

Does Cigarette Smoking Affect the Diagnostic Reliability of Hemoglobin $\alpha 2\delta 2$ (HbA₂)?

Issa S. Tarazi,¹ Mahmoud M. Sirdah,^{2*} Hesham El Jeady,^{1,3} and Rami M. Al Haddad¹

¹Thalassaemia and Haemophilia Centre, Palestine Avenir Foundation, Gaza, Palestine

²Biology Department, Al Azhar University-Gaza, Gaza, Palestine

³European Gaza Hospital, Palestinian Ministry of Health, Gaza, Palestine

Quantitation of hemoglobin $\alpha 2\delta 2$ (HbA₂) is a basic and confirmatory test in diagnosing the carrier state of β -thalassemia. The present study was designed to investigate the effect of cigarette smoking on the diagnostic reliability of HbA₂. A total of 2,867 (654 smokers and 2,213 never smokers) male subjects were involved in the present study. The subjects were categorized into three groups according to their laboratory findings: β -thalassemia minor, iron deficient, and normal groups. Complete blood count (CBC) parameters and HbA₂ levels were compared between smokers and never smokers of each group

according to the independent-samples *t*-test using the SPSS[®] program, significance results were reported at $P < 0.05$. The results showed a significant increase in red blood cell (RBC) mass (RBC count and hematocrit [Hct]) and Hb concentration in smokers of all groups; however, no significant differences were reported in the HbA₂ level between smokers and never smokers in all groups. It was concluded that cigarette smoking does not affect the diagnostic reliability of the HbA₂ test. *J. Clin. Lab. Anal.* 22:119–122, 2008. © 2008 Wiley-Liss, Inc.

Key words: HbA₂; smoking; thalassemia; iron deficiency

INTRODUCTION

Comparative studies between cigarette smokers and never-smokers revealed higher levels of red blood cell (RBC) mass (RBC count and hematocrit [Hct]) and hemoglobin (Hb) in smokers than in never-smokers. Hb level increases as a compensatory mechanism in smokers because the inhaled carbon monoxide of the burning cigarette results in increased carboxyhemoglobin, which has no oxygen-binding affinity. Consequently, different international health parties and centers developed smoking-specific Hb adjustments to define the cutoff values of Hb and Hct in smokers (1,2). Different authors studied and reported the effect of cigarette smoking on Hb subtypes like Hb_{A1C} (3–5), and Hb F (6,7). However, the effect of cigarette smoking on Hb $\alpha 2\delta 2$ (HbA₂) level was never investigated or reported.

β -Thalassemia is a major health problem in the Gaza Strip, Palestine, where 293 patients are currently treated with transfusions and chelation. In September 2000, Palestine adopted a prevention program of obligatory premarital testing for β -thalassemia. The program

considered the HbA₂ quantitation as a major and reliable test for the diagnosis of β -thalassemia minor (8,9). The present study, therefore, was designed and aimed to investigate the effect of cigarette smoking on the diagnostic reliability of HbA₂.

MATERIALS AND METHODS

A total of 2,867 certainly diagnosed male subjects (β -thalassemia minor, iron deficient, and normal), with mean age of 23.72 ± 6.85 years, were involved in the present study. The Gaza strip is a part of the Middle East where oriental conservative traditions and regulations govern the majority of the population. Among these tradition and regulations is the tendency for cigarette smoking, which is very dominant among males and very limited or prohibited among females. Nearly

*Correspondence to: Dr. Mahmoud Sirdah, Biology Department, Al Azhar University-Gaza, PO Box 1277 Gaza, Palestine.
E-mail: msirdah@hotmail.com

Received 13 May 2007; Accepted 12 December 2007

DOI 10.1002/jcla.20228

Published online in Wiley InterScience (www.interscience.wiley.com).

