MICRORNA AS A POTENTIAL BIOMARKER IN THE DIAGNOSIS AND PROGNOSIS OF HODGKIN'S LYMPHOMA: A SYSTEMATIC REVIEW

NIK NURDIYANA NIK IZHAR, BSC
DEPARTMENT OF BIOMEDICAL SCIENCE, KULLIYYAH OF ALLIED HEALTH SCIENCES, INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA, JALAN SULTAN HAJI AHMAD SHAH, 25200 KUANTAN, PAHANG
nk.nurdiyana@gmail.com

MARDHIAH MOHAMMAD, PHD
DEPARTMENT OF BIOMEDICAL SCIENCE, KULLIYYAH OF ALLIED HEALTH SCIENCES, INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA, JALAN SULTAN HAJI AHMAD SHAH, 25200 KUANTAN, PAHANG
mmoh@iium.edu.my

ROZLIN ABDUL RAHMAN, MD, PHD
DEPARTMENT OF PHYSICAL REHABILITATION SCIENCES, KULLIYYAH OF ALLIED HEALTH SCIENCES, INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA, JALAN SULTAN HAJI AHMAD SHAH, 25200 KUANTAN, PAHANG
rozlin@iium.edu.my

NORAFIZA ZAINUDDIN, PHD (CORRESPONDING AUTHOR)
DEPARTMENT OF BIOMEDICAL SCIENCE, KULLIYYAH OF ALLIED HEALTH SCIENCES, INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA, JALAN SULTAN HAJI AHMAD SHAH, 25200 KUANTAN, PAHANG
znorafiza@iium.edu.my

ABSTRACT

Diagnosing Hodgkin's Lymphoma (HL) has become a challenge nowadays and mostly limited to the current therapy. Thus, microRNA is believed to exhibit the best candidate for HL diagnosis and prognosis. To assess the true value of miRNA as a biomarker in HL, a systematic review was conducted to collect and evaluate the current knowledge of miRNAs functioning as diagnostic and prognostic biomarkers in HL. A systematic literature search was conducted to retrieve relevant articles from five different electronic databases up to April 2018. Search terms included 'Hodgkin's lymphoma', 'microRNA', 'prognostic biomarker' and 'diagnostic biomarker'. The selected articles were assessed using CROWE critical appraisal tool. The literature search identified a total of 1362 articles from which a final 20 relevant studies were included. In total, 118 miRNAs with significance to HL were observed. 85 miRNAs were reported with regards to its expression and associated pathogenesis. 66 miRNAs were found to be up-regulated in HL, while 18 were found down-regulated. 20 miRNAs were correlated to prognosis. One study reported higher expression of miRNAs based on the expression in plasma extracellular vesicle, and the finding was found to be reliable for therapy response. Three top most important miRNAs were also depicted in this review, which may have significant role in HL pathogenesis. In conclusion, the relevant information on miRNAs particularly in monitoring treatment response is hoped to benefit the treatment advances in HL in the future.

Keywords: Hodgkin's Lymphoma (HL), microRNA (miR), biomarker, diagnosis, prognosis

INTRODUCTION

Hodgkin's lymphoma is a B-cell abnormality derived from dysregulation of immunoglobulin rearrangement in the germinal centre (Kuppers et al., 1994). Unfortunately, in advanced stages, the relapses and short survival rate after chemotherapy regimen, radiotherapy and autologous stem cell transplantation (ASCT) have resulted in worsened adverse effects such as heart and lung disease, and secondary malignancies (Gotti et al., 2013). Treatment related illness accounts for more deaths than the disease itself (Aleman et al., 2003). An accurate diagnosis should be made for patients' management to be optimally treated and staged (Ansell, 2015). Morphologically, Hodgkin's malignant cells have been well described as multinucleated giant cells called the Reed-Sternberg cells (Küppers & Hansmann, 2005). Medical experts found it very difficult to diagnose HL while it is in advanced stage because of its fewer presence of cancer cells with only 0.1% to 10% of total cells in tissue (Berg et al., 2015). Reliable diagnostic markers should be identified in order to provide information on suitable treatment so that the mechanism of HL related to its pathogenesis could be more comprehended (Zhu et al., 2013). For cases of refractory and relapsed disease, a better tumor-specific target is very crucial. Ansell, (2018) mentioned that the early diagnosis should only be made by biopsy, and fine needle aspiration cytology (FNAC) is not sufficient to provide the underlying mechanism of the malignancies. According to Saraswathi, Bama, & Kumar, (2017), FNAC is a good diagnostic tool as it is significant in screening any enlarged lymph node. However, there were cases reported to have false-negative result in fine-needle aspiration of HL cells (Chhieng et al., 2001). Currently, International Prognostic Score (IPS) is used for prognosis prediction (Moccia et al., 2012) but some scientists agreed that IPS is not very efficient as it does not consider the genetics and epigenetics mechanism (Berg et al., 2015). The knowledge of targeted therapies which is useful in diagnostic and prognostic functioning within HL is very important to overcome the challenges in clinical treatment and treatment failure. Thus, the role of miRNAs as a biomarker is hoped to provide reliable information about the cancer metastasis and outcome risk (Shay, 2017). miRNAs (microRNAs) are short non-coding RNAs occurring naturally which capable in regulating gene expression especially at transcriptional level (Zhu et al., 2013). However, data on miRNAs in HL is very limited and not structured. This systematic review was conducted as to collate all existing studies systematically regarding the correlation of miRNAs with HL disease. Effective diagnostic and prognostic tools using miRNAs could help the current treatment to be shifted into individualized medication.

METHODS

Search Strategy

The databases NCBI, RESEARCH GATE, SCOPUS, EMBASE and PROQUEST were used to collate the existing studies. MeSH terms included to track the relevant articles were the combination of 'Hodgkin's lymphoma' AND 'biomarker' AND 'diagnostic' OR 'prognostic' AND 'microRNA'. No restriction of the search date was set as to include the earliest year microRNA was found as biomarker in HL. Duplicates were identified by using Mendeley software version 1.18. The abstracts and full text were then reviewed based on the inclusion and exclusion criteria. Relevant publications were all sorted out according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram (Figure 1).

