Micro-RNA-371a-3p in Germ Cell Testicular Tumors on Diagnosis: A Prospective Case-Control Study in Turkish Population

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Purpose: We aimed to evaluate the diagnostic sensitivity and specificity of the miRNA-371a-3p for the primary diagnosis of germ cell tumors (GCT) and to investigate its relationship with pathological factors and clinical stage in the Turkish population.

Materials and Methods: In this prospective study, a total of 60 patients with GCTs, and 40 healthy male controls were examined for serum levels of miRNA-371a-3p before orchiectomy and again two weeks after surgery. The miRNA-371a-3p, alpha-fetoprotein (AFP), and beta-human chorionic gonadotropin (bHCG) levels in the preoperative and postoperative periods were compared at different clinical stages. Receiver operating characteristics curve analyses were performed to determine the sensitivity and specificity of miRNA-371a-3p. Clinical and pathological factors such as clinical stage (CS), tumor size, histology, rete testis invasion, and lymphovascular invasion, potentially impacting miRNA-371a-3p expression levels (relative quantity, RQ), were evaluated statistically.

Results: The sensitivity of miR-371a-3p in GCT patients was 98.3%, and the specificity was 95% (AUC = 0.997 [95%Cl:0.99–1], $p < .001$). miR-371a-3p expression was not detected in two patients with teratoma.
The median miR-371a-3p RQ was 489 times in GCT and 2.2 times in the Control group ($p < .001$). In the postoperative period, there was a significant decrease in AFP and bHCG levels in all CS-1 ($p = .01$) and 30% of the other CS ($p = .3$). Throughout this time there was a decrease of 19 times at the miR-371a-3p RQ in CS-1($p < .001$) and 1.6 times in the other CS ($p < .001$). The miR-371a-3p RQs were correlated with tumor size and CS.

Conclusion: The miR-371a-3p seems to have higher diagnostic accuracy than classical serum tumor markers in GCT.

Keywords: biomarker; germ cell tumor; micro-RNA; non-seminomatous germ cell tumor; seminoma; testicular tumor

INTRODUCTION

Testicular cancer is among the most common malignancies in men aged 20–40 years, with an increasing prevalence[1]. The majority of cases consist of testicular germ cell tumors (GCT) divided into seminomas and non-seminomas[2].
Approximately 85% of seminomas and 70% of non-seminomatous germ cell tumors (NSGCT) are present at clinical stage (CS)-1, confined to the testis and epididymis[3,4].
Alpha-fetoprotein (AFP), beta human chorionic gonadotropin (bHCG), and lactate dehydrogenase (LDH) have been the main serum-based biomarkers used in the diagnosis, clinical staging, and follow-up of GCTs[5,6]. However, the most important limitations of these markers are their low sensitivity and specificity. One of these three markers is expressed in only 50% of all GCT patients[7]. These biomarkers could provide insight into testicle cancer subtypes in 60% of all cases. Yet, elevated levels of these biomarkers could also be seen in cases besides GCT. Combined with imaging techniques in GCT patients, the information obtained about the diagnosis and staging may not be conclusive[8,9]. Moreover, in some advanced cases of GCT, the serum levels may be low, and thus expression level and staging are incoherent[10]. Therefore, serum AFP, bHCG, and LDH levels may not provide definitive and conclusive information about diagnosis and staging in GCTs[9]. Considering the inherent limitations of classic tumor markers such as low sensitivity and specificity, new biomarkers with higher sensitivity and specificity are required in GCT diagnosis.
Circulating plasma and serum microribonucleic acids (miRNA/miR), which play with a role in the epigenetic regulation of the genome, have emerged as such a biomarker in many cancer types throughout the last decades[11,12]. These small non-coding RNAs, regulating the translation and transcription of the mRNA, have been associated with all of the hallmark characteristics of various cancers, such as cancer stem cells, genome instability, and treatment response. In several studies conducted within the last decade, specific

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Table 1. Clinical Data of Patients and Control Group

