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The effect of apis Dorsata honey as a complementary therapy to interleukin-6 (IL-6) levels and T lymphocytes of post-chemotherapy breast cancer patients

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Abstract.
BACKGROUND: The overproduction of interleukin-6 (IL-6) in breast cancer cases can aggravate metastases. In comparison, the production of T lymphocytes plays a role in suppressing the development of tumor cells. Honey as a complementary therapy is expected to reduce the overproduction of IL-6 and facilitate the production of T lymphocytes in breast cancer cases.
OBJECTIVE: This study aims to determine the effect of Dorsata honey (DH) as a complementary therapy to IL-6 levels and T lymphocytes of post-chemotherapy in breast cancer.
METHODS: This study was a quasi-experimental approach that employed a pretest-posttest group control design. As many as 30 post-chemotherapy breast cancer patients at the Central Hospital in Eastern Indonesia were randomly selected into a control group of 15 participants. Additionally, an intervention group of 15 participants was given DH orally three times a day at a dose of 15 ml. The blood sample was taken two times, day 0 of the chemotherapy and day 16 (post-chemotherapy). The level of IL-6 was measured by ELISA, while the data were analyzed by Wilcoxon, independent T-test, and Mann–Whitney test.
RESULTS: The results showed that DH did not significantly affect IL-6 levels ($p = 0.17$). However, there was an increase in T lymphocyte levels with statistically significant differences ($p = 0.01$) in intervention groups. There was no difference in Il-6 and T lymphocyte levels between the intervention and control groups ($p > 0.05$).
CONCLUSIONS: Il-6 levels tend to be constant in the intervention group. However, there is a significant increase in the T lymphocyte levels which can indirectly increase the immune system and inhibit tumor cell growth in patients with breast cancer.

Keywords: Dorsata honey, complementary therapy, breast cancer, IL-6, T lymphocytes

1. Introduction

GLOBOCAN data in 2018 ranks breast cancer second with several new cases were more than two million cases, and deaths were more than six thousand people in the world [1]. In Indonesia, breast cancer ranks first with the number of more than 58,000 new cases, and the number of deaths reached 22,000 people [2]. The still high mortality rate suggests the need for progress in the treatment of patients with breast cancer. Honey as a complementary therapy is currently gaining attention for natural cancer therapies. Honey is known to modulate the immune system and has anti-cancer effects through interference with several cell signaling pathways, such as inducing apoptosis, anti-proliferative, anti-inflammatory, tumor necrosis factor (TNF), antioxidant, estrogenic modulatory, and anti-mutagenic pathways [3]. Giving honey in the experiments of animals with breast cancer can inhibit tumor growth so that the size, weight, and multiplicity are generally smaller. Also, the therapy increased the expression of pro-apoptosis protein and decreased anti-apoptotic protein [4–7]. Thus, honey may be used as

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a natural 'cancer alleviating' agent or supplement to chemotherapeutic agents [5].

Honey is useful for antitumor because of its phenolics which include chrysin, luteolin, quercetin, and esters of caffeic that can affect the cancer cells cycles. Honey is also anti-proliferative, pro-cancer apoptosis, anti-angiogenesis, anti-metastasis and acts as a cellular immune cell that works against cancer cells [8–11]. Tualang honey reduces carcinogenesis through hematologic and estrogenic modulation and apoptotic activity in breast cancer when it is done *in vitro* [7]. The nature of honey as an anti-cancer has been widely reported. But, the underlying mechanism and molecular targets still need to be further investigated. A study says that administering Tualang honey can reduce IL-6 production through inhibition of pY-STAT3 [12]. The increase of IL-6 is reported to negatively contribute to therapy, recurrence, aggressive tumor growth, metastasis, morbidity, and therapeutic resistance in cancer cases [13]. Therefore, in this study, we want to determine the effect of Dorsata honey (DH) as a complementary therapy to IL-6 Levels and T lymphocytes as the immune system of post-chemotherapy in breast cancer.

