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Original scientific article

ASSOCIATION OF MICRO RNA EXPRESSIONS WITH PEDIATRIC CELIAC CLINICAL FINDINGS

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There is a need to determine the relationship between the function of the immune system and *miRNA* expression in pediatric celiac disease (pCD). We aimed to describe the expression profiles of *miRNAs* in Turkish pCD patients based on the clinical and pathological findings. This study was conducted on 33 pCD patients and 33 pediatric control subjects with normal biopsy results. Four most common mutations (*DQA1*05,*

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*DQB1*02, DQA1*03, DQB1*03:0.2*) on HLA gene in pCD were screened. Paraffin-embedded biopsy tissue samples were used in *miRNA* isolations followed by cDNA synthesis. Expression of *miRNAs* were evaluated in the groups with qRT-PCR array-method. Significant underexpression of *hsa-miR-194-5p* gene was detected in pCD patients compared to the control group. The *hsa-miR-194-5p* gene was significantly underexpressed in anemic or short stature pCD patients compared to the control. The genes of *hsa-miR-29b-3p, hsa-miR-30e-5p,* and *hsa-miR-146a-5p* were significantly overexpressed in the patients with constipated celiac patients. Significant overexpression of *hsa-miR146a-5p* gene was detected in the Marsh2 and Marsh3a groups. The *hsa-miR-29b-3p, hsa-miR-30e-5p, hsa-let-7a-5p, hsa-miR-27a-3p, hsa-miR141-3p, hsa-miR143-3p,* and *hsa-miR-146a-5p miRNA* genes were significantly overexpressed in the Marsh3b group. Also, the *hsa-miR-194-5p* and *hsa-miR-26a-5p* genes were significantly underexpressed in the comparison of Marsh3c group to the control. These results suggest that m*iRNA* expressions are likely to play a role in the pathogenesis of pCD. It is believed that the current results present valuable inferences that may help understand the genetic boundaries on pCD, which might be further supported by follow up studies on other *miRNAs*.

Keywords: Autoimmunity; chronic enteropathy; epigenetics; *miRNA*; pediatric celiac disease

INTRODUCTION

Celiac disease (CD) is an immuno-dependent systemic disorder in genetically susceptible individuals caused by gluten and related prolamins found in foods such as wheat, barley, rye, and oat. Gluten-dependent clinical manifestations are characterized by the presence of CD-specific antibodies, *HLA-DQ2* or *HLA-DQ8* haplotypes, and enteropathy (HUSBY *et al*., 2012; BASCUÑÁN-GAMBOA *et al*., 2014).

Although CD development is defined as multigenic, the main responsible structures are *HLA-DQ2* and *HLA-DQ8*. *HLA DQ2* or *DQ8* genotype is dominant in type 1 diabetes patients with positive serological tests for CD (SMYTH *et al*., 2008). Again, in CD with Down syndrome, mainly *HLA-DQ2* heterodimer with a carrier rate of approximately 100% was detected. CD is very common in patients with type 1 diabetes, and 50 times more common in people with Down syndrome than in normal individuals. The frequency of CD is observed to be increased in children with Turner or Williams syndrome, selective IgA deficiency or thyroiditis. *HLA-DQ2* homozygosity also increases the risk of T-cell lymphoma with enteropathy (AL-TOMA *et al.*, 2006; ALANAY *et al.*, 2005).

miRNAs play role in the regulation of gene expression, cellular development and differentiation, and physiological function, as well as in the development of tumors, viral infections, and autoimmune diseases (SCHICKEL *et al*., 2008; IBORRA *et al.*, 2012; SONKOLY *et al.*, 2009; DAI *et al*., 2011). In addition, *miRNA* molecules have gradually begun to be accepted as diagnostic and prognostic markers for evaluation of certain diseases. Understanding the molecules involved in the pathogenesis of diseases locally or systemically is important to determine the regulation of *miRNA* in immune cells. It is important to detect the relationship between the immune system function of CD and *miRNA* expressions. While the literature on

miRNA research is rapidly increasing, little is known about the *miRNAs* and pediatric celiac disease (pCD). In the studies, the expression profiles of *miRNAs* in intestinal mucosa were evaluated. Previous studies have shown that *miRNAs* are irregular in intestinal biopsies of CD patients. All mRNA targets of irregular *miRNAs* reported in the literature (i.e., *miR-31-5p, miR-192, miR-194, miR-449a,* and *miR-638*) are bound to several important biological pathways such as Wnt, cell proliferation, differentiation, and adherence junction (CAPUANO *et al*., 2011; VAIRA *et al*., 2014; MAGNI *et al.*, 2014; BUOLI *et al.*, 2015; COMINCINI *et al.*, 2017). There is a need to determine the relationship between the function of the immune system and *miRNA* expression in pCD. In this respect, this study was planned to understand the role of *miRNA* expression in pCD pathogenesis and association with celiac clinical findings.