	GCT Patients (n, %) (Group 1)			Controls (n) (Group 2)	
	CS 1	**CS 2**	**CS 3**		***p***
The number of participants	27 (45)	17 (28)	16 (27)	40	
Median age (years)	31 (21-50)	38 (24-52)	37 (24-56)	36 (27-51)	.16
Seminoma	15 (56%)	12 (70%)	5 (31%)	-	-
Non-seminoma	12 (44%)	5 (30%)	11 (69%)	-	-
Tumor diameter (mm) (IQR)	46 (35-54.5)	50(41-60)	54.5 (40-81.5)	-	.07
pT Stage					
T1	16 (59%)	7 (41%)	3 (19%)	-	-
T2	11 (41%)	8 (47%)	9 (56%)	-	-
T3	-	2 (12%)	4 (25%)	-	-
Rete testis invasion in seminoma	5 (33%)	6 (50%)	2 (40%)	-	-
Lymphovascular invasion in nonseminoma	8 (67%)	4 (80%)	11 (100%)	-	-
IGCCCG prognosis					
Good	-	-	4	-	-
Intermediate	-	-	4	-	-
Poor	-	-	8	-	-
Lymph node metastasis	-	13 (76)	16 (100)	-	-

(**Abbreviations:** CS: Clinical Stage, GCT: testicular germ cell tumor, IGCCCG: International Germ Cell Cancer Collaborative Group, IQR: interquartile range)

miRNA clusters (miRNA-371-3 and miRNA-302/367 clusters) overexpression in GCTs with high sensitivity and specificity have been proposed as a diagnostic indicator[13,14]. High-volume studies have been published supporting especially miRNA-371a-3p as a more accurate biomarker than classical serum tumor markers for diagnosis, clinical staging, and treatment monitoring in GCTs[15,16]. In addition, in the study of Belge et al., it was stated that miRNA-371a-3p was not clearly expressed in non-germ cell gonadal stroma-derived masses and benign testicular lesions[17]. Clinically, the test might be of value in the preoperative characterization, diagnosis, and management of nonpalpable testicular lesions that are not suspicious for cancer or conservative testicular surgery. miRNA-371a-3p is a promising biomarker with the potential to cope with the limitations of classic serum tumor markers. However, issues such as laboratory standardization, availability of testing kits, and prognostic validation need to be resolved for its routine use.

We aimed to evaluate the diagnostic sensitivity and specificity of the miRNA-371a-3p for the primary diagnosis of GCT and to investigate its relationship with pathological factors and clinical stage in the Turkish population.