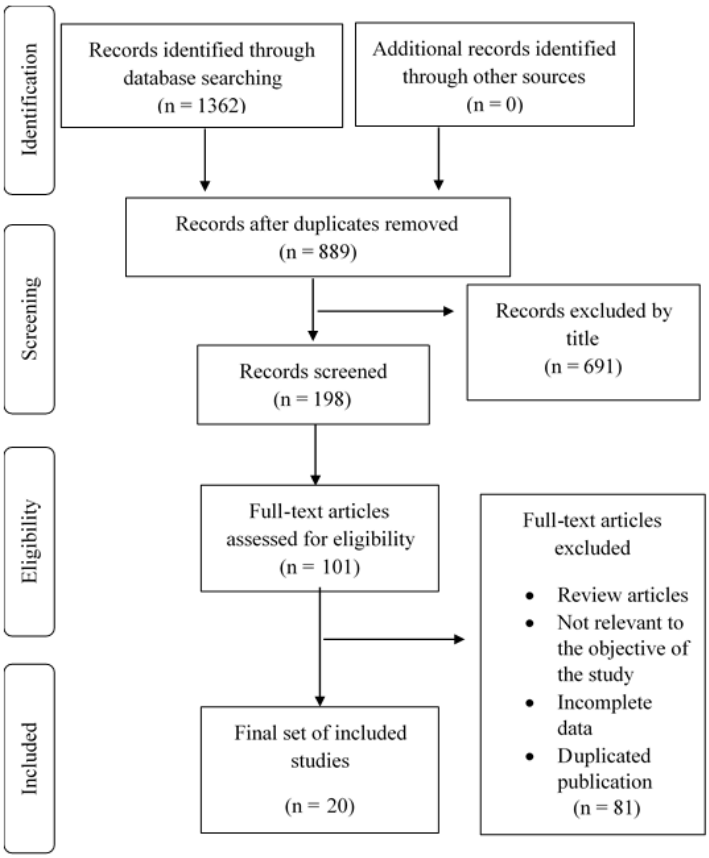

Fig. 1. PRISMA flow diagram showing the resulted articles after being assessed in each stage (source: http://prisma-statement.org)

Study Selection

The selected articles were all original studies and published in English language. All studies reporting on the functions of microRNAs specifically on HL disease were included. Articles which compared the microRNAs signatures in HL disease with other malignancies were also included for comparison and to get a clear view on how HL should be evaluated and treated. Articles that were not relevant to the review questions, have incomplete or missing data as well as duplicated articles were rejected. Articles without full text and review paper were also excluded from this study.

Data Extraction

From the final collection of articles selected, the following information were extracted: author(s)' name(s), year of publication, country of origin of the study, clinical information of the study population (sample size of cases and control, median age), list of up-regulated and down-regulated microRNAs and fold change (if available), type of specimen in which the microRNA was extracted, RNA extraction or isolation, quantification, and quantitative data (significant value on parameter comparisons, if available). Other useful information extracted were the functions, expression and significance of the microRNA types related to the HL pathogenesis. The outcome measurements and the variety of methodology used in the selected studies were heterogeneous. Thus, meta-analysis was not conducted.

Quality Assessment

Assessment was conducted using CROWE Critical Appraisal tools (CCAT) which consists of CCAT form and CCAT user guide (Source: https://conchra.com.au). The form and user guide were used together to avoid compromising the reliability of the scores given. It was assumed that every researcher was familiar with the research designs, the data collection methods, sampling techniques, ethics, and statistical or non-statistical data analysis of all the selected studies. Two independent reviewers were appointed to assess the eligibility criteria of the selected articles. With this assessment, a strict evaluation on the study that fulfilled the requirements and should have met the expectation was achieved.

Analysis of Data

The results from extracted data were tabulated in summary tables and ranked from the earliest studies of microRNA detected in HL to the most recent studies. The significant functions of miRNA in diagnostic or prognostic related to HL pathogenesis, as well as the clinical endpoint of associated miRNAs were extracted. The most studied miRNAs were demonstrated in a bar chart for systematic representation for further review.

RESULTS

Search Result

A total of 1362 articles matched the 'MeSH' keywords. 889 duplicated articles were detected by Mendeley software and excluded from the study. Therefore, 198 full text articles of the resulted articles were scrutinized and 81 of the studies were further eliminated due to the irrelevant content for the review topic, incomplete data, as well as non-original articles. Finally, 20 articles were identified to be eligible and met the inclusion criteria of this study after screening and analysis of the resulted articles and full text (Figure 1).

Study and Patient Characteristics

The general information of 20 included studies was tabulated in Table 1 in an ascending order of the year which were all published between 2005 until 2017. Of the 20 selected studies, six were from the Netherlands, four from Spain and one each from New York, Belgium, Finland, Brazil, Turkey, Tunisia, Australia, China, and Jerusalem. 16 of the studies used formalin-fixed paraffin embedded tissues samples while blood plasma was used in two studies and the remaining studies used serum and plasma extracellular vesicle.

Among the 20 studies, three of it used tonsil tissue as control, six used reactive lymph node (RLN) as control of which one study included both reactive lymph node (RLN) and tonsil tissue as control. One study used tonsil tissue together with miR-122 as positive control and liver-specific miRNA as negative control and another one study which includes four systemic lupus erythematous (SLE) patients as control. 13 of the study used cell lines as the platform either to study the expression of miRNA in HL microenvironment or transfection of cell line and compared the deregulated miRNAs with control. The common cell lines of HL used are L-1236, HDMYZ, KM-H2, L-450 and HDLM-2.