MATERIALS AND METHODS

Ethics and Subjects

Ethical approval for this retrospective study was obtained from the Institutional Ethical Committee (Approval #: 2016/20478486-147). Study patients were composed of 33 pCD patients and 33 pediatric matched control groups. The Europen Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) criteria was used for the diagnosis of CD (HUSBY *et al*., 2012). The age, gender, clinical findings, hematological, and biochemical parameters at diagnosis along with symptoms and signs were recorded. Patients were diagnosed with pCD in the Department of Pediatric Gastroenterology and the control subjects were composed of the children who had gone through upper gastrointestinal endoscopy for non-CD reasons and found to be normal in their endoscopic evaluations and pathological examinations. The biopsy specimens stored in paraffin blocks that had primarily been collected for pathological examinations were used in the study. Patients with chronic illnesses such as diabetes mellitus, autoimmune thyroiditis, other autoimmune diseases, and genetic disorders were excluded from the study. Informed consent from all subjects was obtained prior to any study related procedure. The study was conducted in line with the principles of the Helsinki Declaration.

DNA Isolation

From peripheral blood samples were isolated genomic DNA according to the manufacturer's instructions (Roche, Mannheim, Germany). DNA concentration and purity were determined by absorbance value at 260 nm (A260) and the ratio of A260/A280, respectively, using a spectrophotometer (NanoDrop, Thermo Scientific, USA).

Detecting HLA DQ genotypes

The primary susceptibility genotype for CD is *HLA-DQ2* consisting of *HLA-DQA1*05* and *DQB1*02*. The remainder of the cases were associated with *HLA-DQ8* consisting of *HLA-DQA1*03* and *DQB1*03:02*. Case DNA samples were genotyped for *HLA-DQA1* and *DQB1* according to the SSP-PCR method and as described by ORENAY-BOYACIOGLU *et al.* (2022).

miRNA extraction

Total RNA including short RNA was isolated from endoscopic biopsy specimens by cutting 10 μm slices from formalin-fixed, paraffin-embedded blocks and processed with *miRNeasy* FFPE Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. For the evaluation of RNA concentration and purity, 1.0 μL of the isolated total RNA from each sample was analyzed by spectrophotometry (Thermo Scientific, USA).

miScript miRNA PCR array

The *miRNA* lysates were reverse transcribed with *miScript II RT Kit* (Qiagen,Hilden, Germany) according to the manufacturer's instructions. Expression profiles of *miRNAs* were revealed using *Human miFinder miRNA PCR Array* (Qiagen, MIHS-001Z, Germany) (Table 1). The 96 well Human miFinder miRNA PCR Array RT² Profiler™ PCR Array reactions were then performed on a Rotor-Gene RG-3000 (Corbett Research, Qiagen, Hilden, Germany). PCR conditions were composed of an initial 10 min denaturation at 95°C followed by 40 cycles of denaturation at 95°C for 15 s and a combined annealing/elongation step at 60°C for 30 s. Expression values were normalized with respect to six endogenous *miRNAs* (*SNORD61, SNORD68, SNORD72, SNORD95, SNORD96A,* and *RNU6B/RNU6-2*) and differential expression was calculated using the $2^{-\text{delta delta CT}}$($2^{-\Delta\Delta CT}$) method.