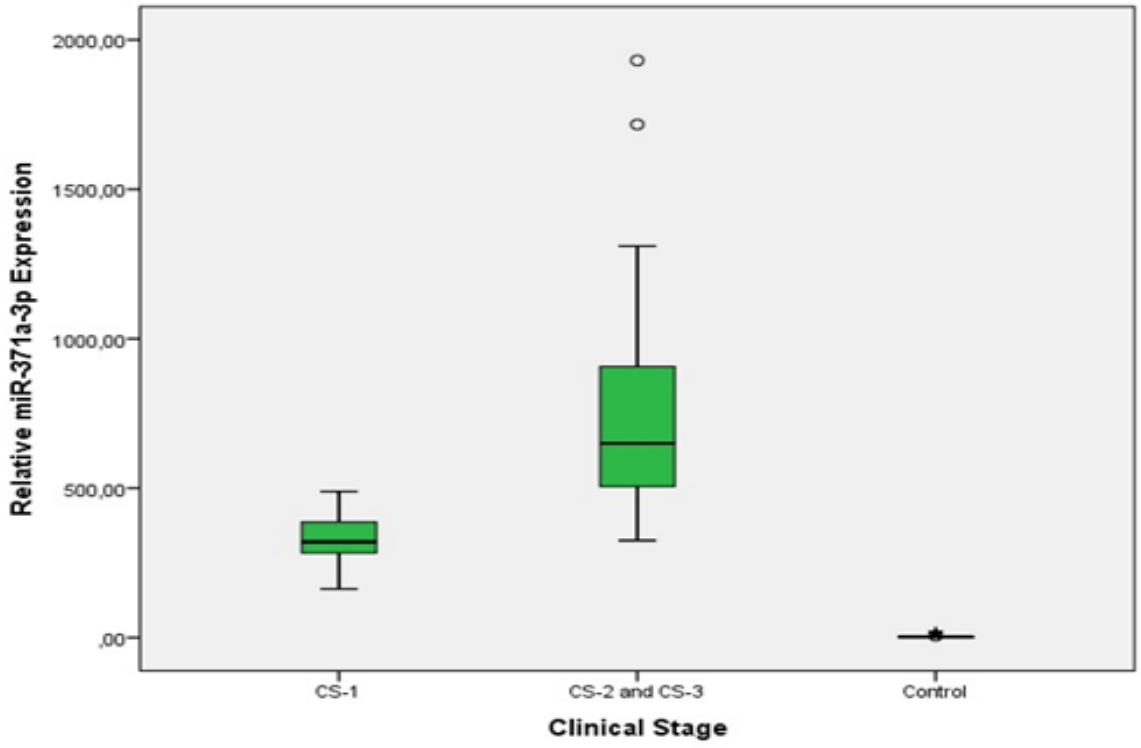

Figure 1. miR-371a-3p levels in CS-1, CS-2 and CS-3, and control groups at the time of diagnosis (CS=Clinical Stage)

Table 2. Preoperative and Postoperative AFP and bHCG Levels in Group 1

	CS-1	CS-2	CS-3
Seminoma			
Pre-operative bHCG (mıu/mL)	0.9 (0-17.6)	6.3 (0.7-274)	1500 (27-34622)
Post-operative bHCG (mıu/mL)	0.3 (0-1.2)	0.3 (0.1-168)	1375 (8.6-22307)
p	.32	.008	.3
Non-Seminoma			
Pre-operative AFP (ng/mL)	12.4 (1.9-57.2)	35 (4-331)	3.9 (1.2-13000)
Post-operative AFP (ng/mL)	2.6 (1.3-4.4)	3.9 (1.5-79.5)	254 (2-20503)
p	.02	.04	.6
Pre-operative bHCG (mıu/mL)	2.7 (0.4-10.9)	29 (2.2-402)	3.8 (1-8327)
Post-operative bHCG (mıu/mL)	0.3 (0-1)	14 (0-60)	1.7 (1-13816)
p	.01	.04	.3

(**Abbreviations:** AFP: alpha-fetoprotein, bHCG: beta-human chorionic gonadotropin, CS: Clinical Stage)

MATERIALS AND METHODS

Patients

In this prospective case-control study, serum samples were collected from 64 consecutive patients between January 2019 and May 2022, before orchiectomy was conducted. The inclusion criteria were the presence of GCT and adulthood. Patients under 18 years of age with a history of any malignancy or chemotherapy and with testicular benign masses (cysts, abscesses, etc.) were excluded from the study. In addition, four patients who underwent orchiectomy were excluded from the study due as the pathological examination revealed non-GCTs. 60 GCT patients were included in the study (Group 1). 40 healthy male blood donors aged over 18 made up the control group (Group 2).

After the physical examination, precedent to orchiectomy, scrotal ultrasound (US), and contrast-enhanced thorax and abdomen tomography (CT) were obtained from all patients in Group 1. Preoperative routine tests and classical tumor markers (AFP, bHCG, and LDH) were measured. In addition, serum levels of miRNA-371a-3p in Group 1 were evaluated before radical inguinal orchiectomy and two weeks after orchiectomy. Serum AFP, bHCG, LDH, and miRNA-371a-3p levels of healthy blood donors in Group 2 were also evaluated. Receiver operating characteristics curve (ROC) analyses were performed to determine the sensitivity and specificity of miRNA-371a-3p. Clinical and pathological factors such as clinical stage, tumor size, histology, rete testis invasion, and lymphovascular invasion (LVI), with a potential impact of miRNA-371a-3p expression levels, were evaluated statistically.

Written consent was received from all participants at the onset of the present study. All procedures in the present study were conducted by the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amend-

