RESEARCH ARTICLE

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A Different Look at Premarital Hemoglobinopathy Screening in Primary Care

ABSTRACT

Objective: Despite the high prevalence of hemoglobinopathies (HBP), the most common single-gene disorders in Turkey, data in some regions are lacking. We aimed to evaluate the effectiveness of the hemoglobinopathy premarital screening program (PMS) and to investigate the contribution of efficient use of complete blood count (CBC) parameters on cost-effectivity.

Methods: HMP diagnosed 49171 subjects in 4 years and CBC of subjects with HMP in a year were evaluated retrospectively.

Results: The total incidence rate of HBP was 3.41%, β-thalassemia trait (β-TT) was 1.98%, HMP incidence in the PMS group was 2.43%, β-TT was 1.08%. Moreover, HbF, HbD, HbC, HbS, HbE and HbJ were detected with the incidences of 0.49%, 0.14%, 0.05%, 0.04%, 0.007% and 0.009%, respectively. RDW/MCH ratio compared to other indices was the most successful for both sexes in diagnostic efficiency of HBP (AUC: male:0.922 - female:0.961) and β-TT (AUC: male;0.928 - female:0.961).

Conclusions: PMS was found to be an effective application program in HMP screening. RDW/MCH ratio was the most useful and easy parameter in detecting HBP and β-TT in PMS and in terms of reducing unnecessary test requests and cost-effectiveness in public health screenings.

Keywords: Hemoglobinopathy, Premarital Screening, β-Thalassemia Trait, RDW, MCH.

Birinci Basamakta Evlilik Öncesi Hemoglobinopati Taramasına Farklı Bir Bakış

ÖZET

Amaç: Türkiye'de en sık görülen tek gen hastalıkları olan hemoglobinopatilerin (HBP) yüksek prevalansına rağmen, bazı bölgelerde veri bulunmamaktadır. Hemoglobinopati evlilik öncesi tarama programının (PMS) etkinliğini değerlendirmeyi ve tam kan sayımı (CBC) parametrelerinin etkin kullanımının maliyet-etkililiğe katkısını araştırmayı amaçladık.

Gereç ve Yöntem: 4 yılda 49,171 HMP tanısı konan olgu ve bir yılda HMP'si olan olguların tam kan sayımları geriye dönük olarak değerlendirildi.

Bulgular: HBP'nin toplam insidans oranı %3.41, β-talasemi taşıyıcılığı (β-TT) %1.98 bulundu. PMS grubunda HMP insidansı %2.43, β-TT %1.08 idi. Ayrıca HbF, HbD, HbC, HbS, HbE ve HbJ sırasıyla %0.49, %0.14, %0.05, %0.04, %0.007 ve %0.009 oranında tespit edildi. ROC analizinde diğer indekslere kıyasla RDW/MCH oranı, HBP (erkek:0.922- kadın:0.961) ve β-TT (erkek;0.928- kadın:0.961) tanısal etkinliğinde her iki cinsiyet için de en başarılıydı.

Sonuç: PMS, HBP taramasında etkili bir uygulama programı olarak bulundu. RDW/MCH oranı; HBP ve β-TT'nin saptanmasında ve halk sağlığı taramalarında gereksiz test isteklerinin azaltılması ve maliyet etkinliği açısından en kullanışlı parametreydi.

Anahtar Kelimeler: Hemoglobinopati, Evlilik Öncesi Tarama, β-Talasemi Taşıyıcılığı, RDW, MCH.

INTRODUCTION

Hemoglobinopathy is a genetic disorder leading to an abnormal globin chain structure in the hemoglobin(Hb) molecule(1). Thalassemia types are more prevalent in the regions of the Mediterranean coastal line (Greece and Italy), the Arabian Peninsula, Iran, Ecuador, Africa, Asia, and Turkey. The genetic prevalence of thalassemia in these regions varies from 2.5% to 15.0%(2).

