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Peripheral T Cell Subpopulation in Patients with Hepatocellular Carcinoma: Relation to Ablation Therapy

Walid El Sherbiny[1], **Raghda E Farag**[1], **Shaker Wagih Shaltout**[1*], **Muhammad Diasty**[1] **and Nashwa Khairat Abousamra**[2]

[1]*Department of Tropical Medicine, Faculty of Medicine, Mansoura University Hospital, Algomhoria Street, Mansoura, Egypt*

[2]*Clinical Pathology Department, Faculty of Medicine, Mansoura University, Egypt*

[*]**Corresponding author:** Shaker Wagih Shaltout, Department of Tropical Medicine, Faculty of Medicine, Mansoura University Hospital, Algomhoria Street, Mansoura 35516, Egypt, Tel: 201001770782; E-mail: Shakershaltout2@yahoo.com

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Abstract

Background: Ablation therapy with advances in its techniques becomes widely used in patients with hepatocellular carcinoma. Ablative techniques can induce tumor cell death and stimulate many immunological responses. These responses can be evaluated through assessment of peripheral immune cells changes in systemic circulation.

Aims: To investigate changes in the peripheral immune cells presented in CD4, CD8 and CD4/CD8 ratio after HCC ablation by different procedures and their relations to ablation result.

Subjects and Methods: This study investigated 73 HCC patients admitted to Department of Tropical Medicine, Mansoura University Hospital, Egypt. The patients were stratified into three groups according to ablative technique used. Radiofrequency ablation was performed for 24 cases, microwave ablation for 24 and transarterial chemoembolization for 25 cases. After history taking, clinical examination, basic investigations, triphasic abdominal computerized tomography before and 4 weeks after the treatment, HCC patients were selected according to EASL guideline. Lymphocyte subset assay using flow cytometry 1 day before, and 4 weeks post ablation was done. The patients subdivided into successful and unsuccessful subgroup according to the result of ablation by CT.

Results: In patients treated with Radiofrequency ablation, significant increase in CD4 count and CD4/CD8 ratio after treatment ($P<0.001$), while $CD8^+$ cells count significantly decreased ($P<0.002$). In HCC patients treated with microwave ablation, $CD4^+$ count and CD4/CD8 ratio significantly increased after treatment ($P<0.001$,<0.007), without significant differences in $CD8^+$ cells count. After transarterial chemoembolization, $CD4^+$ cells count and CD4/CD8 ratio significantly decreased ($P<0.001$) with significant increase of $CD8^+$ cells ($P<0.001$). Changes in CD4, CD8, and CD4/CD8 ratio remained comparable to that occurred in both successfully ablated and cases with residual tumor.

Conclusion: Various ablation procedures of HCC are associated with significant changes in peripheral T cell subpopulation. These changes mostly were due to the ablation of tumor cells but these changes cannot predict the success of ablation or recurrence of previously ablated one.

Keywords: CD4; CD8; CD4/CD8 ratio; Radiofrequency ablation; Transcatheter arterial chemoembolization; MWA

Introduction

Hepatocellular carcinoma (HCC) considers one of the most common and deadly cancers worldwide. It is the sixth most common cancer and the third cancer-related death worldwide [1]. It represents 85% to 90% of primary liver tumors [2].

Despite the great advances in management, HCC patients still have a dreary prognosis, with 5-year survival around 18% as most HCC patients diagnosed at late stages with failure to receive curative treatment [3]. Local ablative therapies produce potential cure for early detected tumors in selected patients [4].

HCC can induce many immune suppressor mechanisms like synthesis of immunosuppressive cytokines, antigen- presenting cells inhibition, changes in regulatory T cells which promote angiogenesis and tumor survival [5].

Normally, lymphocyte subsets remained relatively stable in the peripheral blood and any change in lymphocyte subsets may result in an alteration of immune function [6].

Correlation between patient's survival and immune responses to tumors had been mentioned in many studies. After resection of HCC, $CD4^+$ and $CD8^+$ T cells count infiltrating the tumor has been shown to correlate with improved survival [7]. So, immune-based therapies can be an ideal target for HCC. Knowledge about the immune response peri-ablation therapy may be crucial to get optimum therapeutic response.