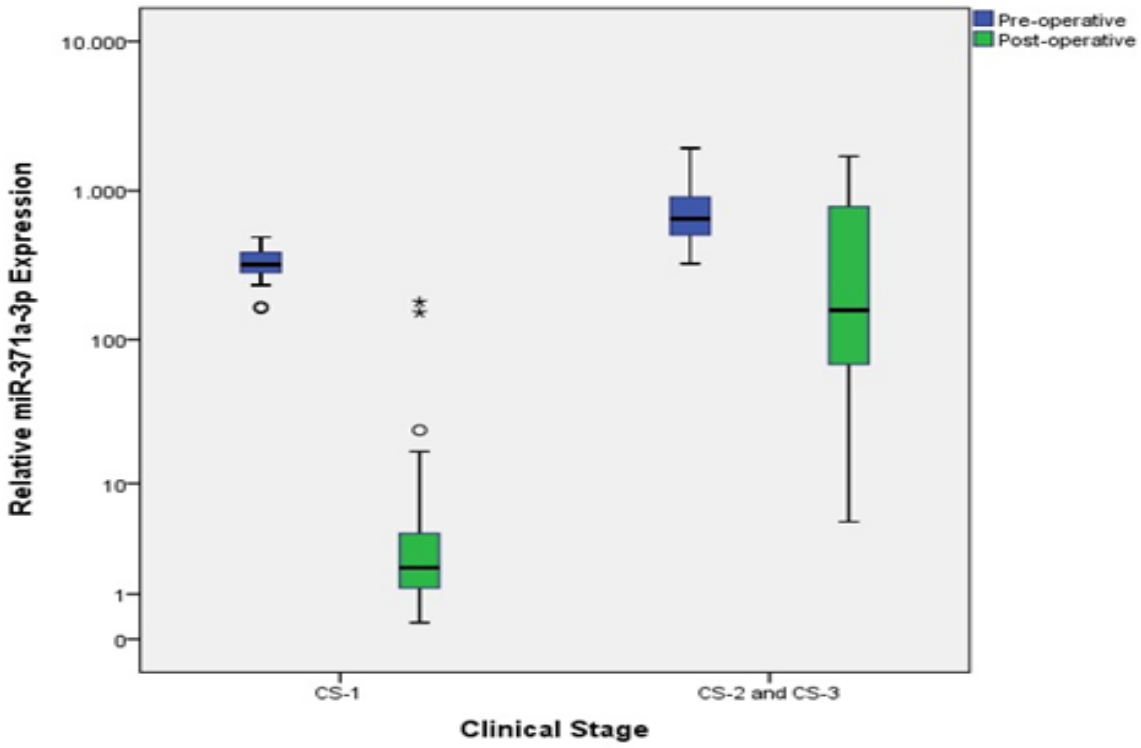

Figure 2: miR-371a-3p levels at preoperative and postoperative second week in different clinical stages (CS=Clinical Stage)

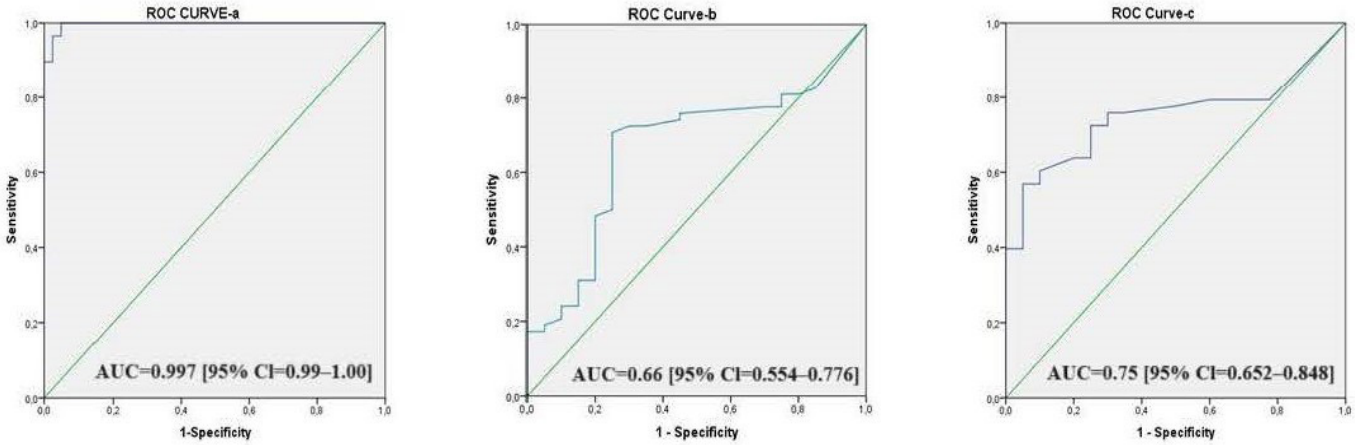

Figure 3. Receiver operating characteristic (ROC) curves of miR-371a-3p, alpha-fetoprotein (AFP), and beta-human chorionic gonadotropin (bHCG) in GCT. (a- miR-371a-3p, b- AFP, c- bHCG, GCT= testicular germ cell tumor)

ments. Consent, according to the Helsinki Declaration was obtained from the Ethics Committee of Necmettin Erbakan University, Meram Faculty of Medicine, before the study (No: 2020/2455).