The diversity and heterogeneous distribution of Hb disorders make it necessary to develop strategies depending on the country. Due to the high rate of marriages between first-degree relatives in our country, a Hemoglobinopathy Control Program with pre-marital screening (PMS) has been initiated to prevent childbirth with HBP(3). As in the examples across the world (Tunisia, Bahrain, Saudi Arabia, Lebanon, United Arab Emirates, Iran, Qatar, and the Gaza Strip), national PMS in Turkey is mandatory and free of charge(4).

PMS and counseling services, in primary health care, aimed to minimize maternal and fetal risks, to prevent birth defects and genetic diseases, to reduce the economic burden on public health and for couples to obtain information about the situations due to potential risks (5). With these programs, carriers can be easily detected by routine haematological methods and pre-warned of reproductive risks (6). In this regard, while the number of couples who had their PMS tests in Turkey in 2003 was 30%, this rate reached 86% in 2013(7).

In this study, we aimed to investigate the prevalence of HMP among individuals who applied to Family Health Centers (FHC) in our province for PMS. Moreover, we planned to retrospectively assess the usefulness of the parameters and formulas based on CBC in detecting abnormal Hb variants among individuals with HBP.

MATERIAL AND METHODS

This study was approved with Ethics Committee Protocol number: 20:09.84.06.05, May 30th, 2019. In this retrospective study, individuals who applied to FHCs for PMS and other reasons were investigated between January 2016 and December 2019, from the public health laboratory electronic archive. CBCs have been measured in Beckman Coulter hematological autoanalyzer and hemoglobin variant analyses have been measured using high-pressure liquid chromatography (HPLC) technique of Arkray Adams A1c HA-8180T analyzer (Arkray, Inc., Kyoto, Japan). All demographic data and test results were taken from patient files retrospectively. Individuals with a recent medical history of systemic and other hematological disorders were excluded. CBC parameters of subjects with HMP (n=393) in 2019 and the healthy control group (n=100) were included in this study for evaluation of indices.

Subjects were considered to have β-TT if they had MCV <80 fl and MCH <27 pg and a hemoglobin A2 level >3.5%. HbF, HbD, HbC, HbS, HbE, HbJ, and other variants were also detected. HbA2 level between 3.1% and 3.5% was considered borderline. The discrimination erythrocyte (RBC) indices and formulas were calculated using the RBC indices as defined following: England&Fraser(8):MCV-RBC-5Hb-3.4 (Cut-off: <0), Mentzer index(9): MCV/RBC count (<13), Shine and Lal (SL-I)(10): MCV2xMCH/100 (Cut-off <1530), Green&King Index (G&K)(11): MCV2xRDW/100xHB (Cut-off <72), RDWI(12):MCV x RDW/RBC (Cut-off <220).

Statistical Analysis: The normal distribution of the variables was determined using the Kolmogorov-Smirnov test. Parametric variables were compared using the Student's t-test. Non-parametric data were analyzed with the Mann–Whitney U test. The AUC was found using a receiver operating characteristics (ROC) analysis to investigate the effectiveness of the methods used in showing marked β-TT and HBP. An AUC of ≤0.05 was evaluated as "the test has no diagnostic value". A p-value of less than 0.05 was considered statistically significant. All statistical analyses were carried out with SPSS IBM 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

In the present study, between January 2016 and December 2019, 44,790 participants of the total 49,171 participants applied for the HMP screening were investigated for PMS, and abnormal hemoglobin variants were detected in 1090 (2.43%) of them. Abnormal Hb variants were detected in 588 of 4381 participants who applied for other reasons (8.91%). The numbers of admissions and abnormal Hb variants' detection rates by years were presented in Table 1.

Table 1. Year-based Thalassemia Screening and Abnormal Hemoglobin Variant Analysis

Year	Total Test Number	Application based on marriage (Number/%)	Abnormal variant analysis as a result of application for marriage (Number/%)	Application for other reasons (Number/%)	Abnormal variant analysis as a result of application for other reasons (Number/%)	Abnormal hemoglobin variant rates in all groups
2016	13.069	12.499 (%95.64)	295 (%2.36)	570 (%4.36)	134 (%23.51)	429 (%3.28)
2017	12.506	11.874 (%94.95)	323 (%2.72)	632 (%5.05)	135 (%21.36)	455 (%3.63)
2018	12.248	11.629 (%94.95)	264 (%2.27)	619 (%5.05)	134 (%21.65)	398 (%3.24)
2019	11.348	8788 (%77.44)	208 (%2.37)	2560 (%22.56)	185 (%7.23)	393 (%3.46)
Total	49.171	44.790 (%91.09)	1090 (%2.43)	4381 (%8.91)	588 (%13.42)	1678 (%3.41)