Table 1 Main characteristics of all the eligible studies with their selection methods of miRNA extraction, quantification and microarray platform. miR: micro-RNA; cHL: classical Hodgkin Lymphoma; RLN: reactive lymph node; LN: lymph node; SLE: systemic lupus erythematous; FFPE: fresh frozen paraffin-embedded; qRT-PCR: quantitative real-time polymerase chain reaction; N/S: not stated

Reference	Country	Patient/control samples	Cell lines	miRNAs examined	miRNAs isolation	miRNA quantification	Microarray platform
Kluiver et al (2005)	Netherlands	Patient tissues selected randomly from tissue bank. RLN and tonsil tissue as controls	L591 KM-H2 L1236 DEV L428* HDLM-2* *No expression observed	miR-155	Total RNA from tissue isolated using Trizol (Invitrogen) Total RNA from cell lines isolated using Absolutely RNA Miniprep Kit (Stratagene)	qRT-PCR	-
Nie et al (2008)	New York	Patient tissues of cHL	L428 KM-H2 L1236	miR-9 Let-7a	N/S	qRT-PCR TaqMan MicroRNA Assays (Applied Biosystems)	-
Navarro et al (2008)	Spain	49 specimens of FFPE lymph nodes of cHL 10RLN as control Validation: 30 FFPE cHL patients, 5RLN	L428 HDMYZ L1236	miR-21 miR-27a miR-147 miR-182 miR-183 miR-216 miR-135a miR-204	RecoverAll Total Nucleic Acid and Trizol total RNA	RT-PCR	BRB Array Tools version 3.5.0

Reference	Country	Patient/control samples	Cell lines	miRNAs examined	miRNAs isolation	miRNA quantification	Microarray platform
Tan et al (2009)	Netherlands	Patient tissues of three HL cases	cell lines from previous study L1236 L428	miR-17-5p miR-106a miR-21 miR-24 miR-146a miR-150 miR-155 miR-181b miR-210	N/S	N/S	-
Gibcus et al (2009)	Netherlands	N/S	L428 KM-H2 L1236	Let-7e miR-155 miR-150 miR-107	Trizol (Invitrogen)	qRT-PCR (TaqMan miRNA)	Agilent Oligo Microarray Kit G4470A
Navarro et al (2009)	Spain	89 tissue of homogenously treated cHL patients	L-428 L-1236 HDMYZ	miR-135a	RNA extracted from cell lines using Trizol total RNA isolation (Invitrogen)	qRT-PCR Taqman miRNA assay	-

Reference	Country	Patient/control samples	Cell lines	miRNAs examined	miRNAs isolation	miRNA quantification	Microarray platform
Vlierberghe (2009)	Belgium	9 cHL tissues	HDLM2 L-450 KMH2 L-1236	miR-21 miR-20a miR-16 miR-155 miR-18a miR-186 miR-374 miR-140 miR-30a-5p miR-196a miR-30b miR-9 miR-614 miR-200a miR-520a	miRNeasy Mini kit	RT-PCR	-
Gibcus et al (2011)	Netherlands	40 tissues of cHL cases. Tonsil tissue & miR-122 (positive control). Liver-specific miRNA negative control	MCF-7 KM-H2 L428 U-HO1 SUDHL4	**miR-17/106b seed family**: miR-17 miR-20a miR-20b miR-93 miR-106a miR-106b	Total RNA isolated using Trizol (Invitrogen)	qRT-PCR	-

Reference	Country	Patient/control samples	Cell lines	miRNAs examined	miRNAs isolation	miRNA quantification	Microarray platform
Huang et al (2011)	China	62 tissue specimens of cHL and RLN hyperplasia	L428 HDLM2	miR-9	Total RNA isolated from cell lines using RNAiso Plus reagent (TaKaRa Bio)	qRT-PCR (All-in-One™ miRNA Q-pcr detection kit)	-
Leucci et al (2012)	Denmark	HL Tissue cells	L428 L540 KM-H2	miR-9	Trizol (Invitrogen)	qRT-PCR	Affymetrix HG-U133 Plus 2.0 arrays
Jones et al (2013)	Australia	42 newly diagnosed cHL blood plasma	-	miR-494 miR-21 miR-1973 miR-638 miR-2861	Total nucleic acid isolation kit (Ambion)	qRT-PCR	miRNA Microarray (Agilent Technologies, version 16.0
Sánchez-Espiridión et al (2013)	Spain	29 advanced cHL cases - discovery set. 168 FFPE - test set.	L428 L540 L1236 HDLM2 HDMYZ	miR-21 miR-92b miR-3z0d miR-30e	Trizol (Invitrogen)	Q-PCR	Agilent 8 9 15K human miRNA platform (Agilent Technologies Inc)

Reference	Country	Patient/control samples	Cell lines	miRNAs examined	miRNAs isolation	miRNA quantification	Microarray platform
Van Den Berg (2015)	Brazil	Peripheral Blood samples Group 1- not treated (n=4) Group 2 treated with ABVD (n=7) Group 3, healthy volunteers (n=14)	-	miR-9 miR-21 miR-26a miR-155	PAXgene Blood RNA kit (Qiagen)	qRT-PCR (TaqMan Universal Master Mix and Taqman miroRNA Assays)	-
Ben Dhiab (2015)	Tunisia	58 HL tissue 15 RLN as control	-	miR9-1 miR9-2 miR-9-3	-	Methylation-specific RT-PCR	-

Reference	Country	Patient/control samples	Cell lines	miRNAs examined	miRNAs isolation	miRNA quantification	Microarray platform
Paydas (2016)	Turkey	32 Paraffin embedded tissue samples cHL 60 cases with RLN as control	-	miR-582-3p miR-525-3p miR-448 miR-512-3p miR-642a-5p miR-876-5p miR-532-3p miR-654-5p miR-128 miR-145-5p miR-15b-5p miR-328 miR-660-5p miR-34a-5p miR-146a-5p miR-93-5p miR-20a-5p miR-324-3p miR-372 **miR-127-3p** miR-155-5p miR-320a miR-370	High Pure miRNA Isolation Kit	qRT-PCR	-