Laboratory methods

Serum samples were drawn from the cubital vein before and 2 weeks after the orchiectomy. The samples were frozen and stored at -80°C until the last stage of the study.

Total Ribonucleic Acid (RNA) was isolated from 100 µl not coagulated serum and stored in the EDTA (Ethylenediamine Tetraacetic Acid) tube serum sample using the Total RNA Purification Kit according to the supplied protocol (Norgen Biotek Corp., Thorold, Canada). For reverse transcription of microRNA into complementary Deoxyribonucleic Acid (cDNA), 1 µl total RNA mixed with 9 µl miR-cDNA Synthesis Kit (A.B.T.™ Laboratory Industry, Ankara, Turkey). The reaction was incubated in the AGS Thermal Cycler (AGS MedTech, Hangzhou, China) for 10 min. at 25 °C, 20 min. at 37 °C, and 5 min. at 85 °C. Preamplification was performed using cDNA and the miR-371a-3p specific miR-qPCR Master Mix (A.B.T.™ Laboratory Industry, Ankara, Turkey).

Real-time PCR was performed on the LightCycler® 480 Real-Time PCR System (Roche Diagnostics, Mannheim, Germany). The PCR conditions were 10 min. at 95 °C, followed by 40 cycles at 95 °C for 10 s and 60 °C for 25 s.

RNU6 snRNA was used as the internal control to measure the relative quantification of serum miRNA-371a-3p. The relative quantification levels were calculated using the 2-ΔΔCT method[18].

Statistical Analysis

To justify the sample size, we performed power analysis using the GPower version 3.1 program. When deciding on the number of samples in the formation of groups, Cohen's ideal effect size assumption of 0.50 and above was accepted[19]. For a statistical power of 0.90, it was determined that a sample size of 98 or above was sufficient in terms of the variables of our study.

SPSS, v.23.0 statistical software (SPSS, Inc., Chicago, IL, USA) package program was utilized for statistical analysis. All quantitative results are expressed as the median and interquartile range (IQR). The Mann-Whitney *U* test was used to compare the independent subgroup data. Whereas the Wilcoxon signed-rank test was used to compare the data of related groups, receiver operating characteristics curve (ROC) analyses were performed to determine the sensitivity and specificity of miRNA-371a-3p, AFP, and bHCG. The Youden index was used to identify the optimal miRNA-371a-3p threshold. An ANOVA test was applied to compare the categorical variable with more than two groups. A Pearson correlation coefficient was used for correlation analysis on normally distributed variables with Kurtosis and Skewness values between - 1.5 and + 1.5. It was observed that the tumor size data met the normal distribution assumptions. However, it was observed that miR-371a-3p RQ values did not meet normal distribution assumptions. Spearman-Brown correlation coefficient was used for correlation analysis of variables that do not comply with normal distribution. A *p*-value of $< .05$ is considered as the threshold level for statistical significance.

The miRNA-371a-3p sensitivity and specificity were evaluated at the time of diagnosis in the GCT patients. Changes in miRNA-371a-3p, AFP, bHCG, and LDH levels secondary to orchiectomy were assessed in all clinical stages.

RESULTS

A total of 100 male patients, 60 in Group 1 and 40 in Group 2 were enrolled in the present prospective case-control study.

A total of 32 patients had seminomas, and 28 patients had NSGCTs. Two patients had teratoma. While the median age was 34 (28-41) years in Group 1, it was 36(27-51) years in Group 2 ($p = 0.16$). In the CS-1, 15 patients had seminomas and 12 patients had NSGCTs. Twelve patients had seminomas and 5 patients had NSGCTs in the CS-2. In the CS-3, 5 patients had seminomas and 11 patients had NSGCTs (**Table 1**). Of the non-seminomas, 2 (7%) were teratomas, 2 (7%) were embryonal carcinomas, 4 (14%) were yolk sac tumors, and 20 (72%) were mixed tumors. Teratoma was detected in five patients with stage 1 mixed tumors (proportions are 10%, 30%, 30%, 60%, and 60%, respectively). In stage 2, teratoma was detected in one patient with a rate of 10%. In addition, teratoma was detected in two patients with a rate of 5%, in stage 3.