TABLE 1. The CBC parameters and HbA₂ levels in the three groups[†]

	Group I (β-thalassemia minor) (N = 1,118)		Group II (iron deficiency) (N = 984)		Group III (normal) (N = 765)	
	Smokers (N = 448)	Never smokers (N = 670)	Smokers (N = 120)	Never smokers (N = 864)	Smokers (N = 86)	Never smokers (N = 679)
WBC (× 10 ⁹ /L)	7.79 (2.43)	7.46 (2.17)	6.69 (1.97)	6.92 (2.02)	7.62 (2.37)	7.11 (1.81)
RBC (× 10 ¹² /L)	6.04 (0.47)*	5.87 (0.61)	5.62 (0.54)*	4.95 (0.64)	5.80 (0.45)*	5.37 (0.63)
Hb (g/dL)	12.97 (1.04)*	12.36 (1.81)	13.06 (1.46)*	11.59 (1.62)	14.41 (0.84)*	13.49 (1.48)
Hct (%)	40.97 (3.32)*	38.84 (4.18)	41.12 (4.91)*	36.43 (5.23)	44.68 (2.68)*	41.51 (4.36)
MCV (fL)	67.16 (5.12)*	65.46 (5.24)	72.18 (5.25)	73.21 (6.25)	76.86 (3.76)	76.96 (4.48)
MCH (pg)	21.24 (1.80)*	20.77 (1.87)	23.11 (2.17)	23.43 (2.25)	24.79 (1.29)	25.02 (1.78)
MCHC (g/dL)	31.62 (1.23)	31.80 (2.32)	32.01 (1.22)	31.87 (1.34)	32.27 (0.68)	32.49 (1.10)
RDW (%)	16.89 (1.12)	17.11 (5.81)	17.15 (2.40)	17.49 (9.75)	15.98 (1.77)	15.64 (1.98)
PLT (× 10 ⁹ /L)	293.08 (68.50)	311.28 (81.06)	300.57 (97.65)	317.81 (81.90)	280.84 (61.89)	293.90 (78.87)
HbA ₂ (%)	5.03 (0.79)	5.04 (0.75)	2.42 (0.44)	2.47 (0.80)	2.46 (0.51)	2.42 (0.49)

[†]Values are means with SDs in parentheses. Statistical analysis was performed between smokers and never smokers of same group.

*Significant, $P < 0.05$.

25–35% of Palestinian males are smokers, while the percentage is about 2.0% in females (10).

The laboratory diagnosis of subject blood samples was achieved according to a definite protocol that was adopted and approved by the scientific and ethical committees at the Thalassaemia and Haemophilia Centre, Palestine Avenir Foundation (9). All the laboratory works and investigations at the Thalassaemia and Haemophilia Centre are carried out in accordance with the Helsinki Declaration of 1975. The subjects were categorized into smokers and never smokers according to a definite questionnaire that filled by each subject involved in the study. The questionnaire also includes personal, demographic information, and a consent statement. All subjects accepted to participate and all signed the consent statement.

The collected blood samples were diagnosed through performing complete blood count (CBC) using an automated Cell-Dyne 1700 Analyzer (Sequoia-Turner Corporation, Santa Clara, CA) and serum ferritin using an IMX System (Abbott, Abbott Park, IL), and quantitation of HbA₂ using ion exchange Hb S-free interference microcolumn chromatography (Biosystem, Barcelona, Spain).

Accordingly, the subjects were categorized into three groups. Group I (microcytic and/or hypochromic, HbA₂ ≥ 3.5% with normal serum ferritin) included 1,118 β-thalassemia minor subjects, group II (microcytic and/or hypochromic, HbA₂ < 3.5% with serum ferritin < 13 g/dL) included 984 iron deficient subjects, and group III (normocytic and normochromic) included 765 normal subjects.

For each group, comparative statistical analysis between smokers and never smokers was performed for hematological parameters and HbA₂ values according

to the independent-samples *t*-test using the SPSS[®] (version 12; SPSS Inc., Chicago, IL) program, significance results were reported at $P < 0.05$.