The incidence of high HbA2, microcytosis, hypochromia, and β-TT was 1.08% in 393 subjects with abnormal hemoglobin variants, detected in PMS of 2019 yearly data. Moreover, HbF, HbD, HbC, HbS, HbE and HbJ were detected with the incidences of 0.49%, 0.14%, 0.05%, 0.04%, 0.007% and 0.009%, respectively. The prevalence rates among subjects applied for other reasons were as follows: 5.98% for β-TT, 0.86% for HbF, 0.16% for HbS, 0.12% for HbD, and 0.12% for HbE. The prevalence of β-TT among all applications was 1.98% (Table 2).

Table 2. Numbers of Abnormal Hemoglobin Variants for 2019

	Abnormal variant analysis as a result of application for marriage	Abnormal variant analysis as a result of application for other reasons	Total
HbA2	122 (% 1.08)	153 (% 5.98)	275 (% 1.98)
HbF Height	56 (% 0.49)	22 (% 0.86)	78 (% 0.56)
HbS Variant	5 (% 0.04)	4 (% 0.16)	9 (% 0.064)
HbC Variant	6 (% 0.05)	-	6 (% 0.043)
HbD Variant	16 (% 0.14)	3 (% 0.12)	19 (% 0.14)
HbJ Variant	1 (% 0.009)	-	1 (% 0.007)
HbE Variant	2 (% 0.017)	3 (% 0.12)	5 (% 0.035)
Total Hb Variant Number	208 (% 1.83)	185 (7.23)	393 (2.83)
Total Screening Person	**11.348**	**2560**	**13.908**

Hb: Hemoglobin

Two different genders were compared concerning CBC and indices in the group with HMPs (n=393) and the healthy control group (n=100). In females, RBC, HB, MCV, MCH, EF index, G&K-I, and SL-I values were significantly lower in the group with HMP than the control group (for all, $p<0.001$), whereas RDW and RDWI values were significantly higher (for both, $p<0.001$). In males, RBC, HB, HCT, MCV, MCH, MCHC, G&K-I, and SL-I values were significantly lower in the group with HMP than the control group (for all, $p<0.001$). On the other hand, RDW, RDWI, and Mentzer index values were significantly higher ($p<0.001$, $p<0.001$, and $p<0.01$, respectively) (Table 3).

