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PARP And BRCA Genes As Representatives Of DNA Repair Pathway In Acute Myeloid Leukemia Patients

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Background: Acute myeloid leukemia (AML) is a heterogenous neoplasm in which myeloblasts are accumulated in bone marrow (BM) causing hematopoietic failure. PARP1 and BRCA1 play a crucial role as DNA damage sensors and contributes to AML progression. This study aimed at analyzing the levels of expression of PARP1 and BRCA1 genes in denovo AML cases.

Patients and Methods: Enrollment consisted of 50 newly diagnosed AML cases and 20 healthy controls. BRCA1 and PARP1 gene expression levels were estimated by (qPCR).

Results: Statistically significant elevation of PARP 1and reduction of BRCA1 expression in AML group compared to control (P=0.047,0.003 respectively). High PARP1 expression level was significant risk predictor for shorter OS and DFS in AML patients (P=0.003,0.001 respectively). However BRCA expression was insignificant risk predictor for both short OS or DFS (P>0.05).Cumulative incidence of DFS was significantly reduced in AML cases with aberrant expression of CD7(p=0.003).

Conclusion: Our results showed enhanced PARP-1 expression in AML patients suggesting it is a risk predictor and a possible therapeutic target for AML treatment. Also, reduction of BRCA1 expression level in AML patients that was insignificant risk predictor for both short OS or DFS,Cumulative incidence of DFS was significantly reduced in AML cases with aberrant expression of CD7.

Key words: AML, PARP1, BRCA1 gene expression, Prognosis, Overall survival, Disease free survival.

1. Introduction

Acute myeloid leukemia (AML) is a genetically heterogeneous neoplasm which results from genetic changes in normal hematopoietic stem cells. Such changes cause disruption of the normal differentiation and/or increased proliferation of abnormal immature leukaemic cells known as blast cells. With the progression of AML, blasts are accumulated in the bone marrow (BM) and blood as well as in organs. They inhibit the production of normal blood cells resulting in fatal infections, hemorrhages, and organ infiltration in the absence of therapy within 12 months after diagnosis (Wu et al., 2023).

AML is the commonest acute leukemia affecting adults and account for 80% of cases (Tsai and Hou, 2024). In Egypt, leukemia accounts for 10% of all cancers, with AML representing 16.9% (Afaf and Mahdia, 2022). AML can develop de novo or secondary to the progression of other diseases or to the use of cytotoxic drugs (Zjablovskaja and Florian, 2019).

The diagnostic workup involves full history taking, clinical picture, peripheral blood smears morphologic evaluation of BM aspirates and flow cytometry (FC), cytogenetic tests, as well as molecular mutation analysis (Haferlach and Schmidts, 2020). About 50% - 60% of newly diagnosed AML have cytogenetic abnormalities (Zhao et al., 2020). The chromosomal abnormalities include translocations, inversions and deletions. Besides, these cytogenetic abnormalities, gene mutations are also key events in AML pathogenesis (Handschuh and Lonetti, 2019).

These alterations can be clustered in three groups: (1) mutations contributing to cell proliferation such as FLT3, c-KIT, RAS, PTPN11 genes; (2) mutations of genes that have a role in myeloid differentiation such as AML1 and CEBPA genes and (3) mutations affecting epigenetic modifiers such as DNTM1, IDH1/2 genes, chromatin modifiers as NPM1, EZH2 genes and tumour suppressor genes like TP53,WT1 (Papayannidis et al., 2020).

The analysis of these alterations at diagnosis allows the identification of prognostically discrete subtypes of AML which in turn enable the adoption of risk-adapted therapeutic strategies. Yet the survival outcome of AML patients remains poor, indicating the need for more effective and less toxic therapeutic agents (Curti et al., 2020).

The DNA damage response (DDR) pathway removes replication errors that occurred from single strand breaks (SSB) or double strand breaks (DSB) and functions as tumor suppressors. Damaged SSB is repaired by base excision repair (BER), nucleotide excision repair (NER), or mismatch repair (MMR) which are dependent mainly on PARP1, PARP2, XRCC1 genes, while homologous recombination (HR), or non- homologous end joining (NHEJ) repair the damaged DSB (Park et al., 2021).

PARP-1 is the most abundantly expressed protein of PARP family, it detects SSB during BER and binds to damaged DNA via the N-terminal zinc finger domain to initiate poly-ADP scaffold formation. The latter recruits other members (e.g. XRCC1) of the BER pathway (Fritz et al., 2021). Blocking of PARP-1 inhibits BER, resulting in accumulation of SSBs and DSBs, which in turn activate HR repair, that is dependent on BRCA1, BRCA2, PALB2, and RAD51 (Keung, Wu, and Vadgama, 2019).

The most critical proteins in HR are BRCA1 and BRCA2 however, these two genes are often mutated in tumors, leading to defects in HR. Without effective HR repair, cells use non-conservative forms of DNA repair such as non-homologous end joining (NHEJ), which may generate large scale genomic rearrangements leading to the lethality of tumor cells (Fritz et al., 2021).

