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COMPARATIVE ANALYSIS ON SEVEN BLOOD BIOMARKERS TO DIAGNOSE COLORECTAL CANCER
KOLOREKTAL KANSERI TEŞHİS İÇİN YEDİ KAN BİYOBELİRTEÇLERİ ÜZERİNDE KARŞILAŞTIRMALI ANALİZ

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ABSTRACT

Cancer has become one of the most important causes of mortality that human beings have faced in this century. Because the digestive system is a region where nutrients are involved and processed in the human body, colorectal cancer (CRC) has been increasing in recent years due to irregular and bad nutrition, stress, immobility and increased environmental pollution. Early detection has become one of the most important ways to stay alive in cancer. In recent years, artificial intelligence studies have begun to be used in the diagnosis and treatment of cancer. In this study, there is a search for early and practical diagnosis by analyzing some blood data acquisition related to colon cancer from different literatures together. Seven different biomarkers and blood-related gene data acquisition were used in the literature and WBC, CRP and CEA type may be used as biomarkers to diagnosis and follow-up for colorectal cancer.

Keywords: Biomarker, blood, cancer, colorectal cancer, diagnosis, gene.

ÖZ

Kanser, insanoğlunun bu yüzyılda karşılaştığı en önemli ölüm nedenlerinden biri haline gelmiştir. Sindirim sistemi, besinlerin insan vücudunda yer aldığı ve işlendiği bir bölge olduğundan, düzensiz ve kötü beslenme, stres, hareketsizlik ve artan çevre kirliliği nedeniyle son yıllarda kolon kanseri artmaktadır. Erken teşhis, kanserde hayatta kalmanın en önemli yollarından biri haline gelmiştir. Son yıllarda, kanser teşhisinde ve tedavisinde yapay zekâ çalışmaları kullanılmaya başlanmıştır. Bu çalışmada, farklı literatürlerden kolon kanseri ile ilgili bazı kan verilerini analiz ederek erken ve pratik tanı için bir çalışma yapılmıştır. Literatürlerden yedi farklı biyobelirteç ve kanla ilgili veri toplama kullanılmış ve WBC, CRP ve CEA adlı biyobelirteçlerin kolon kanseri tespiti ve takibi için kullanılabileceği çıkarımı yapılmıştır.

Anahtar kelimeler: Biyobelirteç, gen, kan, kanser, kolorektal kanser, teşhis.

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INTRODUCTION

During cell growth and division, accumulation of various genetic and epigenetic alterations leads to transformation of a normal cell into a cancer cell. Cells evolve from apoptosis by malign transformation to grow independently and accelerate cell cycle (1) and it is an uncontrolled growth of body cells, which can spread by circulation and affect other parts of the body. Carcinogenesis is a multi-step events chain including transformation, survival, proliferation, invasion, angiogenesis, and tumor metastasis (2). Results from GLOBOCAN (3), Ferlay et al. (3) showed that approximately 14.1 million new cancer cases diagnosed worldwide in 2012 and 8.2 million estimated deaths from cancer. Colorectal cancer (CRC) is the third most common cancer in men and is seen in the second most common women and accounting for about 1.4 million new cases and almost 700 000 deaths in 2012 (3, 4). The most important condition of cancer survival is that the disease is uncovered at an early stage before it spreads. If the cancer is diagnosed early, the chances of treatment and survival increase.

Digital information generated in many areas of life is stored on computers. With the increasing spread of information technology day by day, the resulting data have reached very different and big dimensions. Much data has been gathered to produce meaningful results and to use them when necessary, and analysis of these data has become a need (5). Due to such a need, data mining has begun to develop. Various techniques are used in the health sector with the development of methodologies for collecting data from databases (6). Medical data related to patients are usually stored in unstructured databases where the results obtained from examinations and medical findings are written by a doctor in a text format and have not yet been analyzed in detail (7).