Table 3. Hematological features of subjects at 2019

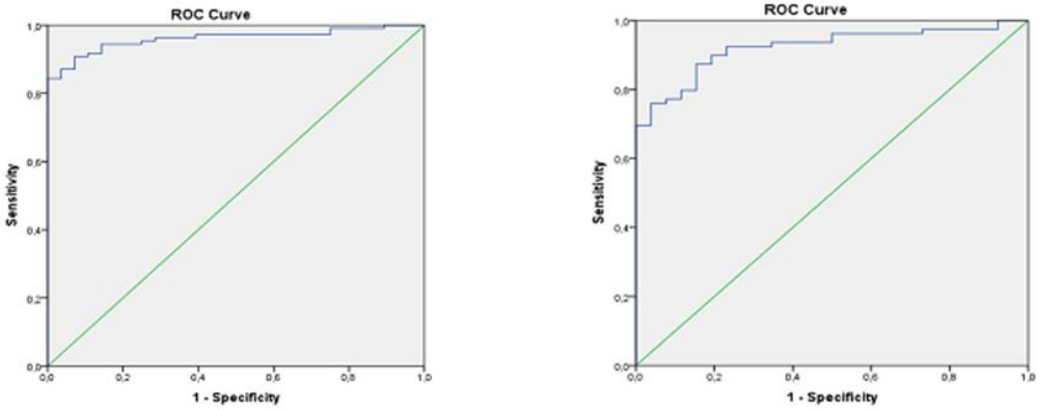
	FEMALE			MALE		
	Healthy Groups (n:52)	HBP Groups (n:210)	*P*	Healthy Groups (n:48)	HBP Groups (n:183)	*P*
Age (years)	33.57 (23 - 69)	30.46 (27 - 77)	0.240	33.15 (23-62)	30.33 (28-73)	0.114
RBC (/mm^3)	6.55 ± 0.42	5.46 ± 0.86	0.000	6.80 ± 0.53	5.25 ± 0.93	0.000
HB (g/dL)	13.11 ± 1.14	11.96 ± 1.77	0.000	15.39 ± 1.05	12.58 ± 1.85	0.000
HCT (%)	38.89 ± 3.01	39.95 ± 3.13	0.705	45.46 ± 3.24	38.82 ± 5.22	0.000
MCV	90.61 ± 6.56	69.08 ± 10.13	0.000	92.26 ± 3.73	75.58 ± 13.11	0.000
MCH	30.95 ± 1.56	22.41 ± 3.96	0.000	32.78 ± 2.26	24.58 ± 4.81	0.000
MCHC	33.08 ± 1.54	34.14 ± 2.4.	0.618	34.96 ± 2.23	32.38 ± 1.25	0.000
RDW	12.78 ± 1.06	17.26 ± 2.63	0.000	12.94 ± 0.94	15.88 ± 2.70	0.000
RDWI	176.84 (138.87 – 418.3)	226.21 (139.1 – 415.3)	0.000	175.46 (131 – 219.1)	230.71 (128.6 – 499.68)	0.000
Mentzer-I	13.87 ± 1.21	13.20 ± 3.98	0.108	13.55 ± 1.33	15.24 ± 5.37	0.007
E- F	15.08 (-1.27- 30.87)	0.42 (-23.1- 32.13)	0.000	5.03 (-5.52 – 14.29)	4.02 (-17.41 – 46.3)	0.586
G&K	13791.89±2210.82	9865.83 ± 3207.71	0.000	17041.85 ± 2569.5	11332.1 ± 3946.5	0.000
SL-I	2555.18±381.84	1148.46 ± 600.19	0.000	2798.29 ± 326.68	1542.22 ± 831.19	0.000
RDW/MCH	0.4±0.04	0.79±0.21	0.000	0.39±0.03	0.69±0.22	0.000

HBP Groups: Haemoglobinopathy Groups. RBC: red blood cells. HB: Hemoglobin. HCT: Hematocrit. MCV: Mean corpuscular volume. MCH: Mean corpuscular hemoglobin. MCHC: Mean corpuscular hemoglobin concentration. RDW: Red cell distribution width. RDWI: Red cell distribution width index. Mentzer-I: Mentzer-Index. E-F: England & Fraser index. G&K: Green and King index. SL-I: Shine Lal Index.

To distinguish HMP and β-TT subjects:

ROC analysis was performed to determine the precision of CBC parameters and indices in the HMP group. AUC values for RDW, MCH and RDW/MCH ratio were acceptable (>70%), concerning diagnostic efficiency for males (0.860, 0.918, 0.922 respectively) and females (0.958, 0.929, 0.961 respectively).

The optimal cut-off value for RDW/MCH ratio in men was 0.44 and the sensitivity was 83%, the specificity was 85% and the PPV was 94%. Besides, at the cut-off value of 0.48 for RDW/MCH ratio, a sensitivity of 91%, a specificity of 93%, and a PPV of 98% were determined in females (Figure 1, Table 4).