In AML; BRCA mutations are uncommon, but low expression of BRCA1/2 were already reported in AML leading to lack of function of these proteins involved in HR pathway or in DSB sensing causing HR-deficiency and confer tumor cells a “BRCAness” phenotype

rendering them extremely sensitive to PARP inhibitors that can be considered as a promising potential treatment for AML (Vikas et al., 2020).

Aim of work was to analyze the median expression levels of PARP1 and BRCA1genes in newly diagnosed AML patients and study the correlation between gene expression patterns and different available prognostic markers including cytogenetic and molecular aberrations, response to treatment, the overall survival and disease-free survival.

2. Patients and methods:

This study is a case-control study, which included 2 groups of adult patients and controls. The patient group included 50 newly diagnosed AML patients (33 male, 17 female), aged 18-85 years . All AML patients were recruited from Oncology center of Mansoura University, from June 2021 to June 2022. Clinical data regarding detailed clinical history, physical examination and laboratory investigations were collected. Patients were treated by induction chemotherapy 7+3 protocol and response assessment were according to ELN recommendation 2022. Patients who achieved complete remission, consolidation therapy by HDA ± Allogeneic stem cell transplant based on risk assessment and donor availability, while relapsed/refractory cases received salvage chemotherapy ± Allogeneic stem cell transplant.

Control group included 20 control group of apparently healthy normal individuals with normal peripheral blood picture, age and sex matched with the patients. The study was approved by the Medical Research Ethics Committee IRB (IRB code no MD.21.04.480).

Specific Molecular Laboratory Investigation were done including estimation of median gene expression level of BRCA1 and PARP1 by qPCR after BM mononuclear cells isolation, RNA extraction and reverse transcription into cDNA.

Mononuclear cells were separated from BM samples of AML patients using Ficoll Hypaque density gradient centrifugation (Lonza, Walkersville, MD, US) (Li et al., 2018), RNA was then isolated from mononuclear cells using miRNeasy Mini kits (Qiagen, Germantown, MD, US). The concentration and purity of RNA were determined with NanoDrop (thermo Scientific, US). cDNA was synthesized from 2μg RNA using high-capacity reverse transcription kit (Applied Biosystems, Foster City, CA, US) based on the manufacturer's guidelines. The qPCR was carried out on StepOne™ using TaqMan gene expression assay for PARP1, BRCA1 genes (Thermo scientific, Life Technologies, Grand Island, NY, US). Housekeeping gene was utilized as internal control. The Ct values were converted to fold changes compared to normal peripheral blood controls and $2^{-\Delta\Delta Ct}$ method was applied for the analysis of fold changes of the AML and measure the relative gene expression (Park et al., 2021).

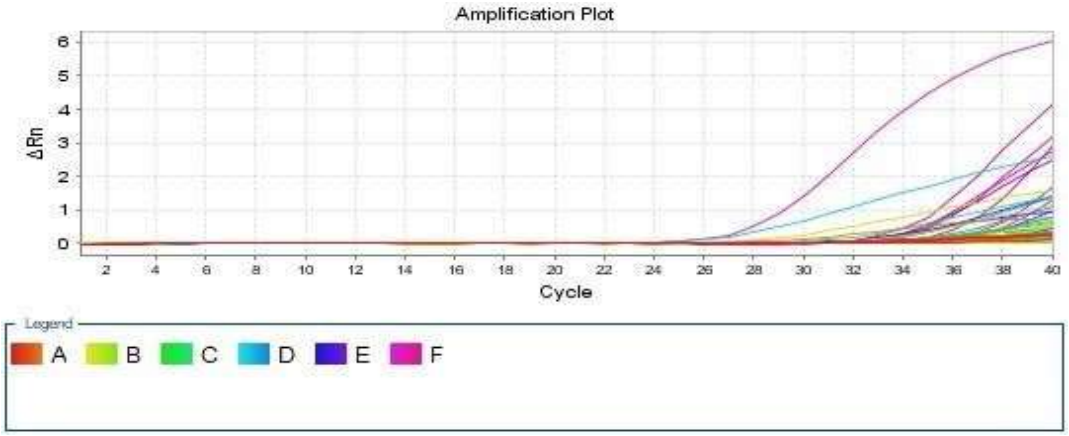


Figure (1): Amplification plot generated by real time PCR.

Sample Size calculation

- Sample size was calculated using online sample size calculator (https://clincalc.com/stats/samplesize.aspx) with calculated mean of PARP-1 expression level (Li et al., 2018), level of alpha error of 5% and study power of 95%.
- A minimal sample size required for the study was calculated to be 45 subjects for AML group. To account for possible, drop out, a total sample of 50 subjects was initially planned to be included in the study for both AML Beside 20 apparently healthy individuals served as control group.

