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Transarterial Chemoembolization in Hepatocellular Carcinoma: A Clinical Efficacy Study of *Ganoderma Lucidum* Extract Polysaccharide Peptide β-Glucan

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a primary liver malignancy with high prevalence rate, associated primarily with the Hepatitis B Virus. HCC is the third leading cause of death worldwide. The prevalence in Indonesia is estimated at 100 per100,000 inhabitants. Treatment is generally ineffective as patients are already at advanced stages when diagnosed. Trans-arterial Chemoembolization (TACE) is a promising treatment, and β-Glucan combined therapy is expected to treat HCC more efficiently. This study evaluated the treatment response of TACE in HCC combined with β-Glucan orally. Methods: A randomized clinical trial was conducted on 63 patients with HCC. The subjects of the study were randomly assigned into two groups: 32 administered TACE plus β-Glucan, and 31 with TACE plus placebo. **Results:** In the β-Glucan group, there were four patients (12.50%) with complete response, 16 (50%) partial response, 3 (9.38%) no response, and 9 (28.12%) in deteriorated response. In the placebo group, there were 0 complete response, 11(35.48%) partial, 2(6.45%) no response, and 18 (58.07%) deteriorated response. The survival rate of the β-Glucan group was longer (median 39 weeks) than the placebo group (median 26 weeks). CD4, CD8 and IL-2 post-TACE increased significantly ($p<0.05$). The placebo group had 4.143 times, significantly higher hazard risk ($p<0.05$).
Conclusion: HCC therapy with a combination of TACE and β-Glucan is beneficial to increase the therapeutic response, immunity and survival rate.

Keywords: Carcinoma survival, HCC, Immunity, TACE, Glucan Therapy
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INTRODUCTION

Subdermal contraceptive implants have been studied and used in humans for over twenty years.[1,2] Contraceptive implants provide long-acting, highly effective reversible contraception. The most recently introduced subdermal implant, Implanon® (N.V. Organon, Oss, the Netherlands), also referred to as the etonogestrel (ENG) implant, is a single rod implant that offers three years of contraceptive efficacy.[3–6] The ENG implant has been used in more than 30 countries, including Australia, Indonesia, and the Netherlands, and was approved by the United States Food and Drug Administration (FDA) in 2006. The ENG implant is an excellent option for women with contraindications to estrogen in addition to any woman who desires long-acting reversible contraception.

The ENG implant is a single-rod implant measuring 40 mm long, and 2 mm in diameter with a solid core of ethylene-vinyl acetate (EVA) impregnated with 68 mg of etonogestrel, the biologically active metabolite of desogestrel.[7,8] The EVA copolymer allows controlled release of hormone over three years of use.[9] Each implant is provided in a disposable sterile inserter for subdermal application.

METHODS

This RCT study was conducted in the radiology department of RSUP Dr. Sardjito Yogyakarta. The research subjects were 63 people, comprised of 32 subjects receiving additional drugs of β-Glucan and 31 subjects not receiving β-Glucan (placebo). The median age of the group with subjects in the placebo group (without treatment) was 56.38 years with a range of 26-78 years, wherein the treatment group was 61.25 years, range 36-84 years. There were no significant differences ($p> 0.05$) for the location of the tumour, tumour size, as well as the condition of the liver and the tumours between both groups. Thus the state of the subject sample validity can be justified. Likewise, the locations of liver tumours were mostly located in the right lobe 26 (81.25%) and 30 (96.77) while there were only some located in the left lobe 6 (18.75%) and 1 (3.23%) in subjects with β-Glucan and subjects with placebo, respectively. There were no significant differences in the location of HCC in the group of subjects given or not given β-Glucan ($p> 0.05$). The study involved randomized clinical trials. The study received ethical clearance approval from the governing institution. Before participation, patients completed informed consent forms.