2. Method

2.1. Setting and subjects

This study employed a quasi-experimental approach by using a pretest-posttest control group experimental design. The study was conducted at the Oncology Surgery Department, Lontara 1 Upper Room, in Dr. Wahidin Sudirohusodo Hospital. IL-6 concentration was determined using the ELISA instrument in Unhas Hospital Laboratory, Makassar, from March to August 2018. The inclusion criteria were women with early and distant metastatic breast cancer without a history of previous therapy. The data were collected, managed, analyzed, and presented in the form of tables and narration. Then, the researchers compared them with the result of other studies.

2.2. Research procedure and materials

The honey that was tested was Dorsata honey. The research was initiated by randomized selection of the controlled and treated group samples. Each subject was first asked to fill an informed consent. The treated group was given 5 ml (a teaspoon) of the tested honey 3 times a day orally (morning, noon, and evening) for 15 days. Then, for the controlled group, patients were suggested to consume vitamin supplements and healthy diets. Closest relatives were asked to supervise the honey consumption to ensure patients' compliance with honey consumption. Blood sampling was performed 2 times, before the treatment (day 0 of chemotherapy) and on the 16th day (post-chemotherapy). IL-6 concentration in the plasma sample was determined using the ELISA method.

2.3. Statistical analysis

The statistical analysis was performed using the Wilcoxon test to analyze each group's pre and post-treatment differences. Mann–Whitney tests were conducted to analyze the difference between both groups. The test results are significant if $p < 0.05$.

3. Results

The average age demography of the respondents in the treated and controlled group was 47.8 and 46.8, respectively. This shows that both groups' age distribution was considered insignificant in its difference ($p = 0.72 > 0.05$). The breast cancer average suffering period of these respondents also shows that there is not any significant difference ($p = 0.28 > 0.05$) although the mean ± SD in the treated group was slightly higher by 2.0 ± 0.9 (Table 1).

There was not any observed significance in the difference of IL-6 concentration before and after honey consumption in the treated group ($p = 0.173$; mean difference of +0.176) and in the controlled group ($p = 0.995$; mean difference of −90.594). However, the researcher found a significant difference ($p = 0.01$) in T lymphocytes in the intervention group (mean difference of +12.1).

There was not any significant difference between the treated and controlled group ($p = 0.467$; mean difference of 36.87) in IL-6 concentration and T lymphocytes concentration ($p = 0.59$; mean difference of 3.4) as well.

4. Discussion

Tumor development is strongly correlated with angiogenesis under the control of growth factors such as vascular endothelial growth factor (VEGF) and cytokines. IL-6 is a pleiotropic cytokine that contributes to the growth and differentiation of tumor cells

Table 1
Demographic characteristics of breast cancer patients treated by chemotherapy in di RSUP Dr. Wahidin Sudirohusodo Makassar (n = 30)

Variable	Treated group (n = 15)	Controlled group (n = 15)	p-value
Age (years old) (mean ± SD)	47.8 ± 6.9	46.8 ± 8.2	0.718*
Min-max	34–61	23–58	
Education level			
Elementary school	6 (15.8%)	5 (13.2%)	
Secondary school	0 (0.0%)	3 (7.9%)	0.872*
High school	6 (15.8%)	4 (10.5%)	
Higher education	3 (7.9%)	3 (7.9%)	
Suffering period (years) (mean ± SD)	2.0 ± 0.9	1.6 ± 0.9	0.282**
Complication			
Positive	0 (0.0%)	0 (0.0%)	0.338*
Negative	15 (39.5%)	15 (39.5%)	
Marital status			
Married	13 (34.2%)	14 (36.8%)	
Widowed	1 (2.6%)	0 (0.0%)	0.785**
Single	1 (2.6%)	1 (2.6%)	

*Dependent t-test, **Wilcoxon.