RESULTS

The CBC parameters and HbA₂ levels for the three groups of the present study are mentioned in Table 1.

Group I (β-Thalassemia Minor, 448 Smokers and 670 Never Smokers)

Statistically significant higher values were reported in the RBC, Hb, Hct, mean cell volume (MCV), and mean cell Hb (MCH) of the β-thalassemia minor smokers as compared to β-thalassemia minor never smokers; the increments were 3.0, 4.8, 5.5, 2.6, and 2.3%, respectively. However, nonsignificant difference was reported in HbA₂ levels, the values of which were $5.03 \pm 0.79\%$ and $5.04 \pm 0.75\%$, respectively.

Group II (Iron Deficiency, 120 Smokers and 864 Never Smokers)

In this group, statistically significant higher values were reported in the RBC, Hb, and Hct of the iron deficient smokers as compared to never smokers; the increments were 13.5, 12.7, and 12.9%, respectively. Nonsignificant difference was reported in the level of HbA₂, the values of which were $2.42 \pm 0.44\%$ and $2.47 \pm 0.80\%$, respectively.

Group III (Normal, 86 Smokers and 679 Never Smokers)

In the normal group, the statistically significant higher values were also reported in the RBC, Hb, and Hct of

smokers as compared to never smokers; the increments were 8.0, 6.8, and 7.4%, respectively. Nonsignificant difference was also reported in the level of HbA₂, the values of which were $2.46 \pm 0.51\%$ and $2.42 \pm 0.49\%$, respectively.

DISCUSSION

Cigarette smoking has been associated with higher levels of RBC mass and total Hb concentration as compared to never or nonsmokers. Consequently, different international health parties and centers developed smoking-specific Hb adjustments to define the cutoff values of Hb and Hct in smokers (1,2). The effects of cigarette smoking were also investigated and reported on Hb subtypes such as Hb_{A1C} (3–5), and Hb F (6,7). However, the effect of cigarette smoking on HbA₂ level was never investigated or reported.

In the Gaza strip Palestine, the premarital tests for β -thalassemia are conducted as an obligatory step and performed according to a definite protocol that was adopted and approved by the scientific and ethical committees at the Thalassaemia and Haemophilia Centre, Palestine Avenir Foundation. The protocol relay on the quantitation of HbA₂ as confirmatory test for β -thalassemia minor (9).

HbA₂ quantitation is a confirmatory test for β -thalassemia minor, and changes in total Hb and Hb subtype levels have been associated with cigarette smoking (1–7); therefore, it could be very helpful and instructive to investigate if there is any effect of cigarette smoking on levels of HbA₂. The results of the investigation may support or direct toward adjusting the working protocol HbA₂ cutoff value.

The present study is considered as the first ever study that discusses the effect of smoking on HbA₂ level. It is worth mentioning that none of the subjects of the present study suffers from any chronic or acute disease or clinical condition. Both β -thalassemia minor and iron-deficient subjects have been identified and diagnosed as a part of the ongoing premarital program.

The present study revealed an elevated RBC mass and total Hb concentrations in smokers in the three groups as compared to their corresponding never smokers. The higher values of RBC mass and Hb concentrations have been also reported and mentioned by other authors for other populations. Adjustments for hematological parameters have been also proposed and recommended when interpreting smokers' laboratory results (1,11,12).

It is important to mention that the increments in the RBC mass and Hb concentration of the iron-deficient smokers are much greater than the increments the smokers of the other groups. For example the increment

in RBC count in β -thalassemia minor smokers was 3.0% and in the normal group it was 8.0%, while in the iron-deficient groups it was 13.5%. The same could be seen in increments of Hb concentration: 4.8% in β -thalassemia minor smokers, 6.8% in normal smokers, while it showed 12.7% in iron-deficient smokers. This should be considered and adjusted when interpreting suspected iron-deficient anemic samples with ferritin level supporting iron deficiency anemia while Hb concentration does not. Another parameter that would be adjusted or at least considered for future studies is the cutoff values for MCV and MCH of smokers in microcytosis/hypochromasia screening programs.