Statistical analysis:

Statistical analysis (SPSS (Version 25.0): A p-value <0.05 was considered significant. mean, SD and median were used for numerical data. Normality was tested with Kolmogorov-Smirnov and Shapiro-Wilk tests. The Mann-Whitney U and Kruskal-Wallis tests were used for group comparisons. Chi-square/Fisher's exact test examined relationships between qualitative variables, and Spearman's correlation assessed associations between quantitative variables. The ROC curve assessed diagnostic sensitivity and specificity, with AUC values indicating test quality.Corp.).

3. Results

The study was conducted on 50 newly diagnosed AML patients. The AML group included 50 patients (17 women and 33 men) aged 18 - 85 years, their median age is 36 years. At diagnosis the median WBCs count of cases was 59 $\times 10^9$/L, the mean of HB was 8.2 gm/dl ±2.21, the median PLT count was 28.5×10^9 /L, median bone marrow blast cells was 90.0%. The patients were classified according to molecular and cytogenetics , 68 % of cases had favorable risk stratification, 22 % of cases were intermediate risk group and 10 % were poor risk group. Complete remission (CR) was achieved in 34 cases (68.0%), partial remission (PR) was achieved in 3 cases (6%), there were 9 cases (18%) with refractory disease (RD) and only 4 cases (8%) were induction death. Of 34 cases with previous complete remission, 15 cases (44.1%) had relapsed Table (1).

Table (1): Demographics, clinical features and laboratory data of acute myeloid leukemia patient group.

AML patients (N=50)		
Median age (years)		36.0 (18-82)
Gender	Males, (N (%))	33 (66.0%)
	Females, (N (%))	17 (34.0%)
WBCS at diagnosis, median (range)		59.0 (0.5-310) $\times 10^9$ /L
Hemoglobin at diagnosis, Mean ± SD		8.2 ± 2.21 g/dl
PLT at diagnosis, median (range)		28.5 (5.5-313) $\times 10^9$ /L
Bone marrow blast (%), median (range)		90 (22-95)
FAB classification	M1, (N (%))	18 (36.0%)
	M2 , (N (%))	9 (18.0%)
	M4, (N (%))	21 (42.0%)
	M5, (N (%))	2 (4.0%)
Molecular and cytogenetics (No positive/No tested (%)	t(8;21) , (N (%))	4/47 (8.5%)
	Inver16, (N (%))	18/47 (38.3%)
	11q23, (N (%))	0/6 (0.0%)
	t(9;22) , (N (%))	0/2 (0.0%)
	FLT3, (N (%))	5/48 (10.4%)
Risk stratification	Favorable, (N (%))	34 (68.0%)
	Intermediate, (N (%))	11 (22.0%)
	Poor, (N (%))	5 (10.0%)
Induction remission response	CR, (N (%))	34 (68.0%)
	PR, (N (%))	3 (6.0%)
	RD, (N (%))	9 (18.0%)
	ID, (N (%))	4 (8.0%)
Relapse (n = 34)	No relapse, (N (%))	19 (55.9%)
	Relapse, (N (%))	15 (44.1%)
Outcome	Alive, (N (%))	20 (40.0%)
	Dead, (N (%))	30 (60.0%)

Table (2) shows PARP1 and BRCA1 expression, there was significant elevation of PARP1 median expression level among AML cases in comparison with control group (P=0.047) and significant reduction of BRCA1 median expression level among AML cases as compared to control group (P=0.003). The median value of expression was utilized to classify patients into low and high expressor groups.

Table (2): Comparison of PARP1 and BRCA1 expressions in studied groups:

	Control (N=20)	AML (N=50)	P
PARP1 Expression	0.277 (0.003-6.467)	0.561 (0.011-79.893)	0.047
BRCA1 expression	4.356 (0.004-55.715)	0.188 (0.001-39.396)	0.003

The WBCs and BM blast were significantly elevated in AML subgroup with high PARP-1 expression in comparison with AML subgroup with low PARP-1 expression (P=0.028, 0.021 respectively). FLT3 mutation, poor risk stratification, relapse and death were significantly more frequent in AML subgroup with high PARP-1 expression in comparison with AML subgroup with low PARP-1 expression (P=0.049, 0.023, 0.001, 0.004 respectively). No significant association existed between BRCA 1 expression level and different clinical and laboratory data in AML patients could be detected as shown in **Table (3)**.