Colorectal cancer is normally not detected in the blood. Only with blood tests doubt about colon cancer may arise. Some types of cancers make themselves visible with changing biomarkers in the blood. These biomarkers were named as carbohydrate antigen (CA 19-9), C-reactive protein (CRP) , Carcinoembryonic antigen CEA (CEA) , White blood cell (WBC) and mean cell volume (MCV). In addition, there are some gene changes in cancer such as Adenomatous polyposis coli (APC) and MLH1 gene for diagnosis and follow-up. Seven biomarkers (contains some traces and gene) of blood-related data were used from the literature. In this study, an attempt was made to search for an answer to the question whether we can detect colorectal cancer by using different blood data in the literature included in the healthy reference range of the colorectal cancer patient and non-reference values.

MATERIAL and METHODS

Blood values related to the patient with colon cancer used in this study were obtained from various literature. Colorectal cancer cases have been more common in recent years. In order to diagnose this type of cancer early, and practically from the blood, seven biomarkers containing important links have been identified in the literature. For this study, 34 articles from 2000 to 2020 were scanned and the results of 6384 patients were examined. This study includes blood data such as gene (APC, MLH1; mutation numbers in related genes were analyzed) and some biomarkers (CA 19-9, CRP, CEA, WBC, MCV) of 6384 colorectal cancer patients. Blood values, an important parameter in the diagnosis and follow-up of diseases, were examined. The values in range and out range on the blood tests of the patients and whether they contain mutations in the two genes were analyzed.

These data acquisition was then extracted, analyzed, and tabulated using Excel software on the PC. Origin Pro2015 and Matlab R2017a software were used for the analysis and correlation of the data acquisition from the patients.

RESULTS

These data were collected from the literature on values included in the healthy reference range of the colon cancer patient and non-reference values. These numbers on seven types biomarkers (gene and some metabolic traces) obtained from colon cancer patients' blood are given in the table below. The biomarkers and genes used in this study are: MLH1 Mut. (mutation) (8-10), APC Mut. (11-14), CRP (15-18), CEA (18-23), CA 19-9 (18, 22, 24), WBC (25), MCV (26-29), and mutation numbers in related genes were analyzed.

In our study, 6384 patient data obtained from 22 articles were used. The number of patients with related age ranges, gender and tumor grades are given in table I. It is seen that the patients in the analysis are generally above middle age. Number of females: 2387, number of males: 3677, number of patients in tumor degrees, (I, II, III and IV) respectively: 469, 1116, 1223, 606. The age, gender and tumor grade information of some patients could not be found from the related literature and this information is indicated under the Table I. The data of biomarkers and genes obtained from the blood of these colon cancer patients are given in other tables.

Figure I, Figure II (a, b,c, d, e) and Table II demonstrates the five biomarkers (CRP, CEA, CA 19-9, WBC, MVC) and their healthy reference values, percent of range in, out of reference values, percent of range out, total CRC patient which were obtained by the blood of the patients and the number of patients that these values were belonged to.

Figure I, Figure II (f, g) and Table III demonstrates two genes (MLH 1 mut., and APC mut.) and their healthy reference values, percent of range in, out of reference values, percent of range out, total CRC patient which were obtained by the blood of the patients and the number of patients that these values were belonged to.

DISCUSSION

Mortality and morbidity in colorectal cancer increase, partly due to early detection of the disease. Non-invasive screening of colorectal cancer can be performed using blood-based biomarkers that will allow early detection of the disease (30). Existing biomarker-based tests used practically for colorectal cancer scanning is strictly limited because most of the laboratory work do not transform into highly sensitive and specific early diagnostic tests (31). Toma and his colleagues stated that while colonoscopy remains an important standard in the diagnosis and treatment of colorectal cancer, other non-invasive options may be required that

Table I. Descriptive statistic of related research (the number of patients, age, gender, and tumor grade)