Female Male

Figure 1. ROC curve analyses of RDW/MCH ratio in both genders with Hemoglobinopathy

Table 4. Comparison of diagnostic accuracy with optimal cut-off values (95% Confidence Interval)

	Sex	Indices	AUC	Cut Off	Sens	Spes	LR^+	LR^-	PPV	NPV	DA
HBP	Men	RDW	0.860	13.85	0.76	0.77	3.29	0.31	0.91	0.51	0.76
		MCH	0.918	30.13	0.78	0.77	3.38	0.29	0.93	0.49	0.78
		RDW/MCH	0.922	0.44	0.83	0.85	5.42	0.20	0.94	0.63	0.84
	Women	RDW	0.958	14.05	0.91	0.89	8.48	0.1	0.97	0.71	0.91
		MCH	0.929	29.17	0.88	0.86	6.15	0.14	0.97	0.6	0.87
		RDW/MCH	0.961	0.48	0.91	0.93	12.7	0.10	0.98	0.72	0.91
β-thalassemia trait	Man	RDW	0.855	13.75	0.78	0.77	3.37	0.29	0.88	0.63	0.78
		MCH	0.921	30.08	0.87	0.86	6.08	0.15	0.97	0.59	0.87
		RDW/MCH	0.928	0.45	0.78	0.85	5.06	0.26	0.91	0.65	0.80
	Women	RDW	0.946	13.94	0.91	0.89	8.48	0.10	0.97	0.71	0.91
		MCH	0.924	29.11	0.87	0.86	6.09	0.15	0.97	0.59	0.87
		RDW/MCH	0.961	0.48	0.91	0.93	12.7	0.10	0.98	0.72	0.91

Sens: sensitivity. Spes: specificity. LR: Likelihood ratio. PPV: Positive predictive value. NPV: Negative predictive value. DA: Diagnostic accuracy. RDW: Red cell distribution width. MCH: Mean corpuscular hemoglobin

When their roles in the detection of β-TT were examined, the AUC values of RDW, MCH, and RDW/MCH ratio parameters were considered significant in diagnostic efficiency with the values of 0.855, 0.921, and 0.928 for males, and 0.946, 0.924, and 0.961 for females, respectively. Regarding RDW/MCH ratio, at the cut-off value of 0.45, a sensitivity of 78%, specificity of 85%, and a PPV of 91% were determined in males. Besides, at the cut-off value of 0.48, a sensitivity of 91%, a specificity of 93%, and a PPV of 98% were determined in females (Table 4).

DISCUSSION

In our study, the prevalence of HMP in whole groups was 3.41% and HMP was detected in 2.43% of PMS in those years (Table 1). To our knowledge, our results are the first concerning revealing the data on HMP prevalence in our city with a population of approximately one million. The overall prevalence of HMP was 2.1%, and this rate reached up to 4.3% in the coastal areas of our country(7). Hence, the results in our province were similar to the HMP average of Turkey. It was noteworthy that the number of HMPs detected in PMS was higher than applications for other causes. This was a good example of how effectively the screening program could be used.

Yearly PMS results of 2019 in our province were analyzed, the prevalence of β-TT was 1.08% and it was 5.98% in people who applied for other reasons. When all applications were considered, the prevalence of β-TT was 1.98% (Table 2). The prevalence of β-thalassemia was reported to be 1.4% (125/8904) and the prevalence of sickle cell anemia was 0.06% (5/8904) in the PMS in our neighboring city, Canakkale(13). Moreover, in a study performed in Kocaeli province, also in the Marmara region, the prevalence of β-TT was 0.89% and sickle cell trait was 0.05%(14). Compared to

some screening programs performed in Turkey, there were significant regional differences. In our country, the highest prevalence of β-TT and HbS were in the Mediterranean region (respectively, 13.1% 10.0%)(15). When we examined the examples across the world, the prevalence of β-TT in Southern Italy, Iran, South Russia, India, and Southeast Asia ranged from 10% to 15%. The rate of β-TT in Greece was 5-15.0%, while it was 4-10.0% in Iran and 3.22% in Saudi Arabia(16). β-TT in our province had a lower prevalence compared to many countries close to average in Turkey.

In our study, analyzed the data of 2019 in PMS, the second-highest HMP was HbF with a rate of 0.49%, and this rate was 0.86% at admission for other reasons. Furthermore, other variants were detected also as seen in Table 2. In the PMS group, HbD was 0.46%, and HbS was 0.09%, HbE was 0.04% and HbD carrier was 0.04% in the Mediterranean region(17). When compared together, HMP rates have lower percentages in our province. Besides that, the HMP rates of our city are below the rates across the world(18). According to our findings, more detection of hemoglobin variants (such as HbC, HbD, and HbJ), less common in PMS compared to those presenting for other reasons, also support the success of the program.