Table (3): Clinical outcome and laboratory data of high and low PARP-BRCA1 expression

Parameter		Low expression PARP-1	High expression PARP-1	P	Low expression BRCA1	High expression BRCA1	P
Age	**Median (Min-Max)**	32.0 (18-82)	46.0 (18-82)	0.097	35.0 (18-82)	37.0 (18-82)	0.662
Gender	**Males**	15 (60.0%)	18 (72.0%)	0.370	17 (68.0%)	16 (64.0%)	0.765
	Females	10 (40.0%)	7 (28.0%)		8 (32.0%)	9 (36.0%)	
Hb g/dl	**Mean ± SD**	8.25 ± 1.87	8.16 ± 2.55	0.888	8.07 ± 2.22	8.35 ± 2.24	0.667
WBCS $\times 10^9$ /L	**Median (Min-Max)**	25.6 (0.5-257)	94.5 (2.8-310.0)	**0.028**	54.3 (0.5-310)	70.7 (2.2-285.0)	0.691
PLT $\times 10^9$ /L	**Median (Min-Max)**	30.0 (6-313)	28.0 (5.5-98.5)	0.541	30.0 (6.0-313)	28.0 (5.5-125.0)	0.560
BM blast %	**Median (Min-Max)**	83.5 (22-95)	90.0 (35-95)	**0.021**	90.0 (22-95)	90.0 (35-95)	0.786
FAB	**M1-M2**	13 (52.0%)	14 (56.0%)	0.777	14 (56.0%)	13 (52.0%)	0.777

	M4-M5	12 (48.0%)	11 (44.0%)		11 (44.0%)	12 (48.0%)	
Molecular and Cytogenetics	**FLT3**	0.0%	5 (20.8%)	0.049	2 (8.7%)	3 (12.0%)	1.00
	Inver16	11 (45.8%)	7 (30.4%)	0.278	9 (39.1%)	9 (37.5%)	0.908
Risk stratification	**Good**	20 (80.0%)	13 (52.0%)	**0.023**	14 (56.0%)	19 (76.0%)	0.326
	Intermediate	5 (20.0%)	6 (24.0%)		7 (28.0%)	4 (16.0%)	
	Poor	0 (0.0%)	6 (24.0%)		4 (16.0%)	2 (8.0%)	
Response	**CR**	20 (80.0%)	14 (56.0%)	0.069	14 (56.0%)	20 (80.0%)	0.069
	Non-CR	5 (20.0%)	11 (44.0%)		11 (44.0%)	5 (20.0%)	
Relapse	**Non Relapsed**	16 (80.0%)	3 (21.4%)	**0.001**	9 (64.3%)	10 (50.0%)	0.409
	Relapsed	4 (20.0%)	11 (78.6%)		5 (35.7%)	10 (50.0%)	
Living status	**Lived**	15 (60.0%)	5 (20.0%)	**0.004**	11 (44.0%)	9 (36.0%)	0.564
	Dead	10 (40.0%)	20 (80.0%)		14 (56.0%)	16 (64.0%)	

ROC analysis was conducted to identify the PARP1 and BRCA1 expressions for prediction of dead cases. PARP1 best cut-off values for prediction of dead cases were above 0.431. The area under the curve (AUC) was 0.707 (P=0.014). BRCA1 best cut-off values for prediction of dead cases were above 0.188. The area under the curve (AUC) was 0.557 (P=0.501) as shown in figure (3)

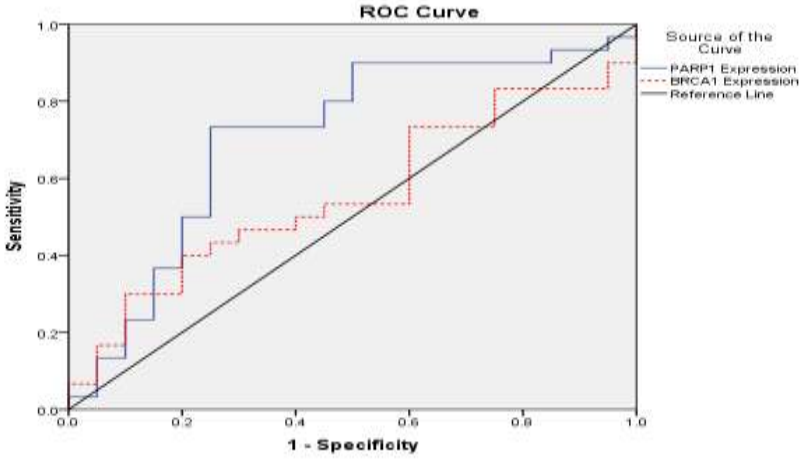


Figure (2): The ROC curve of PARP1 and BRCA1 expression for prediction of death status in AML group.

As shown in Table (4) COX Regression analysis was conducted for predicting factor (s) affecting OS using age, gender, laboratory data, type, risk stratification, response, relapse, PARP1 and BRCA1 as covariates. In AML cases the significant independent factors for shorter OS in AML patients in univariate analysis of AML patients; older cases (P=0.001) intermediate risk stratification (P<0.001), poor risk classification (P=0.004), non-CR (P<0.001), relapsed cases (P=0.006), and high PARP1 expression (P=0.003) were significant independent factors for shorter OS in AML patients in univariate analysis. Moreover, intermediate risk classification, non-CR and high PARP1 expression were significant independent factors for shorter OS in AML patients in multivariate analysis (P 0.025, <0.001, 0.049 respectively). While intermediate risk classification, and high PARP1 expression were significant independent factors for shorter DFS in AML patients in univariate analysis (P=0.037, 0.001 respectively) and multivariate analysis (P=0.005, 0.001 respectively).