Related Research Number	**Age (number of patients)**		**Gender (number of patients)**		**Tumor grade (number of patients)**				**N**
	Age range	**N**	**female (n)**	**male (n)**	**1 (n)**	**2(n)**	**3 (n)**	**4 (n)**	**(Total patients)**
8*	24-80	51.7	30	36	-	-	-	-	66
9*	-	-	-	-	16	86	108	39	249
10*	20-90	62.9	61	69	-	-	-	-	130
	<40	15							
	41-60	40							
	61-80	58							
	>81	17							
11*	23-86	53	44	68	-	26**	45	41	112
	<60	73							
	≥60	39							
12*	median	57	-	-	-	35	8	-	43
	<50	13							
	≥50	30							
13*	median	60.5	18	-	28	-	-	-	46
	36-50	9							
	51-70	28							
	71-84	9							
14*	-	-	50	53	19***	36	31	11	103
15*	31-97	73	232	293	77	183	145	120	525
	<65	152							
	66-79	211							
	>80	162							
16*	median	59.0	364	332	-	-	-	-	696
	<59.5	343							
	≥59.5	353							
17*	<60	98	-	288	-	-	-	-	288
	60-69	114							
	>70	76							
18*	≤70	192	113	222	-	133	112	90	335
	≥70	143							
19*	-	-	66	59	-	-	-	-	125
20*	median	64.2	18	28	3**		-	43	46
21*	<40	56	143	270	8	88	229	88	413
	40-60	147							
	>60	210							
22*	<60	106	122	157	51	117	96	15	279
	≥60	173							
23*	Median	66.6	523	548	-	-	-	-	1071
	51.7-80.3	609							
	53.7-81.1	462							
24*	31-105	63.15	67	49	13	48	44	11	116
25* group 1	<65	161	110	165	60	85	92	38	275
	≥65	114							
25* group 2	<65	120	79	119	54	60	63	21	198
	≥65	78							
25* group 3	<65	69	53	74	26	33	53	15	127
	≥65	58							
26*	48.1-62.2	55.1	-	497	-	-	-	-	497
27*	40-77	64	21	42	9	25	20	9	63
28*	median	68.1	166	190	81	113	115	46	356
	<65	130							
	≥65	226							
29*	28-92	62	107	118	27	74	62	62	225

Related researched was shown by numbers on the table. Respectively, the number of patients in the related age range, the number of patients of the related gender and the number of patients in the related tumor grade. Numbering of the related research was done by using the articles which were indicated the reference section in detailed.
(-) demonstrates that related data was not shown.
** The number of patients with tumor grade is combined.
*** n.a. 6 patients.

Table II. Calculations and values from colorectal cancer patient blood.

References	**(15)**	**(16)**	**(17)**	**(18)**
biomarker	**CRP**	**CRP**	**CRP**	**CRP**
healthy reference values	99	209	94	255
percent of range in	19	30	32.6	76.1
out of reference values	426	487	194	80
percent of range out	81.2	70	67.4	23.9
Total CRC patients	525	696	288	335

References	**(18)**	**(19)**	**(20)**	**(21)**	**(22)**	**(23)**
biomarker	**CEA**	**CEA**	**CEA**	**CEA**	**CEA**	**CEA**
healthy reference values	158	0	18	260	149	462
percent of range in	47	0	39.1	63	53.4	43
out of reference values	173	125	28	153	130	609
percent of range out	53	100	60.9	37	46.6	57
Total CRC patients	335	125	46	413	279	1071

References	**(18)**	**(22)**	**(24)**
Biomarker	**CA 19-9**	**CA 19-9**	**CA 19-9**
healthy reference values	230	238	13
percent of range in	68.7	85.3	11.3
out of reference values	97	41	103
percent of range out	29	14.7	88.7
Total CRC patients	335	279	116

References	**(25)**	**(25)**	**(25)**
biomarker	**WBC**	**WBC**	**WBC**
healthy reference values	20	27	37
percent of range in	7.3	13.6	29.1
out of reference values	255	171	90
percent of range out	92.7	86.4	70.9
Total CRC patients	275	198	127

References	**(26)**	**(27)**	**(28)**	**(29)**
biomarker	**MCV**	**MCV**	**MCV**	**MCV**
healthy reference values	180	25	306	70
percent of range in	51,9	39,7	86	50
out of reference values	167	37	50	70
percent of range out	48,9	60,3	14	50
Total CRC patients	347	63	356	140

Table III. Calculations and values from colorectal cancer patient blood (Gene).