The median miR-371a-3p expression level (relative quantity, RQ) was 489 times higher in Group 1. In group 2, the median miR-371a-3p expression level was 2.2 times ($p < .001$). The RQ of miR-371a-3p was higher in Group 1 at all clinical stages (mean difference 486.8 [95% Cl:374.6–599], $p < .001$). miR-371a-3p was expressed 97 times more in CS-1 (RQ mean difference 319.2 [95% Cl:293.8–344.7], $p < .001$), and 230 times more in other clinical stages (RQ mean difference 759.5 [95% Cl:644.6–874.4], $p < .001$), compared to the Control group (**Figure 1**). The RQ of two patients in stage 3 represents the outliers (1931 and 1717 times). miR-371a-3p expression was not detected in 2 teratoma patients. No correlation was observed between teratoma percentage and miR-371a-3p RQ in mixed tumors, at all stages. (r = -0.31 [95% Cl:-0.64 – 0.02], $p = .11$).

AFP or bHCG was expressed in 53% of the GCT patients. Two weeks after orchiectomy, there was a significant decrease in AFP and bHCG levels in all CS-1 patients, 30% of the other clinical stages ($p = .01$ for CS-1, $p = .3$ for other CS) (**Table 2**). Throughout this time there was a decrease of 19 times at the miR-371a-3p expression levels in CS-1 (preoperative 322.6 (163–489), postoperative 16.6 (0.2–179), $p < .001$), in the other clinical stages a 1.6 times drop was present (preoperative 762.8 (325–1931), postoperative 479.8 (5.1–1700), $p < .001$) (**Figure 2**). The RQ of three patients in stage 1 at the postoperative period represents the outliers (179,152, and 24 times, respectively).

The sensitivity of miR-371a-3p in GCT patients was 98.3% [95% Cl:92.7–98.9%], and the specificity was 95% [95% Cl:89.7–97.6%], (Area Under the Curve (AUC) = 0.997 [95% Cl:0.99–1.00], $p < .001$). The p-value refers to the hypothesis that miR-371a-3p has high diagnostic accuracy on GCT. The highest Youden index and optimal cutoff at an RQ of 13, based on ROC analysis, was obtained. While the sensitivity of AFP is 48% [95% Cl:30.6–65%], and its specificity is 25% [95% Cl:15–40.1%], (AUC = 0.66 [95% Cl:0.554–0.776], p = .006), the sensitivity of bHCG is 56% [95% Cl:39–63%], and its specificity is 46% [95% Cl:31–65%], (AUC = 0.75 [95% Cl:0.652–0.848], $p < .001$) (**Figure 3**).

The comparison of the data obtained revealed that the miR-371a-3p RQ at the time of diagnosis was 1.5–fold higher in NSGCT patients compared to seminoma patients (mean difference 244.6 [95% Cl:66.9–422.3], $p = .008$).

The miR-371a-3p RQs were correlated with tumor size in the CS-1 GCT (r = 0.70 [95% Cl:0.54 – 0.81], $p < .001$). The miR-371a-3p RQ was 1.33–fold higher in tumors larger than 40 mm ($p = .003$). The difference was not observed between the miR-371a-3p RQs of patients with and without rete testis invasion, and LVI ($p = .11$, $p = .54$).

The miR-371a-3p RQs of 20 patients with lymph node metastases were, on average, 1.7-times higher than those of CS-1 patients (mean difference 283.6 [95% Cl:172.6–394,6], $p < .001$).

DISCUSSION

The present prospective study has demonstrated that miR-371a-3p could be an effective marker for the diagnosis of GCTs. It was overexpressed in patients with seminoma and NSGCT. Moreover, the expression levels increased with clinical stage, tumor size, and lymph node involvement. These findings suggest that miR-371a-3p is an important biomarker in the diagnosis and clinical staging of GCTs.