Our study hypothesized that there may be significant changes in peripheral lymphocyte cells after ablation therapy and this may differ according to type of ablation intervention.

Aim:

To investigate changes in the peripheral immune cells subset; CD4, CD8 and CD4/Cd8 ratio after HCC ablation by different procedures (RF, MW and TACE) and the relation between these changes and ablation result.

Patients and method

Patients

This a prospective interventional (Randomized Control Trial) study included 82 patients diagnosed with HCC presenting to the Department of Tropical Medicine, Mansoura University Hospital during the period between May 2015 and April 2016, only 73 patients complete the follow-up period and inclusion criteria and enrolled in this study. According to general condition of the patient, number, site and size of the lesions based on the current accepted guidelines-EASL–EORTC recommendations for treatment of HCC 20128, HCC patients were divided into RFA group includes 24 patients, MWA group includes 24 patients and TACE group (n=25), and they were treated with radio-frequency ablation, micro-wave ablation and TACE, respectively. Then each group subdivided into successful and unsuccessful subgroup according to the result of ablation by CT.

Diagnosis of HCC was confirmed by elevation of α-fetoprotien >400 ng/ml combined with positive one imaging study (triphasic computed tomography or dynamic magnetic resonance. After having informed consent patients and Institutional Review Board approval, primary HCC patient naïve to ablation treatment exhibiting good compliance, with Child-Pugh class A or B and stage A to C Barcelona Clinic Liver Cancer (BCLC) were included in this study. Patients with metastatic liver tumor or liver cirrhosis of Child C were excluded. Each included patient was evaluated by a detailed clinical history and a thorough physical examination. Complete blood picture, complete liver function testes, viral markers (HCV antibodies and HBS antigen by 3rd generation ELISA), serum alpha fetoprotein level (AFP), abdominal ultra-sonography, triphasic abdominal computed tomography with contrast were done for all patients before intervention. For assessment of successful ablation, noncontrast CT was done for patients with TACE after 4 weeks and another one with contrast was performed at 6th week. Patients with RF or MW ablation, contrast enhancing CT were performed 4 weeks after ablation.

Lymphocyte subset assay

Peripheral venous blood samples were taken one day before and four weeks post ablation in EDTA containing tubes. Incubation of blood sample with a mixture of fluorescence labelled anti-CD4, and anti-CD8 monoclonoal antibodies was done for 15 minutes. Flow cytometric analysis was done with isotype control (Mouse IgG, Dakocytoformation, Denmark).

Statistical analysis

SPSS version 21 was used to analyze data. The normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data were described in number and percent. Association between categorical variables was tested using Chi-square test. Continuous variables were presented as mean ± SD (standard deviation). The two groups were compared with Student t test while paired t-test was used to compare paired data.

Results

Patient characteristics

A total of 73 patients with HCC were included in this study. The mean age of the included patients were 54.52 ± 5.62 (range 48-66) years with 41 patients (56.16%) being males. The etiology of cirrhosis and subsequent HCC in all included patients was HCV. According to Child-Turcotte-Pugh Classification, 54 patients (73.97%) were classified as class A and 19 (26.03%) as class B (Table 1).

Variable	RFA (n=24)		MWA (n=24) %		TACE (n=25)		Total	P value
	No	%	No	%	No	%	NO	
Sex								
Male	14	58.3	18	75	20	80	52	P=0.157
Female	10	41.7	6	25	5	20	21	
Age								
Mean ± SD	57.50 ± 6.01		58.33 ± 4.94		57.92 ± 5.13			P=0.728
Min-Max	42-63		50-66		50-66			
Child classification								
A	20	83.3	12	50	22	88	54	P=0.024*
B	4	16.7	12	50	3	12	19	
HCV=hepatitis C virus; HBV=hepatitis B virus								

Table 1: Demographic characteristics of HCC patients.

There were significant decreases in the levels of AFP after treatment in The RFA group and MWA group. (P=0.034) but there were no significant differences in AFP levels after treatment in TACE group (P=0.133) (Table 2).