References	**(8)**	**(9)**	**(10)**
gene	**MLH 1 mut**	**MLH 1 mut**	**MLH 1 mut**
healthy reference values	58	234	95
percent of range in	88	94	73
out of reference values	8	15	35
percent of range out	12	6	27
Total CRC patients	66	249	130

References	**(11)**	**(12)**	**(13)**	**(14)**
gene	**APC mut**	**APC mut**	**APC mut**	**APC mut**
healthy reference values	79	25	20	32
percent of range in	70.5	58	43	31
out of reference values	33	18	26	69
percent of range out	29.5	42	57	71
Total CRC patients	112	43	46	103

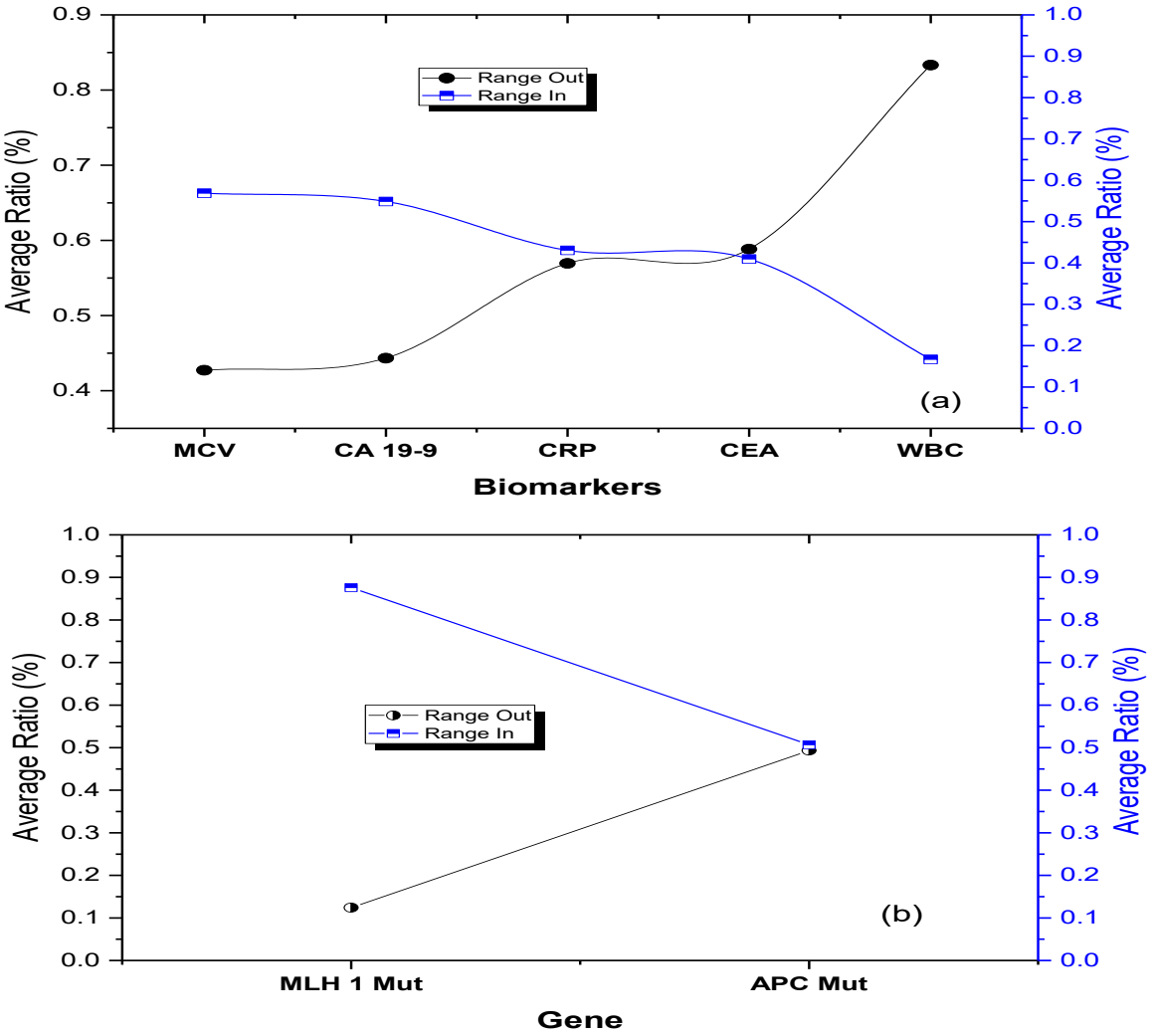

Figure I. Association of changes in patients with colorectal cancer, with reference in and reference out; a) biomarkers, b) gene.