Table 2
Pre and post treatment IL-6 concentration and T lymphocytes analysis

Groups	Mean (pg/ml) ± SD	Mean difference	P*
IL-6			
Treated			
Pre	126.722 ± 119.188	+0.176	0.173
Post	126.898 ± 2013.119		
Controlled			
Pre	187.622 ± 217.875	–90.594	0.995
Post	90.028 ± 35.814		
T lymphocytes			
Treated			
Pre	25.1 ± 8.4	+12.1	0.01
Post	37.2 ± 19.3		
Controlled			
Pre	25.2 ± 12.4	+8.6	0.15
Post	33.8 ± 14.7		

*Wilcoxon.

where IL-6 is found. It tends to increase the worsening of the prognosis in cases of colorectal cancer [14]. Additionally, IL-6 derived from adipocytes promotes the expression of the function of lysyl hydroxylase (PLOD2) by activating the JAK/STAT3 and PI3K/AKT signaling pathways. Hence, it promotes breast cancer metastases [15]. IL-6 role in tumor cell *in vitro* growth is known to have 2 contradicting mechanisms, yet its specific role is still varied and unclear. Some reports show that IL-6 is a growth factor for myeloma/plastocytoma, renal carcinoma, cervical carcinoma, inherited AIDS Kaposi's sarcoma, and certain T and B cell lymphoma [12]. IL-6 responsiveness varied among cell differentiation statuses [16]. It shows that IL-6 regulation is most likely taking part in invasive and metastasis ability through estrogen receptor by suppressing IL-6 expression. Other research suggests that IL-6, IL-8, and TNF-α concentration are correlated with clinical and lymph metastasis stages, as well as with ER and HER2 expression. To be specific, IL-6 and IL-8 seem to have significant potential as cancer biomarkers. Serum cytokine analysis helps in identifying patients' prognoses. Hence it is beneficial in treating a more aggressive disease [12].

Animal studies report that honey administration does not significantly increase mRNA expression of IL-6 [17]. Similar to the findings in this study, the results showed that giving DH to breast cancer patients had not shown any significant effect on IL-6 concentrations, although there was a slight increase of 0.17 after 15 mL of honey treatment every day for 15 days. Manuka honey is found to inhibit STAT3 and thus reduce IL-6 production [12]. The type and source of honey origin may affect decreasing the IL-6 levels. Studies report a significant difference in IL-6 secretion in monofloral honey from arid regions compared to monofloral and heterofloral non-arid regions [18]. Future studies can try to increase the dose of administration to assess at

Table 3
Post treatment IL-6 concentration and T lymphocytes difference analysis between treated and controlled groups

Groups	Mean (pg/ml) ± SD	Mean difference (pg/ml)	P^*
IL-6			
Post	126.898 ± 2013.119	36.87	0.467
Post	90.028 ± 35.814		
T lymphocytes			
Post	37.2 ± 19.3	3.4	0.59
Post	33.8 ± 14.7		

*Mann–Whitney.

what dose DH can affect the levels of Il-6 in breast cancer cases.

Our next goal is to target T lymphocytes as a part of the immune system that can protect the body from infection and help fight cancer. The immune system plays an important role in distinguishing between foreign pathogens and antigens [19]. Post-chemotherapy patients may experience immunosuppression that can inhibit tumor recognition by immune cells and T lymphocyte dysfunction [19], so natural therapy is needed. Although in animal experiments, Manuka honey did not show any significant difference in lymphocyte levels compared to saline [7], in this study, the researchers found that Dorsata honey consumption significantly influences the T lymphocyte count in which improves the immune system post-chemotherapy. $CD8^+$ T lymphocyte cell count is positively correlated with improved breast cancer patient outcomes [20]. It is also positively correlated to breast cancer rates – specific survival (BCSS) in which tumor-infused $CD8^+$ T lymphocytes have the antitumor activity. It is judged from its favorable effect on patients' survival and could potentially be exploited in breast cancer treatment [21]. Several studies have also reported positive implications of increasing T lymphocytes in terms of cytotoxic T lymphocyte antigens (CTLA-4, CD152) by seeing a better prognosis [22,23]. Honey with its various benefits is known to be very selective that only works on breast cancer cells and not on normal cells. Hence, honey is relatively safe for long-term consumption.

5. Conclusion

Giving honey to post-chemotherapy breast cancer patients increases the IL-6 concentration, even though statistically, it is not significant. Other clinical responses show that honey consumption is correlated with higher T lymphocyte count.

Conflicts of interest

None.

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