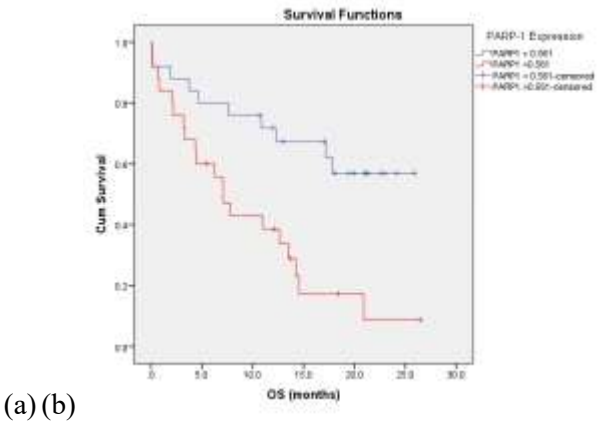
BRCA1 expression is insignificant risk predictor for shorter OS or shorter DFS (P=0.344,0.935 respectively).

Table (4): Cox regression analysis to predict the hazardous factor that could predict overall survival and disease-free survival in AML patients:

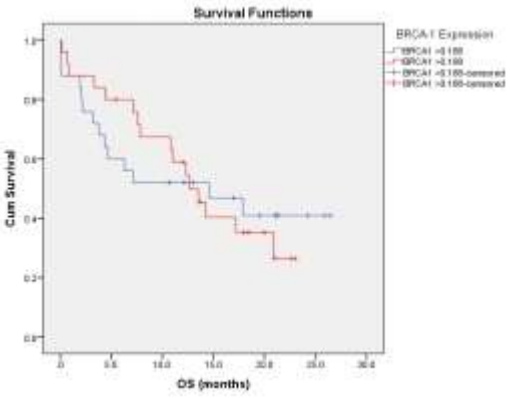
Parameter	Univariable (OS)			Multivariable (OS)		
	P	**HR**	**95 % CI**	**P value**	**HR**	**95 % CI**
Age	**0.001**	**1.032**	**1.012-1.052**	0.866	1.003	0.973-1.034
Gender	0.173	0.568	0.252-1.281			
WBCs	0.415	1.002	0.998-1.006			
Hemoglobin	0.126	0.876	0.739-1.038			
Platelets count	0.791	0.997	0.972-1.022			
LDH	0.310	1.00	0.999-1.00			
Bone marrow blast %	0.546	1.002	0.996-1.008			
FAB classification **M1-M2** **M4-M5**	 R 0.511	 1 0.784	 - 0.379-1.620			
FLT3	0.151	2.184	0.753-6.337			
Inver16	0.332	0.675	0.305-1.494			
Risk stratification **Favorable** **Intermediate** **Poor**	 R **<0.001** **0.004**	 1 **4.463** **4.588**	 - **1.930-10.318** **1.635-12.875**	 R **0.025** 0.984	 1 **7.713** 4.495	 - **1.287-46.233** 0.982-8.293
Response (Non-CR vs CR)	**<0.001**	**14.370**	**6.166-33.494**	**<0.001**	**12.278**	**3.917-38.486**
Relapse	**0.006**	**5.263**	**1.629-17.00**	0.236	2.485	0.552-11.199

(relapsed vs non-relapsed)						
PARP1 expression (high vs low)	**0.003**	**3.234**	**1.486-7.037**	**0.049**	**3.993**	**1.280-18.126**
BRCA1 expression (high vs low)	0.935	0.971	0.473-1.993			
Parameter	**Univariable (DFS)**			**Multivariable (DFS)**		
	P	**HR**	**95 % CI**	**P value**	**HR**	**95 % CI**
Age	0.448	1.012	0.981-1.043			
Gender	0.098	0.343	0.097-1.220			
WBCs	0.655	1.001	0.995-1.007			
Hemoglobin	0.978	0.833	0.706-1.248			
Platelets count	0.896	0.999	0.988-1.010			
LDH	0.787	1.00	0.999-1.001			
Bone marrow blast %	0.672	1.009	0.968-1.052			
FAB classification **M1-M2** **M4-M5**	 R 0.517	 1 1.407	 - 0.500-3.959			
FLT3	0.082	3.873	0.843-17.784			
Inver16	0.331	0.555	0.169-1.820			
Risk stratification **Favorable** **Intermediate** **Poor**	 R **0.037** 0.370	 1 **4.073** 2.576	 - **1.090-15.227** 0.326-20.372	 R **0.005**	 1 **9.505**	 - **2.008-44.990**
PARP1 expression (high vs low)	**0.001**	**9.826**	**2.607-37.036**	**0.001**	**14.818**	**3.544-61.966**
BRCA1 expression (high vs low)	0.344	1.683	0.573-4.945			