Variable	RFA	MWA	TACE
	Median	Median	Median
AFP before	12.8	27.7	17.8
AFP 4 weeks after treatment	9.9	17.4	17.3
Test of sig. p-value	0.034*	0.034*	0.133
AFP=Alpha Fetoprotein			

Table 2: AFP levels before versus after ablation procedures

Changes in the lymphocyte subset after treatment in the groups of HCC patients demonstrated the tendency of partial variables changes. In the RFA group, $CD4^+$ cells and CD4/CD8 ratio remarkably increased after treatment (P<0.001), and the $CD8^+$ cells significantly decreased (P<0.002).

In the MWA group, $CD4^+$ cells markedly increased after treatment (P<0.001), with increase in CD4/CD8 ratio (P<0.007) but there were no significant differences in $CD8^+$ cells. In the TACE group, the $CD4^+$ cells and CD4/CD8 ratio dramatically decreased after treatment

(P<0.001), and the $CD8^+$ cells increased significantly (P<0.001) (Table 3).

Items	Before	After	Test of sig. p-value
	Mean ± SD	Mean ± SD	
RFA group: n=24			
CD4	32.31± 5.68	41.93 ± 4.32	P= ≤ 0.001*
CD8	30.84 ± 6.22	26.17 ± 5.18	P=0.002*
CD4/CD8	1.08 ± 0.24	1.61 ± 0.39	P=0.001*
MWA group:n=24			
CD4	30.6 ± 3.67	38.79 ± 3.98	P= ≤ 0.001*
CD8	29.13 ± 6.23	30.71 ± 6.31	P=0.348
CD4/CD8	1.09 ± 0.26	1.30 ± 0.28	P=0 .007*
TACE group:n=25			
CD4	41.01 ± 6.47	34.4 ± 7.70	P=0.001*
CD8	27.11 ± 6.15	33.86 ± 6.94	P=0.001*
CD4/CD8	1.52 ± 0.45	1.07 ± 0.38	P=0.001*
CD4=cluster of differentiation 4; CD8=cluster of differentiation 8			

Table 3: CD4 and CD8 and their ratio before and after ablation procedures

After that, each group subdivided into successful and unsuccessful subgroup according to the result of ablation by CT. Statistical comparison between subgroups shows significant increase in the levels of $CD4^+$ cells and CD4/CD8 ratio and significant decrease in $CD8^+$cells after treatment in successful and unsuccessful subgroups of RFA group. In both successful and unsuccessful subgroups of MWA group, there were significant increase in $CD4^+$ cells and CD4/CD8 ratio, but there was no significant difference in $CD8^+$ cells after treatment.

After treatment, the TACE group shows significant decrease in $CD4^+$ cells and CD4/CD8 ratio and significant increase in $CD8^+$ cells in both successful and non-successful subgroups. So, there were no difference between successful and unsuccessful subgroups of each group as regard to increase or decrease in $CD4^+$ cells, $CD8^+$ cells and CD4/CD8 ratio (Table 4).

Items	Before	After	Test of sig. p-value
	Mean ± SD	Mean ± SD	
RFA with successful ablation group (n=12)			
CD4	36.1 ± 6.94	43 ± 5.32	P=0.003*
CD8	35.18 ± 7.01	31.8 ± 4.17	P=0.029*
CD4/CD8	1.06 ± 0.33	1.37 ± 0.25	P=0.001*
RFA with Unsuccessful ablation group (n=12)			
CD4	32.72 ± 3.18	44.87 ± 2.98	P ≤ 0.001*
CD8	30.68 ± 4.17	24.75 ± 3.04	P ≤ 0.001*
CD4/CD8	1.05 ± 1.23	1.84 ± 0.36	P ≤ 0.001*
MWA successful ablation (n=16)			
CD4	31.85 ± 3.91	41.11 ± 4.17	P ≤ 0.001*
CD8	29.02 ± 5.76	31.81 ± 6.57	P=0.091
CD4/CD8	1.13 ± 0.25	1.35 ± 031	P=0.001*
MWA Unsuccessful ablation group (n=8)			
CD4	31.4 ± 3.09	37.45 ± 1.60	P ≤ 0.001*
CD8	32.65 ± 6.41	31.98 ± 5.72	P=0.554
CD4/CD8	1 ± 0.24	1.20 ± 0.18	P=0.007*
TACE Successful ablation group (n=10)			
CD4	41.4 ± 6.69	35 ± 6.86	P=0.006*
CD8	25.96 ± 3.06	32.8 ± 3.08	P ≤ 0.001*
CD4/CD8	1.61 ± 0.47	1.09 ± 0.30	P=0.002*
TACE Unsuccessful ablation group (n=15)			
CD4	42.23 ± 6.38	34.81 ± 7.35	P ≤ 0.001*
CD8	31.26 ± 6.85	36.83 ± 7.98	P=0.001*
CD4/CD8	1.42 ± 045	1 ± 0.37	P ≤ 0.001*