Reference	Country	Patient/control samples	Cell lines	miRNAs examined	miRNAs isolation	miRNA quantification	Microarray platform
Van Eijnhoven et al (2016)	Netherlands	20 cHL patients prior treatment Nine healthy individual and four SLE patients as control. Plasma extracellular vesicles	-	miR-21-5p miR-127-3p miR-24-3p let7a-5p miR-155-5p	Trizol-LS (Thermofisher Scientific)	RT-PCR (TaqMan)	-
Cordeiro et al (2016)	Spain	94 cHL tissue LN blood plasma	L-428 L-1236 L-540 HDLM2	piR-651	Total RNA FFPE lymph nodes. RecoverAll. RNA from cHL cell lines (Trizol)	qRT-PCR (TaqMan)	-
Yuan et al (2017)	Netherlands	3 HL tonsil tissue donors of age 2-6 years	L540 KM-H2 L1236 L428 HDLM2 SUPHD1	miR-24-3p	miRNeasy mini kit (Qiagen)	qRT-PCR	Human Whole Genome Oligo Microarray

Reference	Country	Patient/control samples	Cell lines	miRNAs examined	miRNAs isolation	miRNA quantification	Microarray platform
Karihtala et al (2017)	Finland	41 untreated cHL fresh frozen lymph node	-	miR-23b miR-144 miR-383 miR-200a miR-212 miR-28 miR-122	miRNeasy mini kit (Qiagen)	q-PCR	-
Khare et al (2017)	Jerusalem	Blood plasma of 11 HL patients 20 Healthy control	-	miR-182 miR-411 miR-433 miR-25 miR-30a/d miR-26b miR-186 miR-140 miR-125a miR-126-3p miR-144 miR-143 miR-23a miR-122 miR-93	RNeasy MinElute	Qubit 2.0	-

Table 2 presented the clinical characteristics of nine patients eligible for their study. Most of the studies recruited young adult patients aged 13 years old to older patients aged 89 years old. Only one performed their study on patient aged five. Other studies presented on sample characteristics of nodular sclerosis (NS), mixed-cellularity (MC), lymphocyte-rich (LR) and lymphocyte-depleted (LD). Five studies provided records of Epstein - Barr virus (EBV) positive cases in patient samples. Most studies presented patients with first-line treatment of adriamycin, bleomycin, vinblastine, dacarbazine (ABVD) chemo-regimen and seven studies reported on variable period of follow-up to collect treatment response from patients. Two studies underlined HIV positive as their exclusion criterion, while one study excluded HIV positive, hepatitis B and C infection patients and one study ruled out patients of previous malignancy, underwent transplantation and immunodeficiency.

Table 2 Additional information of 9 out of 20 studies which present their patient selection characteristics. M:male; F: Female; EBV: Epstein-Barr virus; ABVD: adriamycin, bleomycin, vinblastine, dacarbazine; MOPPABV: mechlorethamine, vincristine, procarbazine, prednisone/doxorubicin, bleomycin, vincristine; MOPP: mechlorethamine, vincristine, procarbazine, prednisone; BEACOPP: bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone; DHAP: dexamethasone, high dose cytarabine, cisplatine (platinum); SCT: stem-cell transplantation; PBSCT: peripheral blood stem cell transplantation; FFPE: fresh-frozen paraffin embedded; HIV: Human immunodeficiency virus, HEP B/C: hepatitis B/C, NS: nodular sclerosis, MC: mixed cellularity; LR: lymphocyte-rich; LD: lymphocyte-depleted; x: uncategorized; B/AT: Before/After treatment.

First Author (year)	Sample Size (N)	Median age (range)	Sex M:F	Histologic subtype					Stage				EBV +	Treatment	Median follow-up	Exclusion Criterion
				NS	MC	LR	LD	x	I	II	III	IV				
Navarro (2009)	89	29 (13-89)	42:47	70	19	-	-	-	54		35		25	ABVD = (97%)	80 months	-
Navarro (2013)	141	32 (13-89)	72:69	83		58		-	86		54		40	ABVD = 73 MOPPABV = 53 MOPP = 8 OTHER = 7	50 months	-
Sánchez-Espiridión (2013)	29 patients 168 FFPE	18-65	-	143	48	6		1	113		9		-	ABVD	12 months	HIV+
Jones (2014)	42	35.6 (18-79)	22:20	26	4	4	1	7	9		33		-	ABVD = 36 BEACOPP = 2 ABVD+ BEACOPP=2 ChlVP = 2	6 months	HIV +, Hep B/C infection

First Author (year)	Sample Size (N)	Median age (range)	Sex M:F	Histologic subtype				Stage					EBV +	Treatment	Median follow-up	Exclusion Criterion
				NS	MC	LR	LD	x	I	II	III	IV				
Van Den Berg (2015)	11	BT 35 (21-54)	BT 2:2	BT 2	BT 2	-	-	-	BT 3		BT 1		-	ABVD	6.7 months	-
		AT 29 (13-54)	AT 4:3	AT 5	AT 2				AT 5		AT 2					
Dhiab (2015)	-	24 (5-81)	1.2:1	39	13	4	2	-	-		45		19	60% chemotherapy 40% Chemoradiotherapy	39 months	Previous malignancy Trans-plantation Immuno - deficiency
Van Eijnhoven (2016)	20	-	-	-	-	-	-	-	-		-		-	ABVD+ BEACOPP=1; BEACOPP+ rituximab; Allogeneic PBSCT; DHAP+ Brentuximab; vedotin+autologous SCT	-	-

First Author (year)	Sample Size (N)	Median age (range)	Sex M:F	Histologic subtype					Stage				EBV +	Treatment	Median follow-up	Exclusion Criterion
				NS	MC	LR	LD	x	I	II	III	IV				
Cordeiro (2016)	94	34 (15-89)	45:49	61	18	6	2	7	6	51	18	19	28	ABVD = 58 MOPPABV = 27 MOPP = 4 Other = 5	133.5 months	HIV+
Khare (2017)	31	37 (26-63)	8:3	-	-	-	-	-	2	9		-	-	-	-	-

Differentially expressed miRNAs

A total of 118 miRNAs from 20 included studies was tabulated in Table 1. However, only 85 miRNAs were emphasized to be studied as functional miRNAs. Hence, only these 85 miRNAs were reported with regards to its expression and associated pathogenesis as depicted in Table 3 and Table 4. 66 miRNAs showed up-regulation while 18 out of the total miRNAs showed down-regulation. However, miR-150 showed inconsistent expression in two studies. Only 10 studies present their data on miRNA expression in terms of significant value or fold change. Of the 10 studies, the earliest study was conducted in 2005 in which the research observed BIC gene as the pri-miRNA or primary microRNAs for miR-155. On the other hand, one study compared miRNA expression in the CD77+ germinal centre B cells. In addition, there were four studies which compared between HL disease and control, one study that compared HL cell lines with other malignancy, one study that showed positive cytoplasmic staining of expressed miRNAs by in situ hybridization and one study that showed higher expression of miRNAs based on the expression of miRNAs in plasma extracellular vesicle. The later findings were reported to be reliable for therapy response.