RESULTS

The results of therapy with TACE plus β-Glucan drug are as follows: patients who experienced complete response were 4 (12.50%), partial responses were 16 (50%), while no responses were 3 (9.38%), and progressive disease were 9 (28.12%) of 32 subjects. While for the subjects without the addition of β-Glucan, patients who experienced complete response were 0 (0%), partial responses were 11 (35.48%), no responses were 2 (6.45%), and progressive disease was 18 (58.07%) of 31 subjects (Figure 1).

Concerning the results of TACE therapy coupled with β-Glucan drug, patients who experienced complete response were 4 (12.50%) of 32 patients, partial responses were 16 (50%) of 32 patients, no response was 3 (9.38%) of 32 patients, and 9 (28.12%) with a deteriorated response. Whereas in patients without additional β-Glucan who experienced complete response were 0 (0%) of 31 patients, partial responses were 11 (35.48%) of 31 patients, no response was 2 (6.45%) of 31 patients and deteriorated response was 18 (58.07%) of 31 patients. This data shows 20 (62.50%) of 32 patients had complete and partial responses in the group receiving β-Glucan, whereas in the group without β-Glucan yields are lower (11 of 31 patients or 35.48%). Table 1 is clearly showing the effective role and efficiency of β-Glucan with TACE therapy in HCC patients which can provide almost twice as much increased therapeutic response.

From the calculation of this study results, it is concluded that patients with "response to treatment" were as many as 31 patients consisting of 20 (4 complete responses and 16 partial responses) of patients with β-Glucan and 11 patients with placebo (0 complete responses and 11 partial responses) from the total of 63 patients, while patients with "no response to treatment" were

Table 1 Tumour response after TACE therapy in patients with and without β-Glucan

Description	*β-Glucan* (+) N 32 (%)	*β-Glucan* (-) N 31 (%)	*P*-value
The therapeutic response			0.044*
• Full Response	4 (12.50)	0 (0.00)	
• Partial Response	16 (50.00)	11 (35.48)	
• No Response	3 (9.38)	2 (6.45)	
• Deteriorate Response	9 (28.12)	18 (58.07)	

Chi-Square Statistics, * $p < 0.05$ = significant

Table 2 The result of therapy from patients with and without β-Glucan (placebo)

Description of treatment	Outcomes* YES Response to therapy	Outcomes* NO Response to therapy	Total
β-Glucan (+) (event)	20	12	32
β-Glucan (-) (Placebo=Control)	11	20	31
Total	31	32	63

* Two by two table.

Table 3 Table Event Rate, Relative Risk Reduction (RRR), Absolute Risk Reduction (ARR) and Number Needed to Treat (NNT)

Event Rate = No Response Therapy		Relative Risk Reduction (RRR)	Absolute Risk Reduction (ARR)	Number Needed to Treat (NNT)
Control Event Rate (CER) Placebo	Experimental Event Rate (EER) β-Glucan	$\frac{\text{CER-EER}}{\text{CER}}$	CER-EER	$\frac{1}{\text{ARR}}$
20/31 = 64.5%	12/32 = 37.5%	$\frac{64.5\%-37.5\%}{64.5\%} = 42\%$	64.5% - 37.55% = 27%	$\frac{1}{27\%} = 4$
	95% CI	5% to 79%	3.2% to 50.8%	2 to 31

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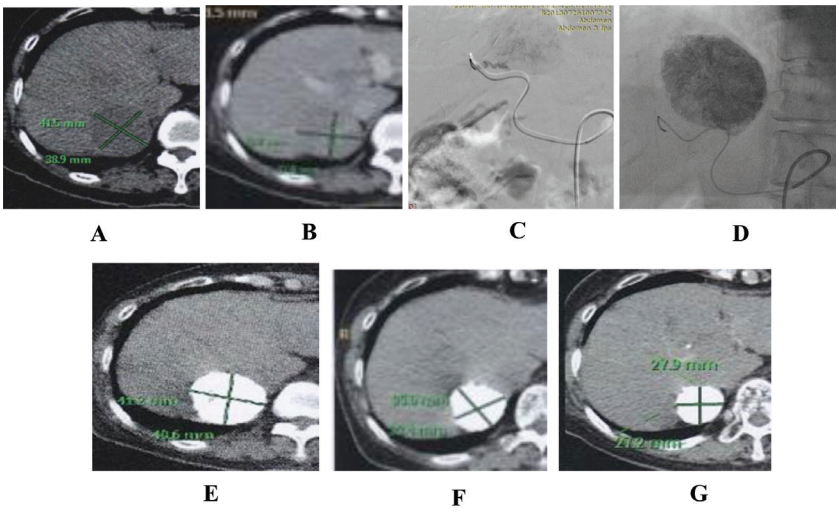

