sensitivity

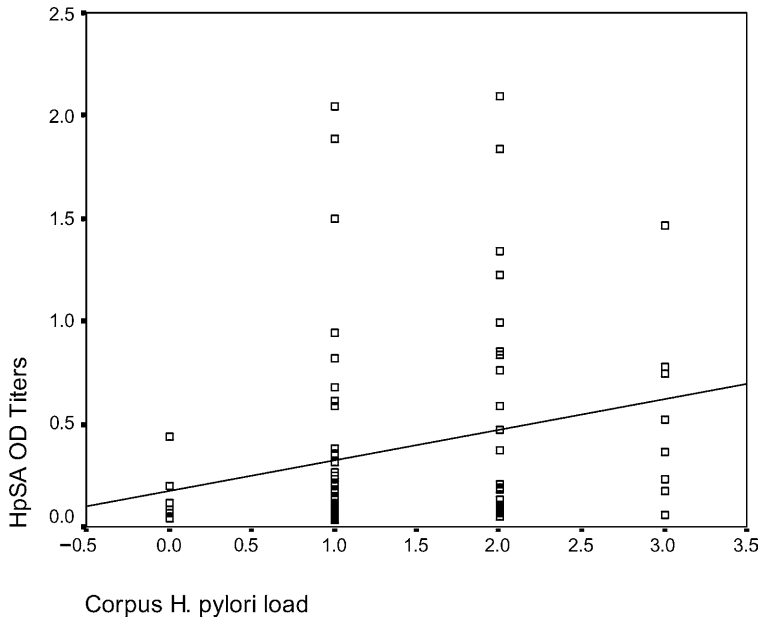

Fig. 4. The significant correlation of the HpSA test optical density (OD) titers with corpus *H. pylori* load ($r = 0.239$, $p = 0.004$).

and specificity. In these studies, the best cut-off values calculated by ROC curve analysis were from OD; 0.024 to 0.3 (Ohkura et al. 2000; Leodolter et al. 2001; Kim et al. 2002; Kato et al. 2003; Syam et al. 2005). In our dyspeptic patients, the best cut-off value calculated by ROC curve analysis was 0.048 and by using this cut-off value, the sensitivity of the test increased from 51.1% to 92% without decreasing the specificity of the test.

The feces amount is a variable which may affect the quantitative assessment of the HpSA test. In our region, patients are generally from rural areas who consume meal with higher fiber content when compared with those from urban areas. People living in rural areas consume whole wheat bread made of local flour whereas people living in urban areas consume bread of white flour (bran free) (Aktan et al. 1984). Wisker et al. (1988) stated that the increase in the intake of dietary fiber results in an increase in stool weight. Stevens et al. (1988) also reported that fiber supplementation decreases transit time and increases daily number of defecations and the wet and dry weights of stool. *H. pylori* may be diluted in the bulky volume of stool in patients who have much more fiber in their diet than western patients (Kamm and Lennard-Jones 1994). This may be one of the reasons for lower number of bacteria in feces which causes lower OD values in HpSA test.

The best cut-off value may also be related partially to the prevalence of *H. pylori* in the population and *H. pylori* load in the patients' stomach. Syam et al. (2005) investigated the diagnostic accuracy of the HpSA test and detected 100% sensitivity and 36.8% specificity using a cut-off value of 0.16 (as recommended by the manufacturer) and 66.7% sensitivity and 78.9% specificity using a cut-off value of 0.274 (calculated by ROC curve analysis). In their patient group 6 of 63 (9.5%) patients were *H. pylori* positive. On the other hand, in another patient group where 31 of 41 (75.6%) patients were *H. pylori* positive, 87.1% sensitivity and 100% specificity were detected according to the manufacturer's cut-off value, and 100% sensitivity and 90% specificity were detected according to the calculated cut-off value of 0.024 by ROC curve analysis (Kim et al. 2002). These two studies may indicate that in low *H. pylori* prevalent populations the cut-off values of HpSA test are higher whereas in high *H. pylori*

prevalent populations the cut-off values are lower which is in accordance with our results. The prevalence of *H. pylori* is very high in Turkey. In our region, *H. pylori* infection is endemic. In our previous study conducted in our region, 95.2% seroprevalence was detected in atherosclerosis patients (Adiloglu et al. 2003). In another serology based Turkish study held in asymptomatic subjects, 30% of the teenagers and 70% of the adults already acquire *H. pylori* infection (Us and Hascelik 1998).