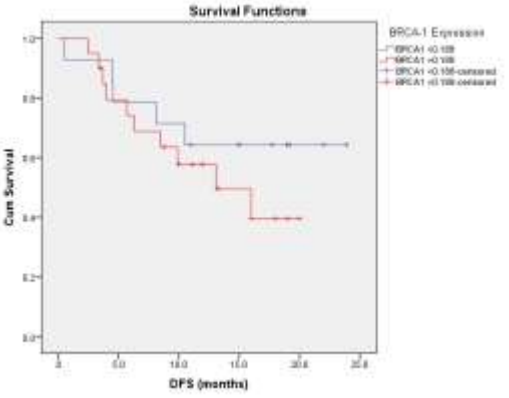
As regard median value of PARP-1 expression ,patients with high PARP1 expression have shorter OS and DFS than those with low PARP1 expression (OS 38.6% vs 71.8% at 12 months interval (P 0.002) , DFS estimates 23.4% vs 85.0% at 11 months interval , also As regard median value of BRCA1 expression, OS and DFS shows no significant difference between high and low BRCA1 expressor groups (OS estimates 58.9% vs 52.0% at 12 months interval (P 0.935),DFS 57.8% vs 64.3% at 11 months interval (P 0.338). as in figure (3)



(a) (b)



(c)



(d)

Figure (3): a- OS profile in AML patients with high and low PARP-1 expression.
b-DFS profile in AML patients with high and low PARP-1 expression**.**
c-OS profile in AML patients with high and low BRCA-1 expression.
d-DFS profile in AML patients with high and low BRCA-1 expression.

Regarding the effect of aberrant expression of CD56 and CD7 on clinical course of AML patients. Cumulative incidence of disease-free survival (DFS) was significantly reduced in AML cases with aberrant expression of CD7 (P=0.003). Otherwise, no significant association between CD7 and CD56 expression on the overall survival (OS) and other parameters of clinical course of AML as demonstrated in Table (5).

Table (5) Effects of expression of CD56 and aberrant expression CD 7 on outcome :

Parameter		CD7		CD56		P[1]	P[2]
		Negative	Positive	Negative	Positive		
Response	CR	26 (66.7%)	8 (72.7%)	30 (73.2%)	4(44.4%)	1.00	0.124

	Non-CR	13 (33.3%)	3 (27.3%)	11 (26.8%)	5 (55.6%)		
Relapse	Non-relapsed	16 (61.5%)	3 (37.5%)	17 (56.7%)	2 (50.0%)	0.417	1.00
	Relapsed	10 (38.5%)	5 (62.5%)	13 (43.3%)	2 (50.0%)		
Outcome	Alive	16 (41.0%)	4 (36.4%)	17 (41.5%)	3 (33.3%)	1.00	0.724
	Dead	23 (59.0%)	7 (63.6%)	24 (58.5%)	6 (66.7%)		
Cumulative incidence of OS*		39.4%	21.2%	35.9%	33.3%	0.634	0.119
Cumulative incidence of DFS*		66.1%	12.5%	51.7%	50.0%	**0.003**	0.562

4. Discussion:

In our study , the aim was to analyze the median expression levels of PARP1 and BRCA1genes in newly diagnosed AML patients and study the correlation between gene expression patterns and different available prognostic markers including cytogenetic and molecular aberrations, response to treatment, the overall survival and disease-free survival.

As regard PARP1 expression, there was significant elevation of PARP1 expression in AML group in comparison with controls (P=0.047) as shown in table (2).

In consistence with our results, Gil-Kulik et al. 2020 evaluated the expression of PARP1,genes in 53 Poladian AML patients, and found that BM cells of AML patients showed higher PARP1 expression than normal controls (P=0.02), that was associated with shorter OS of cases (P=0.003), indicating its prognostic significance in AML.

Our findings also agreed with that reported by Wang et al. 2015 who revealed PARP-1 overexpression among AML cases compared to normal controls. Similarly, Li et al., 2018 examined the expression of PARP-1 in 339 cytogenetically normal AML patients by RT-PCR in China. It was found that PARP-1 expression was significantly increased in AML cases in comparison with normal BM cells, Yaghmaie et al. 2018 also evaluated PARP-1 expression in 65 cases with non-M3 AML and agreed with our results in that AML cases showed higher PARP-1 expression in comparison to control group ($p < 0.001$). Pashaiefar et al. 2018 conducted study on 80 cases of AML patients compared to 19 healthy individuals as control group in Iran to assess relative expression of PARP1 gene and like our results, it was found that AML cases had higher PARP-1 expression in comparison to control group (P=0.0004). Unlike our study, Faraoni et al. 2015 conducted a study evaluated expression of PARP1 in 25 patients with denovo AML and 10 healthy donors and found that PARP1 expression in blast cells did not differ significantly from that in normal cells (P=0.07).

As regard correlation of PARP-1 expression with clinical and laboratory features of AML patients, TLC and BM blast were significantly elevated in AML patients with high PARP-1 expression compared to AML patients with low PARP-1 expression (P<0.05).