The incidence of testicular tumors increases with the rate of self-examination[1]. Testicular cancer usually presents as a painless testicular mass or incidental finding on ultrasound. High-frequency (>10 MHz) testicular US should be used to confirm a testicular tumor to determine the mass, assess its volume and anatomical location, and characterize the contralateral testicle[20]. Its sensitivity in the diagnosis of testicular tumors is over 95%[21]. However, accurate differentiation of the small-sized lesions is not always possible. The introduction of multiparametric US into clinical practice has improved the diagnostic performance of standard US[22]. Aigner et al. demonstrated a sensitivity of 100%, a specificity of 81%, a negative predictive value of 100%, a positive predictive value of 92%, and an accuracy of 94% in the diagnosis of testicular tumors with multiparametric US[23]. Scrotal magnetic resonance imaging (MRI) also provides higher sensitivity and specificity than the US in the diagnosis of testicular tumors[21,24]. MRI proved to be capable of identifying the correct lesion location with sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of 100%, each, with suspected scrotal masses[25]. However, the high costs and low accessibility of these imaging methods are the most important obstacles to routine use.

Testicular tumors have been evaluated with classical serum tumor markers for approximately five decades. However, AFP, bHCG, and LDH levels are elevated only in 60% of GCT patients[13,26]. The classic tumor markers are widely utilized in defining cancer stages. Nowadays, along with classic tumor markers, CT is also used in defining the clinical stage of GCT. However, the sensitivity of CT in the staging of GCT is 67%[10]. The low sensitivity and specificity of analyses in GCT diagnosis and staging make disease management and hence outcomes less successful[27].

The inadequacy of classical serum tumor markers in the evaluation of GCTs indicates the need for new markers. miRNAs have the potential to fill this gap in the diagnosis and management of GCTs. These biomarkers are small, noncoding RNA molecules with approximately 20 nucleotides. miRNAs play a role in the epigenetic regulation of the genome, regulating the expression of approximately one-third of the human genome, and regulating cellular functions, such as cell cycle, cell growth, proliferation, apoptosis, and differentiation[28]. The expression of miR-132 and miR-212 in prostate cancer and the demonstration of down-regulation of some genes by RNA sequence analysis indicate that miRNAs will become important in urological cancers[29,30].

miR-371-3 clusters are expressed from embryonic stem cells and play a role in pluripotency control[31]. They can be detected at very low levels in normal testicular tissue[32]. GCTs of the testis also originate from undifferentiated embryonal cells. It has been stated that miRNA-371a-3p is a GCT marker with a sensitivity and specificity of over 90% in the diagnosis[7,11,33]. The inability to detect this marker in other malignities and the presence of it at higher rates in the testicular vein than in the peripheral circulation in GCT increases its specificity[34]. Moreover, the lack of miRNA-371a-3p in non-GCT tumors is another factor contributing to

its specificity[17]. The probable reason for the elevated expression level and thus the sensitivity and specificity of miR-371a-3 in GCT is its similarity with embryonic stem cells[35].

The present study has defined the sensitivity of miR-371a-3p as 98.3% and the specificity as 95% in GCTs. The study of Dieckmann et al., with the largest number of patients in the literature, has also reported the sensitivity of miR-371a-3p as 90.1% and the specificity as 94.0% in 616 GCTs[15]. They have stated that AFP, bHCG, and LDH accuracy rates were less than 50%[15]. Likewise, in the present study, the sensitivity and specificity of AFP and bHCG were lower than miR-371a-3p. These findings reveal that miR-371a-3p is more effective in GCT diagnosis compared to classical serum tumor markers.

A further factor increasing miR-371a-3p specificity is the lack of expression in non-GCT cases[17]. GCTs of the testis originate from undifferentiated embryonal cells which are expressed in the miR-371-3 clusters[31]. Non-GCTs are biologically quite different from embryonic stem cells[17]. The differential clonal origin of miR-371a-3p explains the lack of expression in non-GCT. However, as stated in previous studies, the lack of expression of the marker in teratomas is a significant clinical limitation[16]. This can be attributed to the fact that the teratoma is a mature neoplasm much more differentiated than embryonic stem cells[33]. No obvious miR-371a-3p expression in four non-GCT patients and two teratoma patients was observed in the present study. In addition, we found that the teratoma proportion in mixed tumors was not correlated with the miR-371a-3p RQ. This may be due to the low total number of NSGCT patients in the study.

The expression level of miR-371a-3p was higher in non-seminoma than in seminoma patients. This may be due to the close biological similarities of undifferentiated stem cells expressing miR-371-3 clusters to embryonal carcinoma cells (hence NSGCT). Previous studies have reported that the expression level of miR-371a-3p is higher at less differentiated cellular levels in NSGCT compared to seminomas[15].