Table 1. Contents of human miFinder miRNA PCR Array plate

hsa-miR-142-5p	*hsa-miR-9-5p*	*hsa-miR-150-5p*	*hsa-miR-27b-3p*	*hsa-miR-101-3p*	*hsa-let-7d-5p*	*hsa-miR-103a-3p*	*hsa-miR-16-5p*	*hsa-miR-26a-5p*	*hsa-miR-32-5p*	*hsa-miR-26b-5p*	*hsa-let-7g-5p*
hsa-miR-30c-5p	*hsa-miR-96-5p*	*hsa-miR-185-5p*	*hsa-miR-142-3p*	*hsa-miR-24-3p*	*hsa-miR-155-5p*	*hsa-miR-146a-5p*	*hsa-miR-425-5p*	*hsa-miR-181b-5p*	*hsa-miR-302b-3p*	*hsa-miR-30b-5p*	*hsa-miR-21-5p*
hsa-miR-30e-5p	*hsa-miR-200c-3p*	*hsa-miR-15b-5p*	*hsa-miR-223-3p*	*hsa-miR-194-5p*	*hsa-miR-210-3p*	*hsa-miR-15a-5p*	*hsa-miR-181a-5p*	*hsa-miR-125b-5p*	*hsa-miR-99a-5p*	*hsa-miR-28-5p*	*hsa-miR-320a*
hsa-miR-125a-5p	*hsa-miR-29b-3p*	*hsa-miR-29a-3p*	*hsa-miR-141-3p*	*hsa-miR-19a-3p*	*hsa-miR-18a-5p*	*hsa-miR-374a-5p*	*hsa-miR-423-5p*	*hsa-let-7a-5p*	*hsa-miR-124-3p*	*hsa-miR-92a-3p*	*hsa-miR-23a-3p*
hsa-miR-25-3p	*hsa-let-7e-5p*	*hsa-miR-376c-3p*	*hsa-miR-126-3p*	*hsa-miR-144-3p*	*hsa-miR-424-5p*	*hsa-miR-30a-5p*	*hsa-miR-23b-3p*	*hsa-miR-151a-5p*	*hsa-miR-195-5p*	*hsa-miR-143-3p*	*hsa-miR-30d-5p*
hsa-miR-191-5p	*hsa-let-7i-5p*	*hsa-miR-302a-3p*	*hsa-miR-222-3p*	*hsa-let-7b-5p*	*hsa-miR-19b-3p*	*hsa-miR-17-5p*	*hsa-miR-93-5p*	*hsa-miR-186-5p*	*hsa-miR-196b-5p*	*hsa-miR-27a-3p*	*hsa-miR-22-3p*
hsa-miR-130a-3p	*hsa-let-7c-5p*	*hsa-miR-29c-3p*	*hsa-miR-140-3p*	*hsa-miR-128-3p*	*hsa-let-7f-5p*	*hsa-miR-122-5p*	*hsa-miR-20a-5p*	*hsa-miR-106b-5p*	*hsa-miR-7-5p*	*hsa-miR-100-5p*	*hsa-miR-302c-3p*
cel-miR-39-3p	*cel-miR-39-3p*	*SNORD61*	*SNORD68*	*SNORD72*	*SNORD95*	*SNORD96A*	*RNU6-6P*	*miRTC*	*miRTC*	*PPC*	*PPC*

Array data analysis

Array data analysis was performed using the manufacturer-provided online software at http://www.sabiosciences.com/pcrarraydataanalysis.php. According to the data analysis results, overexpressed and underexpressed 10 *miRNA* genes and a housekeeping gene (*SNORD95*) were selected for validation with qRT-PCR.

Validation of miRNA profile by qRT-PCR

Complementary DNA (cDNA) was amplified with *mi*Script SYBR Green PCR Kit using commercially available *hsa-miR-29b-3p*, *hsa-miR-30e-5p*, *hsa-let-7a-5p*, *hsa-miR-103a-3p*, *hsa-miR-27a-3p*, *hsa-miR141-3p*, *hsa-miR143-3p*, *hsa-miR-146a-5p*, *hsa-miR-194-5p*, and *hsa-miR-26a-5p mi*Script Primer Assays (Qiagen, Hilden, Germany). Also, *SNORD95* was amplified as reference *miRNA*. The PCR reactions were prepared by mixing 2.2 µl of cDNA sample with 12.5 µl of SYBR Green Master Mix and 10 pmol of primer per assay. Final volumes were brought to 25 µl by adding dH_2O and the PCR reactions were conducted in Rotor-Gene RG-3000 (Corbett Research, Qiagen, Germany). PCR conditions were composed of an initial 5 min denaturation at 95°C followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 40 s, and elongation at 72°C for 1 min with a final elongation step at 72°C for 2 min. Cycle threshold (Ct) values of the assays were normalized against the reference *SNORD95* using Relative Expression Software Tool (REST V.2.0.13) in standard mode.