Table 3 miRNAs expression and its significance as compared to other microenvironment; RLN: reactive lymph node; LCL: germinal centre B-cell derived lymphoblastoid BL: Burkitt's lymphoma; PMBL: primary mediastinal B-cell lymphoma cl: cell lines; N/A: not available

Reference	miRNA	p-value	miRNA expression	Description
Kluiver (2005)	miR-155	0.0068	Up-regulated	Level of BIC in paraffin and frozen tissue as pri-miRNA
		0.007		
Nie (2008)	miR-9	0.03	Up-regulated	miRNAs level in HL cell line by direct cloning
	Let-7a	0.004	Up-regulated	
Navarro	miR-21	N/A	Up-regulated	
(2008)	miR-27a	N/A	Up-regulated	
	miR-147	N/A	Up-regulated	4-fold change in cell lines
	miR-182	N/A	Up-regulated	compared with mean
	miR-216	N/A	Up-regulated	expression of RLN
	miR-126	N/A	Down-regulated	
	miR-135a	N/A	Down-regulated	
	miR-204	N/A	Down-regulated	

Reference	miRNA	p-value	miRNA expression	Description
Vlierberghe (2009)				Fold change:
	miR-21	N/A	Up-regulated	6.13
	miR-20a	N/A	Up-regulated	6.41
	miR-16	N/A	Up-regulated	3.83
	miR-155	N/A	Up-regulated	5.08
	miR-18a	N/A	Up-regulated	3.79
	miR-186	N/A	Up-regulated	4.57
	miR-374	N/A	Up-regulated	4.63
	miR-140	N/A	Up-regulated	4.87
	miR-30a-5p	N/A	Up-regulated	4.9
	miR-196a	N/A	Up-regulated	5.24
	miR-30b	N/A	Up-regulated	5.46
	miR-9	N/A	Up-regulated	3.92
	miR-614	N/A	Down-regulated	-4.94
	miR-200a	N/A	Down-regulated	-4.65
	miR-520a	N/A	Down-regulated	-2.81

Reference	miRNA	p-value	miRNA expression	Description

Gibcus (2009)	let-7e	0.0004	Up-regulated	HLcl vs LCLcl
	miR-155	0.1	Up-regulated	HLcl vs BLcl
	miR-107	0.007	Up-regulated	HLcl vs BLcl
	miR-150	0.5	Down-regulated	HLcl vs PMBLcl
		0.05	Down-regulated	HLcl vs LCLcl
		0.01	Down-regulated	HLcl vs PMBLcl
		0.02	Down-regulated	HLcl vs BLcl
Tan (2009)	miR-181b	N/A	Up-regulated	-Positive cytoplasmic staining
	miR-155	N/A	Up-regulated	-Positive cytoplasmic staining
	miR-146a	N/A	Up-regulated	-Positive cytoplasmic staining
	miR-210	N/A	Up-regulated	
	miR-21	N/A	Up-regulated	-Positive staining observed surrounding HRS cells
	miR-150	N/A	Up-regulated	-Relatively low in HL cell lines but strong cytoplasmic staining observed in HRS cells
	miR-24	N/A	Up-regulated	

Reference	miRNA	p-value	miRNA expression	Description
Jones	miR-494	0.0001	Up-regulated	qRT-PCR result correlated
(2013)	miR-21	N/S	Up-regulated	with matched microarray
	miR-1973	0.0035	Up-regulated	results of HL disease compared with control
	miR-638	0.0027	Up-regulated	
	miR-2861	0.0002	Up-regulated	
Van den Berg	miR-9	0.025	Up-regulated	Comparison between healthy control (fold-change)
(2015)	miR-21	0.0043	Up-regulated	miR-9 = 5.5
	miR-26a	0.011	Down-regulated	miR-21 = 5.23
	miR-155	0.0184	Reduced expression	miR-26a = 1.35
				miR-155 = 2.36
				Compared with patients underwent ABVD treatment (not differing from control)
	miR-9	0.025		
	miR-21	0.019		miR-9 = 62 –
	miR-155	0.019		miR-21 = 2.48 –
				miR-155 = 2.05 –

Reference	miRNA	p-value	miRNA expression	Description
Van Eijnhoven (2016)	miR-127-3p	0.0019	Up-regulated	Prior treatment in total plasma vs. plasma EV in HL cases
	miR-155-5p	<0.0001	Up-regulated	
	miR21-5p	0.010	Up-regulated	
	Let7a-5p	0.0009	Up-regulated	
	miR24-3p	0.0017	Up-regulated	
Karihtala et al (2017)	miR-383	0.019	Up-regulated	Expression of miRNAs in histology
	miR-200a	0.044	Up-regulated	
	miR-23b	0.021	Up-regulated	Expressed in advanced stages
		0.025	Up-regulated	Associated with B symptoms
	miR-212	0.049	Up-regulated	No correlation with clinicopathological parameters
	miR-510	0.058	(Undefined)	No correlation with clinicopathological parameters

Reference	miRNA	p-value	miRNA expression	Description
Khare (2017)	**miR-182**	0.001	Up-regulated	Expression of miRNAs as compared to control sample **miR-182** and **miR-140** are the most up-regulated miRNA in HL compared to control **miR-144** and **miR-143** are the most down-regulated miRNA in HL compared to control
	miR-411	0.006	Up-regulated	
	miR-433	0.006	Up-regulated	
	miR-25	0.00002	Up-regulated	
	miR-30a	0.0004	Up-regulated	
	miR-30d	0	Up-regulated	
	miR-26b	0.0449	Up-regulated	
	miR-186	0	Up-regulated	
	miR-140	0	Up-regulated	
	miR-125a	0.001	Up-regulated	
	miR-126-3p	0	Down-regulated	
	miR-144	0	Down-regulated	
	miR-143	0.007	Down-regulated	
	miR-23a	0.001	Down-regulated	
	miR-122	0.0007	Down-regulated	
	miR-93		Down-regulated	