Differentiation of abnormal HMPs from the healthy population has crucial clinical implications. Electrophoresis or chromatographic techniques should be employed for identification. Since the use of these methods in the field is limited, screening the whole society with these methods is challenging and expensive. Hence, it was suggested to use a series of algorithms that combine routine RBC parameters and indices to determine the people to be screened or avoid unnecessary tests on healthy people(19). The clue for thalassemia was low MCV or low MCH. The same results suggest iron deficiency anemia, which was likely to indicate thalassemia in ethnic populated regions of countries at risk(20). In the literature, specifically, MCH, MCV, and RDW parameters were used in the differentiation of HMPs(21–24). In our study, MCV, MCH, and RDW were significantly different in the groups with HMP in both genders than the control group. These results were consistent with the findings obtained in the previous studies(20,25–27). Particularly, RDWI, G&K, and SL-I derived indices from RBC parameters, were significantly different in both genders in the group with HMP, while the E-F index was significantly different in males and the Mentzer index in females than the control group (Table 3). Thus, these parameters could be used in determining subjects with HMP.

In our study, RDW/MCH ratio was diagnostically efficient for both genders in ROC analysis to determine the precision in detecting HMP (Figure 1). Upon literature review, similar studies have shown that the RDWI is an efficient parameter in differentiating beta-thalassemia patients from patients with iron deficiency anemia(11,28). Velasco-Rodrígue et al. (2017) revealed that RDW could differentiate Hb E- β -thal from β -thal (22) and suggested that it would be the most likely diagnosis at RDW ≥ 20% and/or MCH <19 pg in the differentiation of α-thalassemia and HbH disease. When evaluated together with our results, RDW/MCH ratio could play a role in HMP screening.

Likewise, the diagnostic efficiency of RDW/MCH ratio was significant in both genders in determining β-TT (Table 4). Contrary to our findings, Sudman AA et al. (2012)(25) examined the efficiency of the MCH and MCV parameters in the ROC analysis in differentiating those with β-TT from those with non-HMP and found the sensitivities to be 100% and the specificities between 64.0% and 71.0%. Compared to our study, more efficient and effective process management could be achieved than MCV and MCH if individuals above the cut-off values of RDW/MCH ratio in both genders were screened. Based on this, it was a remarkable finding that the number of subjects requiring further examinations for HMP diagnosis in the community would decrease considerably. Indeed, supporting our findings, Chatterjee et al. (2015)(29) revealed that MCH is superior to MCV for thalassemia screening (as it varies greatly in different HMP conditions). Charoenkwan P. et al.(30) found MCV below 95 fl with 100% sensitivity and 92.4% specificity in the demonstration of HMP presence in α-thalassemia screening.

One may solely perform CBC tests at the first step of a thalassemia screening program and effectively use the proposed indices. As already known, HPLC is 10-15 times more expensive than CBC. Variant analysis could be performed with HPLC test at the 2nd step, not only in PMS, but in all patients for the first step, by distinguishing the patients with the RDW/MCH ratio with an accuracy of 91% in women and 84% in men. With this algorithm, a cheaper and easier diagnosis could be made.

Conclusions: In the PMS data of Tekirdağ province, HMP prevalence was 2.43% and the prevalence of β-TT was 1.08%. The number of HMP s and rare variants (such as HbC, HbD) detected in people presenting with PMS were higher than those applicating with other causes. The use of MCH in men and RDW in women may confuse the diagnosis. Instead, RDW/MCH ratio would be more useful and simpler in decision making. RDW/MCH ratio was diagnostically efficient for both genders to determine the precision of detecting HMP and β-TT. It could be applied by calculating with automation like NLR as those days of COVİD19 pandemics. Using a single ratio and cut-off provides a more efficient application.

As a result, RDW/MCH ratio would both reduce unnecessary test requests and facilitate the selection of individuals who require further examination for a definitive diagnosis in community screenings with limited healthcare equipment and resources. It could be the basis for earlier, inexpensive and effective thalassemia screening in all patients, not just PMS.

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