Data Analysis

The relative expression of each *miRNA* was calculated by the comparative $2^{-\Delta\Delta CT}$ method and normalized against *SNORD95* as endogenous control. Values higher than 2.0 were defined as increased expression and those less than 0.5 were defined as decreased expression.

RESULTS

Sociodemographics and clinical results

Sociodemographic and clinical characteristics of the pCD is given in Table 2. Of the 33 celiac patients 20 (60.6%) were girls and 13 (39.4%) were boys while in control group 22(66.7%) were girls and 11(33.3%) boys. The mean age was 12.27±4.93 years in pCD groups and 12.63±4.49 years in the control groups. There was no significant difference between two groups regarding sex and age distribution. The most common findings in pCD patients were abdominal pain (87.9%), failure to thrive (63.6%), iron deficiency anemia (63.6%), and *HLA-DQ2* positivity (57.6%). When the clinical findings of the patients were evaluated according to the Marsh classification, the prevalence of iron deficiency anemia in Marsh3c patients was significantly higher compared to other Marsh classes (P=0.010). Although the short stature was mostly seen in Marsh3c patients, it was statistically insignificant (P=0.089).

Table 2. Socio-demographical and clinical comparisons of groups

	pCD Groups (n=33)
Age(years, mean±SD)	12.27±4.93
Gender (Male/Female)(n)(%)	13 (39.4%)/20 (60.6%)
Diarrhea (Yes/No)(n)	7/26
Underweight (Yes/No)((n)	21/12
Abdominal pain (Yes/No)((n)	29/4
Vomiting (Yes/No)((n)	3/30
Short stature(Yes/No)((n)	10/23
Constipation(Yes/No)((n)	9/24
Iron deficiency anemia(Yes/No)((n)(n)	21/12
Marsh 1(n)	4
Marsh 2(n)	3
Marsh 3a(n)	6
Marsh 3b(n)	7
Marsh 3c(n)	13
DTGA (Positive/Negative)(n)(%)	21 (36.4 %)/12 (63.6 %)
EMA(Positive/Negative)(n)(%)	21 (36.4 %)/12 (63.6 %)
HLA DQ2(Positive) (n)(%)	19(57.6%)
HLA DQ8(Positive)(n)(%)	5(15.2%)

miRNA array results

Significant underexpression of 9 *miRNA* genes (*hsa-miR-103a-3p, hsa-miR-29b-3p, hsa-miR-30e-5p, hsa-let-7a-5p, hsa-miR141-3p, hsa-miR143-3p, hsa-miR-146a-5p, hsa-miR-194-5p, hsa-miR-26a-5p,* and *hsa-miR-27a-3p*) were detected in pCD patients compared to the control group.

miRNA primer assay results

No statistically significant difference was detected in *miRNA* gene expressions when the pCD boys and girls were compared to the control group (P>0.05). Against the control group, *hsa-miR-194-5p* gene was significantly underexpressed both in pCD patients (P=0.016) (Table 3) and anemic pCD patients (P=0.008). A statistically significant underexpression was detected in the *hsa-miR-194-5p* gene of pCD patients with short stature when compared to the control group (P=0.008). No statistically significant *miRNA* gene expression was detected between the pCD patients with or without diarrhea and the control group (P>0.05). Comparing against the control group, statistically significant overexpression in *hsa-miR-29b-3p, hsa-miR-30e-5p,* and *hsa-miR-146a-5p* genes were detected in pCD patients with constipation (P=0.036, 0.047, and 0.019, respectively). When the weight gain was controlled for, the pCD patients did not show statistically significant gene expression against the control group (P>0.05).