Figure 1 CT before TACE pre-contrast the nodule is relative hypodense (A, 41.5x38.9mm) and relatively enhanced in post-contrast (B, 41.7x37.8mm), C-D procedure of TACE. After TACE, the chemoembolant covered entire tumor as a full response of treatment (E= TACE1, 41.2x40.6mm; F=TACE2, after 6m from TACE1, 35.0x30.4mm; G=TACE3 after 8m from TACE2 or 14 m from TACE2, 27.0x27.2mm

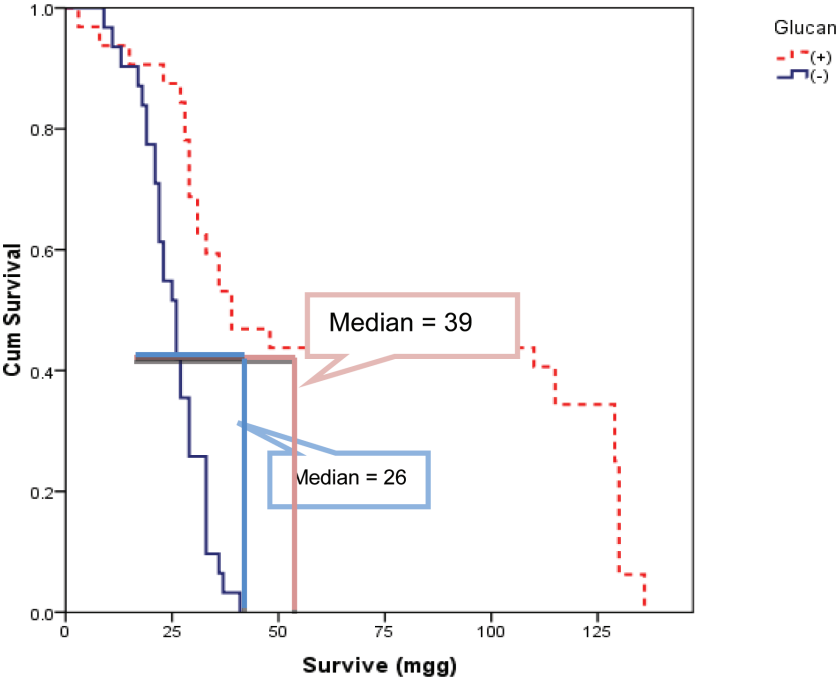

Figure 2 Graphs of Kaplan-Meier survival in patients with β-Glucan (dotted line) and without β-Glucan (continuous line). It appears the patient with β-Glucan can extend his life (> 125 weeks) with a median of 39 weeks compared to patients without β-Glucan (placebo) (survival < 50 weeks, median 26 weeks).

32 patients consisting of patients with β-Glucan as many as 12 patients (3 patients no response and 9 patients with deteriorated responses) and placebo as many as 20 patients (2 no response and 18 deteriorated responses) (Table 2).