Table 4 miRNAs observed to be associated with HL pathogenesis

miRNA ID	Role of miRNA	miRNA expression in HL	Mechanism	First Author	Year
miR 155	Oncogene	Up-regulated	Correlated with **BIC** expression which is a pri-miRNA that can be processed to miR-155	Kluiver	2005
miR-9	Oncogene	Up-regulated	miR-9 overexpression inhibits the levels of **PRDM1/Blimp-1** (tumor-suppressor gene)	Nie	2008
Let-7a	Oncogene	Up-regulated	Let-7a overexpression inhibit the levels of **PRDM1/Blimp-1** (tumor-suppressor gene)	Nie	2008
miR-17-5p	Oncogene	Up-regulated	May have contributed to **NF-kB** activation, disturbed cell cycle and anti-apoptotic features	Tan	2009
miR-106a	Oncogene	Up-regulated	May have contributed to **NF-kB** activation, disturbed cell cycle and anti-apoptotic features	Tan	2009
miR-135a	Tumor-supressor	Down-regulated	Lower level of miR-135a contribute to higher probability of relapse by increasing mRNA and protein levels of anti-apoptotic gene **Bcl-xL** by targeting **JAK2**	Navarro	2009
miR-17/106b	Oncogene	Up-regulated	Inhibition of miR-17/106b seed family enhance p21 protein levels and restore cell cycle control in HL cells by targeting **CDKN1A**	Gibcus	2011
miR-9	Oncogene	Up-regulated	miR-9 expression is controlled by CD99 which directly targeting **PRDM1** (regulator of plasma-cell differentiation)	Huang	2011

miRNA ID	Role of miRNA	miRNA expression in HL	Mechanism	First Author	Year
miR-9	Oncogene	Up-regulated	miR-9 decrease protein level of **HuR** and **DICER1** resulted in increased tumour outgrowth	Leucci	2012
miR-21	Oncogene	Up-regulated	Overexpression causes apoptosis inhibition (increased **BCL2/BAX & BCL2L1/BAX ratios**) contributed to clinical resistance to therapy in cHL patients	Sánchez-Espiridión	2013
miR-30d	Oncogene	Up-regulated	Up-regulation causes decreased **CDKN1A** levels associated with lower **TP53** protein levels	Sánchez-Espiridión	2013
miR-9	Oncogene	Up-regulated	miR-9 methylation especially miR-9-2 undergoes methylation located on 5q14.3 which is the main locus affected in HL	Ben Dhiab	2015
miR-889	Oncogene	Up-regulated	miR-889 found to be related to B symptom	Paydas	2016
miR-127	Oncogene	Up-regulated	miR-127 found to be related to prevalent in nodular sclerosis type	Paydas	2016
miR 24-3p	Oncogene	Up-regulated	Apoptosis inhibition by targeting **CDKN1B/pP27^{kip1}** and **MYC**	Yuan	2017
miR-23b	Oncogene	Up-regulated	Correlated inversely with CD3 and CD20 Associated with advance stages and B symptoms	Karihtala	2017
miR-144	Oncogene	Up-regulated	Correlated with CD3, CD20 and CD30 inversely	Karihtala	2017

miRNA ID	Role of miRNA	miRNA expression in HL	Mechanism	First Author	Year
miR-383	Oncogone	Up-regulated	Down-regulate mitochondrial isoform of enzyme PRDX family (PRDX III) Frequently expressed in nodular sclerosis, followed by mixed cellularity, least expressed in classical lymphocyte-rich	Karihtala	2017
miR-23b	Oncogene	Up-regulated	Down-regulate mitochondrial isoform of enzyme PRDX family (PRDX III) Associated with advanced stage and B symptoms	Karihtala	2017
miR-200a	Oncogene	Up-regulated	Most frequent expressed in tumors with nodular sclerosis, followed by mixed cellularity, least expressed in tumor classical lymphocyte-rich	Karihtala	2017
miR-28	Oncogene	Up-regulated	Elevated in advanced stage and B symptoms	Karihtala	2017
miR-122	Oncogene	Up-regulated	Associated with presence of B symptoms	Karihatala	2017
miR-182	Oncogene	Up-regulated	Inactivation leads to pro-tumor inflammation	Khare	2017
miR-140	Oncogene	Up-regulated	Inactivation leads to pro-tumor inflammation	Khare	2017

miRNAs associated with clinical outcome of HL

In the present review, two studies that were not highlighted in the previous narrative review by Cordeiro et al., (2017) were reported. In total, there were nine studies presenting significant outcome related to HL disease, as summarized in Table 5.

Table 5 miRNAs associated with clinical outcome in cHL. LN: lymph nodes

Reference	miRNAs	Samples	Patients	Clinical outcome
Navarro (2009)	miR-135a	LN	89 cHL	Disease-free survival
Huang (2011)	miR-9	LN	62 cHL	Treatment response
Sánchez-Espiridión (2013)	miR-21 miR-92b-5p miR-30d	LN	29 cHL patients with 168 HIV advanced cHL	Failure-free survival
Jones (2013)	miR-30e miR-494 miR-21 miR-1973	Plasma	42 HIV negative, HCV negative and HBV negative cHL	Relapse and interim therapy response
Van den Berg (2015)	miR-9 miR-21 miR-155	Peripheral blood	Four before treatment and seven after treatment	Treatment response
Van Eijnhoven (2016)	miR-21-5p miR-127-3-p miR-1555-5p let-7a-5p	Plasma extracellular vesicles	20 cHL before treatment (13 primary and 7 relapsed) 7 after treatment	Complete metabolic response
Cordeiro (2016)	piR-651	Serum	94 HIV-CHL	Treatment response, Disease-free survival, Overall survival
Karihtala (2017)	miR-122 miR-212 miR-510	LN	41 cHL	Poor disease-specific survival Poor relapse-free survival

Prevalence of miRNAs studied in existing research

miR-9 and miR-21 were the current most studied miRNAs of HL reported in six studies followed by five studies that reported the deregulation of miR-155. Table 6 shows the number and percentages of the mentioned miRNAs constituted in current studies. miR-9 were most reported to inhibit genes that are crucial to induce plasma cell differentiation while both miR-21 and miR-155 overexpression increase the tumor growth ability which is also induced by apoptosis inhibition (References as stated in Table 6). On the other hand, miR-20a, miR-24-3p, miR-30d, miR-93, miR-106a, miR-122, miR-127-3p, miR-140, miR-144, miR-135a, miR-155-5p, miR-182, miR-186, miR-200a each were reported in two studies.