Table 3. Comparison of pCD patients to the control group

Genes	pCD and control groups		
	Fold Regulation	p-value	FDR
hsa-miR-29b-3p	1.4922	0.163965	0.46
hsa-miR-30e-5p	1.0025	0.265089	0.48
hsa-let-7a-5p	-1.2608	0.207365	0.47
hsa-miR-103a-3p	-1.1294	0.681075	0.47
hsa-miR-27a-3p	1.0734	0.287853	0.48
hsa-miR141-3p	1.0117	0.305235	0.48
hsa-miR143-3p	1.2351	0.295591	0.47
hsa-miR-146a-5p	1.6751	0.086318	0.45
hsa-miR-194-5p	-1.5383*	0.015763*	0.49
hsa-miR-26a-5p	-1.4704	0.934452	0.48
SNORD95	1	0	

FDR: False Detection Rate

Table 4. Comparison of Marsh 1, Marsh 2, Marsh 3a, Marsh 3b, and Marsh 3c pCD patients to the control group.

Genes	Control Group									
	Marsh 1 pCD patients		Marsh 2 pCD patients		Marsh 3a pCD patients		Marsh 3b pCD patients		Marsh 3c pCD patients	
	Fold Regulation	p-value	Fold Regulation	p-value	Fold Regulation	p-value	Fold Regulation	p-value	Fold Regulation	**p-value**
hsa-miR-29b-3p	-1.0684	0.6742	1.3017	0.7333	1.2185	0.7239	4.0357*	0.0033*	1.1475	0.1077
hsa-miR-30e-5p	-1.0831	0.7172	-1.5675	0.4000	-1.3169	0.3958	4.1181*	0.0095*	-1.6231	0.0896
hsa-let-7a-5p	-1.0308	0.9177	-3.9303	0.1413	-2.113	0.1345	3.3374*	0.0032*	-1.7855	0.0826
hsa-miR-103a-3p	1.2338	0.6697	1.7835	0.1660	4.0729	0.2686	-3.0415	0.9212	-1.5162	0.5980
hsa-miR-27a-3p	-1.0613	0.6387	1.0656	0.7996	-1.0355	0.7594	2.7378*	0.0061*	-1.3781	0.3210
hsa-miR141-3p	-1.1584	0.6091	-1.2258	0.5003	-1.0921	0.5710	2.2591*	0.0017*	-1.3781	0.2883
hsa-miR143-3p	1.0262	0.7696	1.1062	0.9915	-1.0194	0.7798	3.5504*	0.0044*	-1.171	0.6523
hsa-miR-146a-5p	1.3175	0.3298	2.0472*	0.0044*	2.0999*	0.0003*	3.7589*	0.0018*	1.0583	0.0958
hsa-miR-194-5p	-2.2848	0.0668	-1.1517	0.3670	1.0118	0.6046	1.0271	0.7358	-2.152*	0.0152*
hsa-miR-26a-5p	-1.1189	0.7081	-1.3601	0.2648	-1.5725	0.0897	1.1729*	0.0365*	-2.0725*	0.0020*
SNORD95	1	0	1	0	1	0	1	0	1	0

***:** Fold regulation values <-2and p<0.05 and Significant p<0.05

When pCD patients diagnosed according to the Marsh classification were compared against the control group, significant overexpression of *hsa-miR-146a-5p, hsa-miR-29b-3p, hsa-miR-30e-5p, hsa-let-7a-5p, hsa-miR-27a-3p, hsa-miR141-3p, hsa-miR143-3p* and significant underexpression *of hsa-miR-26a-5p, hsa-miR-194-5p* were detected in pCD patients (P≤0.05) (Table 4).

No significant correlation was detected between *miRNA* expressions of *HLA-DQ2* positive, *HLA-DQ8* positive, *HLA-DQ2/DQ8* negative pCD, and the control groups.

DISCUSSION

The demographic characteristics and the complaints of 33 pCD patients were evaluated in the current study. Female/male ratio of the study subjects was 1.5, which is correlated with the previous reports (KULOGLU *et al.,* 2009; KHATIB *et al.,* 2016). In literature, *HLA-DQ2* positivity of CD patients was reported to be higher than *HLA-DQ8* positivity. In our study, *HLA-DQ2* positivity was 57.6% and *HLA-DQ8* positivity was 15.2%. Similarly, in other studies conducted in Turkey the frequencies of *HLA-DQ2* and *HLA-DQ8* positivities were 84.7% and 15.3% (KULOGLU *et al.,* 2008) and 52.59% and 14.87% (KURTOGLU *et al.,* 2018). The most common findings in pCD patients in the current study were abdominal pain, failure to thrive, and iron deficiency anemia. Diarrhea, abdominal pain, short stature, and constipation have been reported as frequent complaints since the clinical findings of the disease cover a wide spectrum in different studies (KULOGLU *et al.,* 2009; KHATIB *et al.,* 2016; IWAŃCZAK *et al.,* 2013; FASANO, 2005). When the clinical findings of the patients were evaluated according to the Marsh classification, the prevalence of iron deficiency anemia in Marsh3c patients appeared to be significantly high. Due to the presence of total villous atrophy in these patients, anemia occurs frequently. Although it was mostly seen in Marsh3c patients, the short stature was not significantly correlated with the Marsh3c group. Inability to obtain adequate nutritional support in chronic absorption disorder is also expected to result in short stature and failure to thrive.