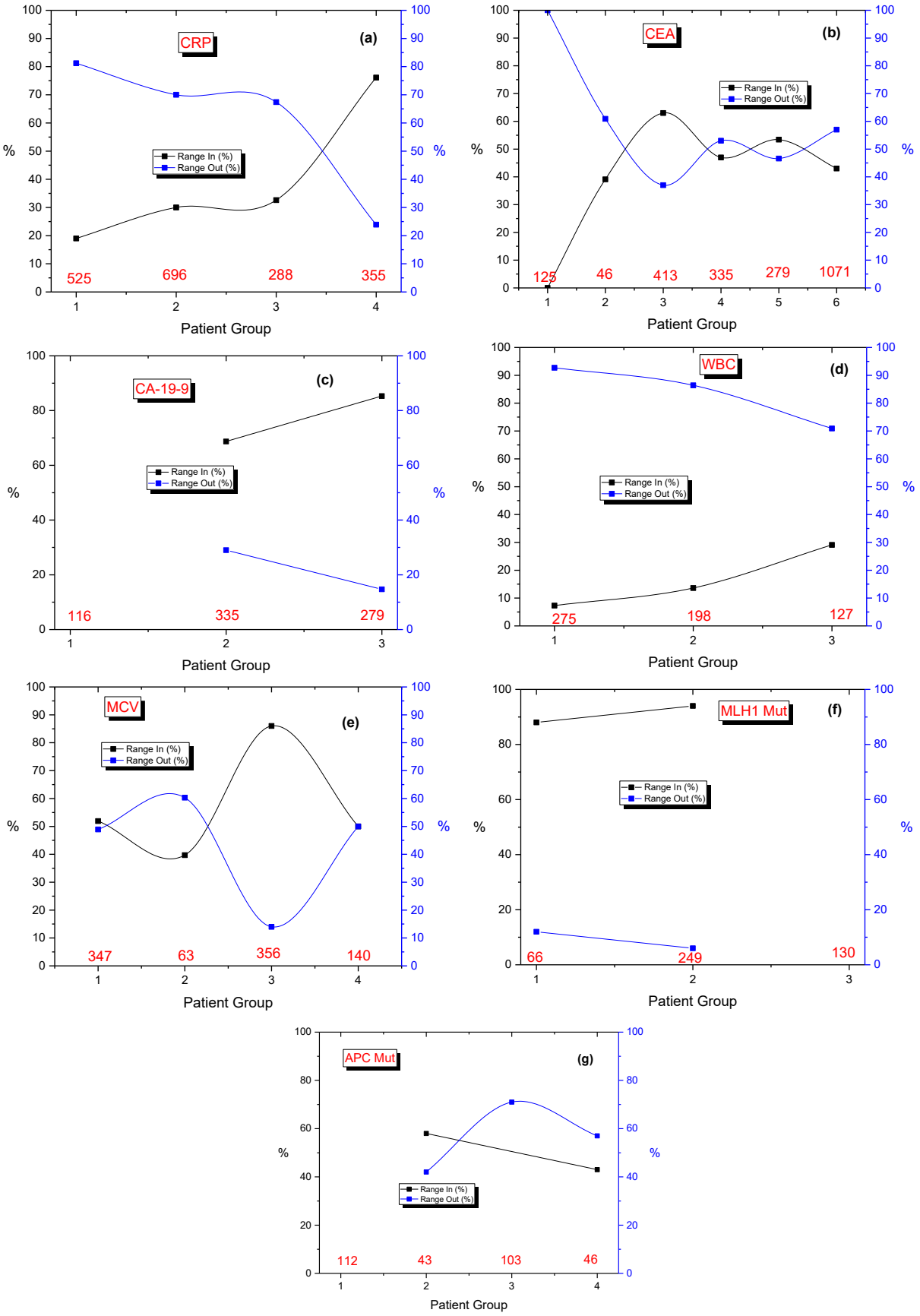

Figure II. Association of gene/ biomarkers changes in patients group with colorectal cancer, percentage with reference in and reference out: a) CRP, b) CEA, c) CA 19-9, d) WBC, e) MCV, f) MLH 1 Mut, g) APC Mut.