Air pollution may be considered as a confusing or overwhelming factor in smoking-related studies. It is worthwhile to mention that the Gaza strip, Palestine, is a narrow piece (365 km²) of land along the Mediterranean coast. Just 40 km long and 10 km wide. Neither major nor minor industries exist in the Gaza strip. The people there depend on fishing and growing crops. Therefore, sources for air pollution are very limited and we believe that it should not be considered as an overwhelming factor in a region like the Gaza strip. However, it could be considered an effective factor in developed and industrialized countries.

Regarding the core of the present work, which was the effect of cigarette smoking of the level of HbA₂, the comparative treatments of results in the three groups showed nonsignificant differences between the level of HbA₂ in smokers and never smokers, nevertheless, of the higher level of total Hb in the smokers of the three groups. The present finding therefore supports the diagnostic reliability of HbA₂ quantitation even in smokers. So, no adjustment is required for the HbA₂ cutoff value between smokers and never or nonsmokers.

It was concluded that, for hematological laboratories, smoking is not an interrupting factor for the diagnostic reliability of HbA₂. However, for total Hb concentration and RBC mass cutoff values, especially in iron-deficient samples, smoking should be considered, as it increases these parameters considerably.

REFERENCES

- Centers for Disease Control and Prevention (CDC). Recommendations to prevent and control iron deficiency in the United States. *MMWR Recomm Rep* 1998;47:1–36.
- Yun SH, Choi YH, Moon YS, Ahn SH, Kim TG. Difference in hemoglobin between smokers and non-smokers. *J Korean Acad Fam Med* 2002;23:80–86.
- Nilsson PM, Lind L, Pollare T, Berne C, Lithell HO. Increased level of hemoglobin A1c, but not impaired insulin sensitivity,

- found in hypertensive and normotensive smokers. *Metabolism* 1995;44:557–561.
4. Gulliford MC, Ukoumunne OC. Determinants of glycosylated haemoglobin in the general population: associations with diet, alcohol and cigarette smoking. *Eur J Clin Nutr* 2001; 55:615–623.
 5. McCulloch P, Lee S, Higgins R, McCall K, Schade DS. Effect of smoking on hemoglobin A1c and body mass index in patients with type 2 diabetes mellitus. *J Investig Med* 2002;50:284–287.
 6. Bureau MA, Shapcott D, Berthiaume Y, et al. Maternal cigarette smoking and fetal oxygen transport: a study of P50, 2,3-diphosphoglycerate, total hemoglobin, hematocrit, and type F hemoglobin in fetal blood. *Pediatrics* 1983;72:22–26.
 7. Fagan DG, Lancashire RJ, Walker A, Sorahan T. Determinants of fetal haemoglobin in newborn infants. *Arch Dis Child Fetal Neonatal Ed* 1995;72:111–114.
 8. Palestine Avenir Foundation. Management and Control of Thalassaemia in Gaza. In: Newsletter of the Thalassaemia and Haemophilia Center-Gaza. Gaza Strip, Palestine: Palestine Avenir Foundation; 2005. p 2–4.
 9. Tarazi I, Al Najjar E, Lulu N, Sirdah M. Obligatory premarital tests for β -thalassaemia in the Gaza Strip: evaluation and recommendations. *Int J Lab Hematol* 2007;29:111–118.
 10. Palestinian Central Bureau of Statistics. Smoking in the Palestinian territory: press release on the world day to stop smoking. Palestinian National Authority; 2007. p P1–P2.
 11. Galea G, Davidson RJ. Haematological and haemorheological changes associated with cigarette smoking. *J Clin Pathol* 1985;38:978–984.
 12. Alton I. Iron deficiency anemia. In: Stang J, Story M, editors. Guidelines for adolescent nutrition services. Minneapolis, MN: University of Minnesota Press; 2005. p 101–108.