While the number of *miRNA* studies is rapidly increasing, small amount of data is available about *miRNAs* and pCD. The researchers have identified the distribution of *miRNAs* for intestinal equilibrium and have established the expression profile of total *miRNAs* present in the intestinal mucosa. In a study, *miRNA* expression was analyzed in a group of untreated adult CD patients, a group of treated patients, and control cases. Researchers have reported deregulation of 7 *miRNAs* (*miR-31-5p, miR-192-3p, miR-194-5p, miR-551a, miR-551b-5p, miR-638,* and *miR-1290*) in CD patients with different clinical phenotypes compared to the control cases. Both, in the group with classic clinical symptoms (diarrhea, abdominal pain) and the group with iron deficiency, sizable downregulation of *miR-31-5p* and *miR-192-3p* and upregulation of *miR-1290, miR-638,* and *miR-551b-5p* was detected compared to the control group. Also, it was found that *miR-194-5p* was downregulated in patients with CD in comparison to the control cases. Interestingly, downregulation of *miR-194-5p* or upregulation of *miR-638* also occurs when CD patients with classic clinical symptoms were compared to the CD patients with anemia. Conversely, overexpression of *miR-1290* and downregulation of *miR-31-5p* and *miR-192-3p* were found to be associated with CD regardless of clinical symptoms (VAIRA *et al*., 2014). In our study, a total of nine *miRNA* genes were investigated by qRT-PCR in pCD and control group. Similar to the findings of VAIRA *et al*. (2014), significant underexpression of *hsa-miR-194-5p*

gene was detected in the comparison of pCD patients with the control group. In addition, *miRNA* expressions of pCD patients with different clinical symptoms were evaluated in our study. Significant results were found in patients with anemia, short stature, and constipation symptoms. In comparison of pCD patients with iron deficiency anemia to the control group, *hsa-miR-194-5p* gene was significantly underexpressed. Also significant underexpression of *hsa-miR-194-5p* gene was detected in the pCD patients with short stature compared to the control group. Significant overexpressions of *hsa-miR-29b-3p, hsa-miR-30e-5p,* and *hsa-miR-146a-5p* genes were detected when the pCD patients with constipation were compared to the control group. CAPUANO *et al.* (2011) studied small intestine biopsies of pCD patients regardless of whether the disease was active, and found that approximately 20% of the expressions of *miRNAs* tested were different when compared to children in the control group. That study showed that there was an inverse relationship between the expression of *miR-449a* in the small intestine of pCD, which is thought to be a characteristic feature, and the NOTCH1 signaling pathway, and production of goblet cells. In a recent study, in which circulating *miRNAs* in pCD patients (treated and untreated) were investigated, underexpression of circulating *mir-31*, overexpression of circulating *mir-21* in patients with untreated pCD, and overexpression of circulating *mir-21* in the patients with tTG-IgA positive pCD patients were determined (AMR *et al*., 2019) The results in the current study are different from the results of AMR *et al*. (2019) and CAPUANO *et al.* (2011) as different *miRNAs* were investigated.