Adverse molecular risk as FLT3 mutation, poor risk stratification, relapse and death were significantly more frequent in AML patients with high PARP-1 expression compared to AML patients with low PARP-1 expression (P<0.05). We found no statistically significant difference between patients with high PARP-1 expression compared to AML patients with low PARP-1 expression regarding age, gender, hemoglobin, platelet count, distribution of FAB classification, adverse cytogenic risk as well as the rates of CR after induction therapy (P>0.05) as mentioned in table (3).

In agreement with our results, Li et al., 2018 has found that patients with high PARP-1 expression had more BM blasts (p=0.003), elevated peripheral blood WBC levels p=0.008) and a higher incidence of FLT3-ITD mutation (P =0.031). Yaghmaie et al. 2018 also found no statistically significant difference between PARP-1high and PARP-1low patients according to the age, gender, hemoglobin, platelet count, as well as in distribution of FAB classification (P <0.05), also found that Patients with adverse cytogenetic risk have higher PARP-1 expression than other cytogenetic risk groups (p = 0.004). Like our results, Pashaiefar et al. 2018 has found that PARP1 was significantly upregulated in poor prognosis AML patient group with adverse cytogenetic and molecular risk when compared with the good or intermediate prognosis subgroup (P=0.01).

Unlike our results the study conducted by Yaghmaie et al., 2018 found no statistically significant difference between PARP-1 high and PARP-1 low patients according to the peripheral white blood cells (WBCs) and percentage of BM blasts but revealed a statistically significant difference between the two groups of patients in the rates of CR after induction therapy (p = 0.04).

In our study on AML cases we found that older cases >50, intermediate and poor risk classification, non-CR, relapsed cases, and high PARP1 expression were significant independent factors for shorter OS in AML patients in univariate analysis (P<0.05) .Moreover, intermediate risk classification, non-CR, relapsed cases, and high PARP1 expression were significant independent factors for shorter OS in AML patients in multivariate analysis (P<0.05). Also, intermediate risk classification, and high PARP1 expression were significant independent factors for shorter DFS in AML patients in univariate analysis and multivariate analysis (P<0.05) as mentioned in table (4).

In agreement with our study, the study conducted by Li et al., 2018 on 339 Chinese AML patients assessed their PARP-1 expression by real-time PCR and relation between PARP1 expression and the clinical characteristics and prognosis of the patients has found that overall survival (OS) and event free survival (EFS) were significantly shorter in older AML patients >60 years, AML patients with FLT3-ITD mutation (P<0.001) and patients with high PARP1expression (OS, P =.005 and EFS, P=.004). Yaghmaie et al., 2018 agreed with our study and found that high expression levels of PARP-1 were associated with worse overall survival (OS) (p = 0.01) and relapse-free survival (RFS) (p = 0.005) than low expressor group.

The PARP1 overexpression in AML patients can be explained by that the induced replication and oxidative stress in AML leading to accumulation of DNA damage in AML blast cells. Therefore, PARP-1expression is induced to detect DNA breaks. It then enhances the covalent binding of ADP-ribose units to itself (automodification) or other nuclear proteins

(heteromodification). This highlights the importance of PARP-1 in the pathogenic process and progression of hematological cancers (Trombetti et al., 2021).

The abnormal DNA repair resulting from increased PARP-1 expression might increase the antiapoptotic properties of AML blasts as PARP-1 upregulates the expression of myeloproliferative leukaemia virus oncogene via poly ADP-ribosylation, which in turn induces PI3K-AKT and MAPK pathways, induces the proliferation of AML blasts and inhibits apoptosis. This explains decreased response to chemotherapeutic agents and short OS. This finding suggested that inhibition of PARP-1 might be an effective option to overcome chemoresistance in AML and prolong OS (Kontandreopoulou et al., 2021).

In addition, PARP-1 has a role in DNA methylation as it regulates the expression levels and protein activity of DNA methyltransferase-1. Of note, AML cells have abnormal patterns of DNA methylation, and AML cases may benefit from combinations of hypomethylating agents and PARP inhibitors (Yaghmaie et al. 2018).

Also, PARP overexpression has been linked to poor clinical outcomes in AML cases. In AML cells, PARP contributes to immune evasion by 2 mechanisms: (1) apoptosis of anti-tumor immune cells, and (2) inhibition of the expression of NK-cell activating receptor-ligand on AML cells making them unrecognized by NKs and T lymphocytes (Vago and Gojo, 2020).

In our study, as regard BRCA1 expression, there was significant reduction of BRCA1 expression among AML cases in comparison with controls (P=0.003) as in table (2), BRCA expression has no effect and was insignificant independent factor for shorter overall survival and DFS in AML patients as in table (4)

This result, on its own, is particularly significant since several PARP inhibitors can induce synthetic lethality in BRCA1 or BRCA2 mutated cancers with a defective repair of DNA double strand breaks (DSB) by homologous recombination and have been well-tolerated in clinical trials in BRCA1,2 deficient breast and ovarian cancers and may therefore be potential candidates and promising target therapy for AML with low expression of BRCA1,2 genes (Park et al., 2021).