Table 6 Three top most miRNAs in existing studies

	miR-9	**miR-21**	**miR-155**
Total Number	6	6	5
Percentages (%)	13	13	11
References	1. Nie et al (2008) 2. Vlierberghe et al (2009) 3. Huang et al (2011) 4. Leucci et al (2012) 5. Van Den Berg et al (2015) 6. Ben Dhiab et al (2015)	1. Navarro et al (2008) 2. Tan et al (2009) 3. Vlierberghe et al (2009) 4. Jones et al (2013) 5. Sánchez-Espiridión et al (2013) 6. Van Den Berg et al (2015)	1. Kluiver et al (2005) 2. Tan et al (2009) 3. Gibcus et al (2009) 4. Vlierberghe et al (2009) 5. Van Den Berg et al (2015)

CONCLUSION

In total, 118 miRNAs with significance to HL were observed. 85 miRNAs were reported with regards to its expression and associated pathogenesis. 66 miRNAs were found to be up-regulated in HL, while 18 were found down-regulated. 20 miRNAs were correlated to prognosis. One study reported higher expression of miRNAs based on the expression in plasma extracellular vesicle, and the finding was found to be reliable for therapy response. Three top most important miRNAs were also depicted in this review, which may have significant role in HL pathogenesis. In conclusion, the relevant information on miRNAs particularly in monitoring treatment response is hoped to benefit the treatment advances in HL in the future.

ACKNOWLEDGEMENT

The authors would like to thank Assoc. Prof. Dr. Ahmad Aidil Arafat Dzulkarnain, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia for his helpful advice on various technical issues examined in this paper. The authors however bear full responsibility on the paper.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

This research was financially self-supported.

Author Contributions

NNNI wrote the main body of the paper. MM, RAR and NZ provided feedback on the draft paper and approved the drafted manuscript. All authors read and approved the final manuscript.

REFERENCES

Aleman, B. M., Belt-Dusebout, A. W., Klokman, W. J., Veer, M. B., Bartelink, H., & Leeuwen, F. E. (2003). Long-Term Cause-Specific Mortality of Patients Treated for Hodgkin's Disease. Journal of Clinical Oncology, 21(18), 3431-3439. doi:10.1200/jco.2003.07.131

Ansell, S. M. (2015). Hodgkin Lymphoma: Diagnosis and Treatment. Mayo Clinic Proceedings,90(11), 1574-1583. doi:10.1016/j.mayocp.2015.07.005

Ansell, S. M. (2018). Hodgkin lymphoma: 2018 update on diagnosis, risk-stratification, and management. American Journal of Hematology, 93(5), 704-715. doi:10.1002/ajh.25071

Berg, A. V., Magalhães, L., & Vidal, A. F. (2015). MicroRNAs as Biomarkers of the Response to Treatment with ABVD Scheme in Hodgkin Lymphoma. Journal of Leukemia, 03(04). doi:10.4172/2329-6917.1000200

Chhieng, D. C., Cangiarella, J. F., Symmans, W. F., & Cohen, J. (2001). Fine-needle aspiration cytology of Hodgkin disease. Cancer, 93(1), 52-59. doi:10.1002/1097-0142(20010225)93:13.0.co;2-3

Cordeiro, A., Monzó, M., & Navarro, A. (2017). Non-Coding RNAs in Hodgkin Lymphoma. International Journal of Molecular Sciences, 18(6), 1154. doi:10.3390/ijms18061154

Dhiab, M. B., Ziadi, S., Louhichi, T., Gacem, R. B., Ksiaa, F., & Trimeche, M. (2015). Investigation of miR9-1, miR9-2 and miR9-3 Methylation in Hodgkin Lymphoma. Pathobiology, 82(5), 195-202. doi:10.1159/000432402

Eijndhoven, M. A., Zijlstra, J. M., Groenewegen, N. J., Drees, E. E., Niele, S. V., Baglio, S. R., . . . Pegtel, D. M. (2016). Plasma vesicle miRNAs for therapy response monitoring in Hodgkin lymphoma patients. JCI Insight, 1(19). doi:10.1172/jci.insight.89631

Gibcus, J. H., Kroesen, B., Koster, R., Halsema, N., Jong, D. D., Jong, S. D., . . . Berg, A. V. (2011). MiR-17/106b seed family regulates p21 in Hodgkins lymphoma. The Journal of Pathology, 225(4), 609-617. doi:10.1002/path.2958

Gibcus, J. H., Tan, L. P., Harms, G., Schakel, R. N., Jong, D. D., Blokzijl, T., . . . Berg, A. V. (2009). Hodgkin Lymphoma Cell Lines Are Characterized by a Specific miRNA Expression Profile. Neoplasia, 11(2). doi:10.1593/neo.08980

Gotti, M., Fiaccadori, V., Bono, E., Landini, B., Varettoni, M., Arcaini, L., & Bonfichi, M. (2013). Therapy-Related Late Adverse Events in Hodgkin's Lymphoma. Lymphoma, 2013, 1-7. doi:10.1155/2013/952698

Huang, X., Zhou, X., Wang, Z., Li, F., Liu, F., Zhong, L., . . . Zhao, T. (2011). CD99 triggers upregulation of miR-9-modulated PRDM1/BLIMP1 in Hodgkin/Reed-Sternberg cells and induces redifferentiation. International Journal of Cancer, 131(4). doi:10.1002/ijc.26503