It can also be seen from the results that the incident of no response to treatment in this study was 32 out of a total of 63 patients, which are comprised of the incidence of no response to treatment groups with β-Glucan (12 of 32 patients) and the incidence of no therapeutic response in the placebo group (20 of 31 patients). Calculation of Control Event Rate (CER) = 20/31 = 64.5% is the proportion of no therapeutic response in the control group without β-Glucan (placebo) and Experimental Event Rate (EER) = 12/32 = 37.5% is the proportion of no response to treatment in the intervention group (β-Glucan) to obtain the value of the Relative Risk (RR) of no response to treatment in the intervention group (β-Glucan), which is = (12/32): 20/31) = 0.58 compared with the placebo group.

Table 3 shows Relative Risk Reduction (RRR) = 42% (95% CI: 5% - 79%), Absolute Risk Reduction (ARR) = 27% (95% CI: 3.2% - 50.8%) and Number Needed to Treat (NNT) = 4 patients (95% CI: 2-31). Relative Risk Reduction (RRR) equal to 42% means that the results of this study showed a relative risk reduction of no response to therapy with β-Glucan group compared with the placebo group at 42%, which in other words the results of this study are relatively useful.

The study shows survival rates were significantly increased ($p<0.05$). Survival median in patients given β-Glucan was 39.0 weeks (95% CI: 22.401 to 55.559), while those not given β-Glucan its median survival was 26.0 weeks (95% CI: 22.410 to 29.590). These data indicate β-Glucan administration may clinically increase the median survival significantly. The use of the mean value as an estimate of relative survival is limited due to the vast distribution of survival of patients if it is censored. Calculation log-rank (Mantel-Cox) was used to assess the equality of survival distributions providing results of 23.98 (Figure 2).

DISCUSSION

From the result of the study, as seen in Figure 1, it can be seen that there were no significant differences ($p>0.05$) in tumour size before TACE therapy in both groups. Still, after TACE, there was a significant difference in HCC tumour size between both groups ($p<0.05$). Thus, it can be concluded that the addition of β-Glucan medication extracted from GLPP can increase the effects of TACE therapy on HCC.

Number Needed to Treat (NNT) is equal to 4. NNT equal to 4 means that it takes four patients who need to be given HCC medication of β-Glucan to prevent one patient further with no response to treatment in patients who underwent TACE.

Interpretation of NNT is very dependent on what the outcome is used for and on the condition or disease. For patients with severe outcomes, the NNT can have a reasonably wide range because the positive effects of this therapy are expected. In patients who underwent TACE for HCC with no response to treatment outcome, with NNT equal to 4 it is very rational to be accepted as an essential result so it should be considered to be applied to the patient pool.

If it is determined that the patient has a doubled risk of "no response to therapy" if he/she does not get additional medication such as β-Glucan in the control group, then ft = 2 and NNT / ft ≈ 2. This result means that it is needed to provide additional β-Glucan medication in two high-risk patients to prevent one event of no additional therapeutic response.

There was no significant difference in tumour size before TACE therapy ($p>0.05$). Still, after TACE, there were significant differences in tumour size ($p<0.05$) between the groups of patients receiving β-Glucan and without β-Glucan. The number of patients who had tumours shrink (<50 cm^2) increased from 4 patients (12.50%) before therapy to 8 patients (25.00%) following treatment in patients with β-Glucan administration group. While the patients in the group without β-Glucan, there was an increase in tumour size from 21 (67.74%) to 23 (74.19%) patients after therapy. These findings support the conclusion that the addition of β-Glucan drugs can increase the effects of TACE therapy on HCC.

It has been proposed by Zhou and Gao in 2002 [19] that β-glucan administered will bind to the surface of leukocytes or in specific serum proteins which then can activate macrophages, T-helper lymphocytes, Natural Killer (NK) cells, and other effector cells. All of these will increase the production of cytokines by activating effector cells, for example, Tumor Necrosis Factor Alpha (TNF-α), Interleukins (IL), Interferons (IFN), Nitric Oxide (NO) and antibodies. Cao and Lin in 2004 [20] also proposed in addition to the anti-tumour effects of Glucan, it also has an anti-angiogenic effect on tumours *in vivo*, which consists of "direct and indirect" angiogenesis inhibitors. Direct angiogenesis inhibitors prevent proliferation, migration or eliminate the cell's response to a spectrum of pro-angiogenic protein vascular endothelial cells, while indirect angiogenesis inhibitors generally prevent the expression or block the activity of tumour proteins which activate tumour angiogenesis. Thus, as a result, no tumour angiogenesis occurs after Glucan treatment.