In previous studies, the sensitivity and specificity of the HpSA test were found to be comparable to the UBT (Casswall et al. 1999; McNamara et al. 1999; Braden et al. 2000), but there are only a few studies investigating the correlation between HpSA OD values and gastric inflammation scores or *H. pylori* load. The reaction velocity of the biopsy urease test, the $\Delta\ ^{13}C$ value of the UBT and the OD value of the HpSA test correlated well with *H. pylori* density in the stomach (Labenz et al. 1996; Chou et al. 1997; Chang et al. 2002). In another study, HpSA OD highly correlated with gastric antrum *H. pylori* load evaluated histologically (Gallo et al. 2001). In our study, gastric corpus, but not antrum *H. pylori* load significantly correlated with HpSA OD values (Fig. 4). It was suggested that the UBT can be used to predict both intragastric bacterial load and severity of related gastritis (Perri et al. 1998). However, Matthews et al. (2005) detected that the ^{13}C-UBT results significantly correlated with urease and myeloperoxidase activity and severity of gastritis but not with bacterial load. There are also studies which failed to establish a significant correlation between gastric mucosa *H. pylori* load and C-UBT or urease test (Graham et al. 1987; Logan et al. 1991).

To our knowledge there is only one study that investigates the correlation between HpSA test and gastric inflammation evaluated by updated Sydney System quantitatively. Chang et al. (2002) detected that both the level of $\Delta\ ^{13}CO_2$ of ^{13}C-UBT and the OD value of the HpSA test correlated well with the gastric mucosal inflammatory activity diagnosed by updated Sydney System. In our study, antral inflammation degree (Fig. 2), gastric corpus PMNL density (Fig. 3) and antrum PMNL density significantly correlated with HpSA OD values. The HpSA test is a helpful tool to estimate the severity of gastric inflammatory activity and may predict the need for further invasive procedures. In this point of view, the HpSA test may be an alternative to gastric endoscopy especially in children and some patients who are unable to tolerate endoscopic examination. Moreover, HpSA test may also be an alternative to serological tests for epidemiological researches and it may be used in the screening of asymptomatic individuals who have high risk of gastric malignancies such as young people with a family history of gastric cancer.

In our study, it was found that gastritis scores and bacterial load significantly correlated with HpSA OD values. Denser *H. pylori* infection causes more severe corpus gastritis (Chuang et al. 2004). It is also expected that denser *H. pylori* infection in gastrointestinal tract causes denser antigen in the feces. Matsuda et al. (2003) detected a positive correlation between HpSA OD values and the number of *H. pylori* isolated by quantitative culture of biopsy specimens.

In our study, *H. pylori* negative patients were fewer in number which might seem to be a handicap but the high specificity obtained by HpSA test (100%), both with the manufacturer's and calculated cut-off values reduced this effect of this handicap.

In conclusion, by adjusting HpSA cut-off values according to our population, the test sensitivity increased without decreasing the specificity. Therefore, the cut-off values of the HpSA test must be calculated for every population separately to obtain the highest sensitivity and specificity. We also concluded that HpSA OD values reflect the severity of gastritis and gastric mucosal *H. pylori* load. As a result, the HpSA test is a helpful tool to evaluate the severity of gastric inflammatory activity and gastric *H. pylori* load and may replace further invasive procedures in some patient groups.

Acknowledgments

We thank Mrs. Beture Memedova, Associate

Professor of English Literature in Suleyman Demirel University Faculty of Letters and Sciences, for her assistance in English edition. We also thank Mr. A. Nesimi Kisioglu, Associate Professor of Public Health in Suleyman Demirel University Medical School for his assistance in statistical analysis.

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