The *miRNA* expression patterns in duodenal biopsies of CD adults with Marsh3c pathology were assessed by microarray analysis, as the expression profile of *miRNAs* may change due to the severity of the intestinal damage. The researchers showed that the expression of *miR-192-5p, miR-194-5p, miR-31-5p, miR-338-3p,* and *miR-197* significantly decreased in biopsies obtained from CD patients and especially those with Marsh3c pathology. Expressions of *miR-338-3p* and *miR-197* were significantly reduced in CD regardless of the severity of mucosal impairment (MAGNI *et al.,* 2014). Similarly, in our study a significant underexpression of *hsa-miR-194-5p* was detected in the comparison of pCD diagnosed with Marsh3c to the control group. The current study adds to the current knowledge by evaluating the data of pCD patients. In a different study comparing pCD patients to the control group, the duodenal biopsies of pCD patients classified as Marsh3a, 3b, and 3c were evaluated. In the study, both *miRNAs* and mRNA target genes were evaluated in pCD patients to assess whether *miRNAs* expressed differently in adults were present in children. Similar to previous investigations, significant downregulation of *miR-31-5p* and *miR-338-3p* was observed. It was found that upregulated *miR-21-5p* and the other *miRNAs* play role in the regulation of immune response in the duodenum of Marsh3c pCD patients but not in adult CD patients. Although the *miR-486-5p* level was found to be upregulated in the biopsies from pCD patients, it was not statistically significant (BUOLI *et al.*, 2015). In the current study, significant overexpression of *hsa-miR-146a-5* was detected in Marsh3a pCD patients. Also, significant overexpressions of *hsa-miR-29b-3p, hsa-miR-30e-5p, hsa-let-7a-5p, hsa-miR-27a-3p, hsa-miR141-3p,* and *hsa-146a-5p* were detected in Marsh3b pCD patients, while significant underexpression of *hsa-miR-194-5p* and *hsa-miR-26a-5p* were present in Marsh3c pCD patients. In our study, the results of Marsh3a, 3b, and 3c were different according to studies in the literature. This may be due to the fact that different *miRNA* genes were screened in our study.

Our current preliminary study suggests that various sets of *miRNAs* can be seen with different clinical findings and Marsh classifications in pCD. In addition, more reliable markers are still needed for pCD diagnosis. In this respect, larger studies are also needed to determine the potential of *miRNAs* to be used as biomarkers.

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ASOCIJACIJA EKSPRESIJE MIKRO RNK SA CELIJAKIČNIM NALAZOM DECE

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Izvod

Postoji potreba da se utvrdi odnos između funkcije imunog sistema i ekspresije miRNA u pedijatrijskoj celijakiji (pCD). Cilj nam je bio da opišemo profile ekspresije miRNA kod turskih pCD pacijenata na osnovu kliničkih i patoloških nalaza. Ova studija je sprovedena na 33 pCD pacijenata i 33 pedijatrijska kontrolna ispitanika sa normalnim rezultatima biopsije. Proverene su četiri najčešće mutacije (DQA1*05, DQB1*02, DQA1*03, DQB1*03:0.2) na HLA genu u pCD. Uzorci biopsijskog tkiva ugrađeni u parafin korišćeni su u izolaciji miRNA nakon čega je usledila sinteza cDNK. Ekspresija miRNA je procenjena u grupama metodom qRT-PCR. Utvrđena je značajna podekspresija *hsa-miR-194-5p* gena kod pCD pacijenata u poređenju sa kontrolnom grupom. Gen *hsa-miR-194-5p* bio je nedovoljno eksprimiran kod pacijenata sa anemijom ili pCD niskog rasta u poređenju sa kontrolom. Geni *hsa-miR-29b-3p, hsa-miR-30e-5p* i *hsa-miR-146a-5p* bili su značajno izraženi kod pacijenata sa celijakijskom konstipacijom. Značajna ekspresija *hsa-miR146a-5p* gena otkrivena je u grupama Marsh2 i Marsh3a. *hsa-miR-29b-3p, hsa-miR-30e-5p, hsa-let-7a-5p, hsa-miR-27a-3p, hsa-miR141-3p, hsa-miR143-3p* i *hsa-miR-146a-5p* miRNA geni su bili veoma izraženi u Marsh3b grupi. Takođe, geni *hsa-miR-194-5p* i *hsa-miR-26a-5p* bili su nedovoljno izraženi u poređenju Marsh3c grupe sa kontrolom. Ovi rezultati sugerišu da ekspresije miRNA verovatno igraju ulogu u patogenezi pCD. Dobijeni rezultati mogu pomoći u razumievanju genetskih granica na pCD, što bi moglo biti potvrđeno naknadnim studijama o drugim miRNA.

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