Jafri, M. A., Al-Qahtani, M. H., & Shay, J. W. (2017). Role of miRNAs in human cancer metastasis: Implications for therapeutic intervention. Seminars in Cancer Biology, 44, 117-131. doi:10.1016/j.semcancer.2017.02.004

Jones, K., Nourse, J. P., Keane, C., Bhatnagar, A., & Gandhi, M. K. (2013). Plasma MicroRNA Are Disease Response Biomarkers in Classical Hodgkin Lymphoma. Clinical Cancer Research, 20(1), 253-264. doi:10.1158/1078-0432.ccr-13-1024

Karihtala, P., Porvari, K., Soini, Y., & Haapasaari, K. (2017). Redox Regulating Enzymes and Connected MicroRNA Regulators Have Prognostic Value in Classical Hodgkin Lymphomas. Oxidative Medicine and Cellular Longevity, 2017, 1-8. doi:10.1155/2017/2696071

Khare, D., Goldschmidt, N., Bardugo, A., Gur-Wahnon, D., Ben-Dov, I. Z., & Avni, B. (2017). Plasma microRNA profiling: Exploring better biomarkers for lymphoma surveillance. Plos One,12(11). doi:10.1371/journal.pone.0187722

Kluiver, J., Poppema, S., Jong, D. D., Blokzijl, T., Harms, G., Jacobs, S., . . . Berg, A. V. (2005). BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. The Journal of Pathology, 207(2), 243-249. doi:10.1002/path.1825

Küppers, R., & Hansmann, M. (2005). The Hodgkin and Reed/Sternberg cell. The International Journal of Biochemistry & Cell Biology, 37(3), 511-517. doi:10.1016/j.biocel.2003.10.025

Kuppers, R., Rajewsky, K., Zhao, M., Simons, G., Laumann, R., Fischer, R., & Hansmann, M. L. (1994). Hodgkin disease: Hodgkin and Reed-Sternberg cells picked from histological sections show clonal immunoglobulin gene rearrangements and appear to be derived from B cells at various stages of development. Proceedings of the National Academy of Sciences, 91(23), 10962-10966. doi:10.1073/pnas.91.23.10962

Leucci, E., Zriwil, A., Gregersen, L. H., Jensen, K. T., Obad, S., Bellan, C., . . . Lund, A. H. (2012). Inhibition of miR-9 de-represses HuR and DICER1 and impairs Hodgkin lymphoma tumour outgrowth in vivo. Oncogene, 31(49), 5081-5089. doi:10.1038/onc.2012.15

Moccia, A. A., Donaldson, J., Chhanabhai, M., Hoskins, P. J., Klasa, R. J., Savage, K. J., . . . Sehn, L. H. (2012). International Prognostic Score in Advanced-Stage Hodgkins Lymphoma: Altered Utility in the Modern Era. Journal of Clinical Oncology, 30(27), 3383-3388. doi:10.1200/jco.2011.41.0910

Navarro, A., Diaz, T., Martinez, A., Gaya, A., Pons, A., Gel, B., . . . Monzo, M. (2009). Regulation of JAK2 by miR-135a: Prognostic impact in classic Hodgkin lymphoma. Blood, 114(14), 2945-2951. doi:10.1182/blood-2009-02-204842

Navarro, A., Gaya, A., Martinez, A., Urbano-ispizua, A., Pons, A., Balague, O., &

Gel, B. (2008). MicroRNA expression profiling in classic Hodgkin lymphoma.

Blood, 111(5), 2825–2832. doi.org/10.1182/blood-2007-06-096784.

Nie, K., Gomez, M., Landgraf, P., Garcia, J., Liu, Y., Tan, L. H., . . . Tam, W. (2008). MicroRNA-Mediated Down-Regulation of PRDM1/Blimp-1 in Hodgkin/Reed-Sternberg Cells: A Potential Pathogenetic Lesion in Hodgkin Lymphomas. The American Journal of Pathology, 173(1), 242-252. doi:10.2353/ajpath.2008.080009

Paydas, S., Acikalin, A., Ergin, M., Celik, H., Yavuz, B., & Tanriverdi, K. (2016). Micro-RNA (miRNA) profile in Hodgkin lymphoma: Association between clinical and pathological variables. Medical Oncology, 33(4). doi:10.1007/s12032-016-0749-5

Sánchez-Espiridión, B., Martín-Moreno, A. M., Montalbán, C., Figueroa, V., Vega, F., Younes, A., . . . Garcia, J. F. (2013). MicroRNA signatures and treatment response in patients with advanced classical Hodgkin lymphoma. British Journal of Haematology, 162(3), 336-347. doi:10.1111/bjh.12390

Saraswathi, D., Bama, D. S., & Kumar, R. A. (2017). Diagnostic Challenges in Cytodiagnosis of Hodgkin Lymphoma – An Experience in Rural Based Tertiary Care Hospitals. Int J Sci Res. 6(9), 305–307.

Vlierberghe, P. V., Weer, A. D., Mestdagh, P., Feys, T., Preter, K. D., Paepe, P. D., . . . Speleman, F. (2009). Comparison of miRNA profiles of microdissected Hodgkin/Reed-Sternberg cells and Hodgkin cell linesversusCD77 B-cells reveals a distinct subset of differentially expressed miRNAs. British Journal of Haematology, 147(5), 686-690. doi:10.1111/j.1365-2141.2009.07909.x

Yuan, Y., Kluiver, J., Koerts, J., Jong, D. D., Rutgers, B., Razak, F. R., . . . Berg, A. V. (2017). MiR-24-3p Is Overexpressed in Hodgkin Lymphoma and Protects Hodgkin and Reed-Sternberg Cells from Apoptosis. The American Journal of Pathology, 187(6), 1343-1355. doi:10.1016/j.ajpath.2017.02.016

Zhu, M., Xu, Z., Wang, K., Wang, N., Zhu, M., & Wang, S. (2013). MicroRNA and gene networks in human Hodgkin's lymphoma. Molecular Medicine Reports, 8(6), 1747-1754. doi:10.3892/mmr.2013.1741