The present study has determined that the expression level of miR-371a-3p is related to the clinical stage of the GCT. The RQ increased statistically significantly with the clinical stage. Dieckmann et al. stated that there was a more than 10-fold increase in miR-371a-3p expression level between CS-3 and CS-1, and the miR-371a-3p discriminated localized CS-1 disease from systemic disease[15]. Similarly, Myklebust et al. reported that miR-371a-3p was associated with the clinical stage and they achieved the highest diagnostic accuracy, especially in CS-1 disease[36]. The increase of miR-371a-3p expression with clinical stage could indicate a non-localized disease and adjuvant treatment needs.

In the present study, the decrease in miR-371a-3p RQ, two weeks after orchiectomy was greater in CS-1 than in other clinical stages. This may be due to the increased burden of disease (number of tumor cells) with clinical stage. The increase in tumor size in CS-1, parallel to RQ, supports the hypothesis of the present study. Another support to the fact that GCT affects marker level is the study of Leão et al. in which RQ was close to zero after chemotherapy in CS-2 patients whose miR-371a-3p expressions continued after orchiectomy[35].

The predictive value of classical serum tumor markers and imaging methods used in GCT clinical staging is unclear. Uncertainty remains about staging using CT in 20% of CS-2 patients[37]. As the currently employed tests do not provide clear information in clinical staging, miR-371a-3p has been investigated as a potential solution. In the study of Leão et al., high miR-371a-3p levels persisted in 13.3% of CS-1 patients after orchiectomy and miR-371a-3p levels returned to normal levels in 10% of CS-2 patients, indicating the inadequacy of radiology in staging GCT[35]. In the present study, higher miR-371a-3p RQs were maintained in 11.1% of the CS-1 patients after orchiectomy. In 11.2% of the CS-2 patients, the RQs decreased to the normal levels. The findings suggest that the use of miR-371a-3p may contribute to the accuracy of disease staging.

There might be another clinical significance for the fact that miR-371a-3p levels do not return to normal in advanced clinical stages. Whereas a 19-fold decrease in miR-371a-3p expression levels was observed in the second postoperative week in CS-1, only a 1.6-fold decrease has been determined in other clinical stages in the present study. AFP and bHCG levels decreased in all CS-1 patients and 30% in all advanced-stage patients. Similar findings have also been observed in other studies[7,38]. Dieckmann et al. reported that the miR-371a-3p RQ decreased to normal levels after orchiectomy in 91.77% of the 371 patients with CS-1, but remained at high levels in 82.41% of the 70 patients with CS-3 after orchiectomy. Therefore, the miR-371a-3p level should be carefully monitored in the postoperative period since it could be a predictor of occult metastatic disease.

The most important limitation of the present study is the low total number of patients, the low number of metastatic patients, and the heterogeneity of the groups. In addition, the lack of long-term observational data on the patients is an important limitation. The serum samples were frozen and stored at -80°C until the last stage of the study; thus, measurement results may be affected due to hemolysis and waiting time.

A clear algorithm for the routine use of miR-371a-3p, a potential new marker in GCT, and its use with imaging methods in diagnosis and staging, has not yet been defined. According to our best knowledge, the present study is the first to evaluate the diagnostic accuracy of miR-371a-3p in GCT, factors affecting its expression level, and its relationship with clinical stage in a Turkish population. The data obtained has the potential to contribute to the existing literature and support the importance of miR-371a-3p in GCT diagnosis and staging. Thus, miR-371a-3p, together with classical diagnostic tools, may prevent waste of time in advanced cases before adjuvant therapy onset and prevent unnecessary adjuvant treatments in localized cases.

CONCLUSIONS

The findings of the present study support the data in the literature by proving that miR-371a-3p has over 90% sensitivity and specificity in the diagnosis of GCT. Another significant aspect of the present study is the definition that the expression level is related to the clinical stage and high expression levels continue in advanced disease. In addition to existing diagnostic tools in the post-orchiectomy period, monitoring miR-371a-3p expression levels may contribute to disease staging, detection of occult metastatic disease, and treatment management and thus become a valuable marker in GCT. It

may provide an idea for differential diagnosis in non-palpable, small testicular masses and avoid unnecessary surgery. Data in the literature indicate that this marker with high diagnostic accuracy will find wide use. However, limited access and high costs are still significant issues for routine clinical use.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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