Table 4: Lymphocyte subsets in different subgroups before and after treatment

Discussion

Surgical resection, liver transplantation and various locoregional treatments including radiofrequency and microwave ablation are currently considered curative treatment modalities for HCC [8,9]. Also, TACE is typically used to treat patients with intermediate-stage HCC [10]. Ablative therapies can improve general condition with lower cost due to its ability to be performed outpatient, in addition to repeatability [11].

Interventional therapeutic procedures leads to immunogenic hepatocyte death, formation of necrotic tissue and establishment of chemical debris, this stimulating the release of certain chemicals and cytokines which stimulates the immune system and causes maturation and migration of DCs and cross-priming for T cells leading to struggle of further tumour growth and tumour cell death [12].

Our results found that there were significant changes in peripheral lymphocyte count after ablation therapy. In patients who perform RFA, $CD4^+$ cells count and the CD4/CD8 ratio markedly increased and $CD8^+$ cells decreased. This immunological changes following RFA could be explained by release of what is called "danger signals" which activate antigen presenting cells that triggers effective adaptive T-cell responses.

These danger signals consist mainly a of heatshock protein (HSPs) which activates dendritic, natural killer cells and macrophages [13]. Hansler et al. suggested that the thermal effect of RFA by necrotic cells induction can stimulate non-specific inflammatory reaction that reverses immune tolerance or anergy against tumour acting as "in vivo tumour vaccination" [14].

In our study we reported that $CD4^+$ cells and CD4/ CD8 ratio has significantly increases ($p<0.001$) in MWA group. These changes were supported by Dong et al. who studied the pathological and immunogenic sequences of microwave ablation and reported a maximal changes on the third day and he also noticed a lower rate of recurrence with high degree of infiltration [5]. Microwave ablation can be more superior than RF in certain cases due to higher intratumoral temperatures with larger tumor ablation volume [15,16].

TACE has shown improvement in the last years and is widely applied in clinical practice. The mechanism of this treatment relies on the observation that local chemotherapy induced tumour cell necrosis following vessel embolization can lead to a series of pathological and physiological changes. Recent studies proved the efficacy of TACE in induction of apoptosis, which provides theoretical evidence at the molecular level for the therapeutic effect of TACE [17].

In our study we confirmed the pervious finding of Guan et al. who reported that both $CD4^+$ count and the CD4/CD8 ratio markedly decreased while $CD8^+$ cells increased in the TACE group, suggesting that immunologic function was compromised shortly after treatment [18], and this may be a transient effect of injected chemotherapy. After follow-up of patients according to the result of ablation by CT, the changes in CD4, CD8, and CD4/CD8 ratio remained comparable to that occurred in both successfully ablated and cases with residual tumor cells. So we believed that these changes were resulted by the maneuver of ablation rather than the result of ablation and these changes cannot predict the outcome of ablation.

At present, an increasing number of studies focus on immunotherapy of HCC [19-21]. Immunotherapy trials for HCC use different mechanisms to potentiate the immune cells functions. Injection of cytokine, infusion of tumor infiltrating lymphocytes, or antigen presenting cells are tried to stimulate immune cells. The results of these different trials are controversial [22-24]. To date, there has been no breakthrough so far in the immunotherapy of HCC.

The small sample size is one of limitation in this study. So, studies with large sample size are recommended.

Conclusion

Significant changes have been observed in peripheral immune cells after performing various ablation procedures of HCC. It is believed that these changes were resulted by the ablation of tumor but cannot predict the success of ablation.

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