It was already reported that AML cells with low expression of RAD51, ATM, BRCA1, and BRCA2, displayed a significant response to PARP inhibitors (Li et al., 2022). In agreement with our results, Faraoni et al. 2015 examined 25 denovo AML cases and 10 healthy donors to evaluate BRCA1 transcription. It has found that AML blast cells expressed lower BRCA1/2 levels than BM cells from healthy controls supporting a less effective HR repair that may account for the sensitivity to PARP inhibitor Olaparib.

Another similar study done by Poh et al. 2019 found that BRCA1 expression levels were lower in AML cases than levels in normal BM cells (P<0.0001) due to promotor Hypermethylation of BRCA1 gene that is associated with lower mRNA transcription and protein expression. Another study done by Podszywalow-Bartnicka et al. 2014 has also revealed TIAR-dependent downregulation of BRCA1 levels in leukemic blasts.

Unlike our results, Park et al. 2021 examined170 AML cases in Japan (15 APL cases and 155 non-APLcases). They found increased expression levels of BRCA1 in non-APL

patents more than APL patients. In agreement with our results, A study conducted on 339 patients of AML in China and asses the relation between BRCA 1 expression and overall survival in that the expression of BRCA1 in AML patients had no significant correlation with prognosis or short overall survival (Li et al., 2018).

Unlike our results, a Comprehensive analysis of expression of BRCA1,2 repair genes was conducted in Japan on 170 AML patients included 15 APL and 155 non-APL AML patients found The BRCA overexpressed group showed lower CR rate, shorter overall survival and early death rate than the non-overexpressed group in non- APL AML patients (P=0.0286,P=0.0012, 0.0378) respectively and explained this by that BRCA is DNA repair gene so its upregulation in AML patients may allow error prone DNA to repair in cancer cells, leading to consistently survive and may exert resistance to chemotherapy (Park et al., 2021). In our study, Among the 50 cases studied, CD7 was the most frequently aberrant expressed in 11 AML cases (8 cases were AML M1,2 and 3 AML 4) .CD56 was expressed in 9 cases (6 cases of them were AML 1,2 and 3 AML M4,5) as shown in table (5).

A study done by Gupta et al. 2021 agreed with our results and showed that CD7 was the most frequently expressed Ag in 15 cases (32.6%) (11 cases of these 15 cases were AML M 1,2 and 4 cases were AML M4) followed by CD56 which was found in 10 patients (21.7%) (6 cases were M1,2 and 4 cases were AML M4).

Most of the aberrantly expressed nonmyeloid markers investigated were detected in the acute myeloblastic leukemia cases (M1,2). This can be explained by the fact that these markers were expressed early in hematopoietic ontogeny in the lesser-differentiated acute myeloid leukemia subtypes, including FAB M0, M1, and M2 (Gupta et al., 2021).

Bai et al., 2024 explained that CD7 expression in AML is associated with the expression of the immature antigens CD34, HLA-DR, and terminal deoxynucleotidyl transferase (TdT). they also found a diminished response to a standard therapeutic regimen and Therefore, contend that AML with CD7 may originate from early hematopoietic precursors and indicate biologic aggressiveness in a significant proportion of patients.

In our study, the cumulative incidence of DFS was significantly reduced in AML cases with aberrant expression of CD7(P=0.003) with no effect on remission rate or overall survival (P>0.05). No significant association between CD56 expression and clinical outcome of AML cases either remission rate, OS and DFS (P>0.05) as shown in table (4).

A study conducted by Jaddaoui et al., 2022 agreed to our results and showed that the CD7+ phenotype was significant adverse prognostic factor for AML clinical outcome. They suggested an origin from common progenitors between the NK cell and the myeloid lineage They concluded that the CD7+ and CD56+ myeloid/NK cell precursor acute leukemia might constitute a distinct biological and clinical disease entity.

A study done by Gupta et al. 2021 showed that CD7 and CD56 expression at diagnosis associated with a low remission rate and biological aggressiveness in AML patients (P=0.004) They suggested that CD7 and CD56 expression be evaluated in all patients with acute leukemias at the time of diagnosis in view of the poor clinical outcome.

Unlike our results, A study was done by Hussein and Jawad, 2021

has found that CD7 expression in AML has no effect on OS or DFS but was associated with decreased complete response rates (P=0.03)

5. Conclusion

- There was significant elevation of the median expression level of PARP1 in acute myeloid leukemia (AML) group compared to control group and significant reduction of BRCA1 median expression level in AML group compared to control group.
- Patients with intermediate and poor risk classification, non-complete remission cases, relapsed cases, and high PARP1 expression were significant adverse independent factors for shorter OS and DFS in AML patients.
- BRCA1 expression was insignificant risk predictor for both short overall survival and disease free survival
- Cumulative incidence of DFS was significantly reduced in AML cases with aberrant expression of CD7.

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