may suggest new methods to early diagnosis (32). Multiple blood tests have become an important point in becoming a faster and more practical blood test alternative to the fecal occult blood test for early diagnosis of this disease. Werner and his colleagues' study stated that CEA and anti-p53 can contribute to the development of a multi-marker blood-based test for early detection of colorectal cancer (33).

Normally, colorectal cancer was not detected in the blood. Only with blood tests doubts about colon cancer may arise. In this study, an attempt was made to search for an answer to the question whether we can detect colorectal cancer by using different blood data in the literature. For the diagnosis of colorectal cancer and guidance in follow-up; it is aimed to search for serious clues by going out from some kind of data. However, it is a fact that there is no certainty in this quest. Perhaps together with several different markers, could be a clue to this diagnosis and follow-up guidance. Due to the reason that these indicators can change in other diseases and physiological events.

Elevated inflammatory biomarkers and gene mutations are associated with increased CRC risk, particularly colorectal cancer. However, over time, changes in biomarkers and gene mutations do not suggest that they deserve to be considered early detection markers for CRC. This study supports a role for inflammation in CRC, but also demonstrates that these markers are not useful for early detection of CRC (34). Gao his colleagues reported that combined serum markers can not only be used to diagnose colorectal cancer but also can be used to guide treatment and assess the tumor status for patients' prognosis (22).

Seven different biomarkers and gene mutations ratios of 6384 colorectal cancer patients obtained from the literature were examined (see Table II and Table III) and the correlation between the numbers of people inside and outside the healthy reference range has been tried to be analyzed by various computer programs (Excel, Origin Pro 2015, Matlab R2017a).

In this study with various gene and biomarkers data in the literature; MLH1 Mut, MCV, CA 19-9, APC Mut, CRP, CEA, and WBC were evaluated for the values of 6384 CRC patients. In this regard, it has been discussed which type of gene and biomarkers is more prominent in the detection of the cancer concerned, using data obtained from the blood of CRC patients.

In a study of Paik and colleagues on colorectal cancer, it was reported that the number of WBCs increased with increasing lesions (25). In three separate studies with a total of 600 patients; with the WBC out of the healthy reference range being 83.31% associated with CRC (see Figure I). If the WBC score is outside of the healthy reference interval, it can be considered that the patient has cancer with probability of 83.31% (see Figure I). In this case, the other gene and biomarkers strains are sorted (see Figure I) in terms of the relative proportions; 2144 patients had CEA (58%) and 3783 patients had CRP (56%) (see Figure Ia). According to the results, the three of the biomarkers which were WBC, CRP and CEA were more effective in early detection of colorectal cancer (CRC) compared to the other two biomarkers; CA 19-9 and MCV (see Figure I). On the other hand, there were no significant differences between the patient in healthy reference range and out of the range in terms of MLH1 mut. and APC mut. (See Figure Ib).

There are several studies on the interaction of the CEA tumor marker with colorectal cancer. CEA is a tumor marker that is overexpressed, especially in colorectal cancer, and there are many conflicting findings about the sensitivity and usefulness of this tumor marker (19, 20**)**. The percentages of the individuals included in the reference range of 7 different gene and biomarkers were compared (see Figure II). Based on the percentages of patients in the healthy reference range, f (MLH1 mut), a (CRP), b (CEA) gene/biomarker graphs are similar (see Figure II). According to the results, the most efficient ones were CRP, CEA, and WBC (see Figure II).

Conclusion

It may be said that by going out of these results; WBC, CRP and CEA together can be used as biomarkers for colorectal cancer detection and follow-up for colorectal cancer.

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