According to Cao and Lin[20] β-Glucan has the effect of anti-tumour and anti-angiogenesis, whereas according to Zhou and Gao[19] β-Glucan has the ability to attack cancer cells by activating the immune cells, either cellular or humoral, and inhibit the growth of cancer cells. CD4 cells in subjects who received β-Glucan in post-TACE appear higher (486.022) significantly ($p<0.05$) than in subjects who did not receive the drug Glucan (354.977). Cytotoxic CD8 cells appear higher on a post-TACE in patients who receive β-Glucan (320.922) compared to subjects who did not receive β-Glucan (243.694) significantly ($p<0.05$). The CD4/CD8 ratio in post-TACE subjects appears higher in subjects with β-Glucan (1.608) compared to subjects without β-Glucan (1.535) even though it was not statistically significant ($p>0.05$).

The IL-2 in subjects with β-Glucan is higher (17.662) than subjects without β-Glucan (9.753) significantly ($p<0.05$). This finding suggests that there is an influence of β-Glucan to trigger the production of cytokine, Interleukin (IL)-2. IL-2 is a pleomorphic cytokine that controls the growth of T-cells, induces differentiation of regulatory T-cells (T-regs) and mediates activation-induced cell death. β-Glucan has a role against cancer, by activating not only macrophages, but also NK-cell, Killer T-cells, lymphokines and IL-1 and -2 cells.[21]

From this study, it can be concluded that the addition of β-Glucan medication for HCC therapy with TACE method can provide better HCC therapeutic response significantly ($p<0.05$) and also increase the survival rate of patients significantly ($p<0.05$). The addition of β-Glucan extract enhances the body's immune cells, including CD4, CD8 and IL-2 against hepatocellular carcinoma significantly ($p<0.05$), which can increase the success of HCC therapy with TACE.

Subjects who were given β-Glucan had increased survival rate significantly ($p<0.05$). For further the success of HCC therapy, in addition to TACE, it needs to be coupled with β-Glucan drugs to improve the therapeutic response of HCC. Univariate Cox Hazard Risk Analysis revealed that patients without β-Glucan have a hazard risk (risk of death) 4.143 times greater than patients given β-Glucan before TACE therapy. Based on the above results, we recommend for the addition of β-Glucan drugs to improve the effectiveness of treatment in HCC patients who undergo the TACE method to improve patient survival. We concluded that subjects with TACE therapy supplemented with β-Glucan drugs have a more prolonged survival (> 125 weeks) while those not given β-Glucan have a survival of fewer than 50 weeks.

CONCLUSION

From the description of the research and discussion above, it can be concluded that transarterial

chemoembolisation (TACE) by giving the chemoembolant drug selectively through catheterization on the feeding artery of HCC tumour, coupled with β-Glucan drug therapy is a potent method, has a high success rate and is very useful in HCC therapy providing a positive and significant therapeutic response ($p<0.05$). This study demonstrated the addition of β-Glucan before TACE therapy method successfully increased complete and partial response to treatment in 62.50% of patients, while doubly increasing the number of patients with shrinkage of tumor the size to <50cm^2 (from 12% to 25%), increasing CD4, CD8 and IL-2 cells, improving the mean survival rate, and having a lower hazard risk of death. The result in patients without β-Glucan showed the contrary result.

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ETHICAL CLEARANCE

This study has obtained ethics approval from the Ethics Committee of FKKMK Universitas Gadjahmada, prior to the study conducted.

CONFLICT OF INTERESTS

The authors declare that there were no conflicts of interest in the process of this study.

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AUTHOR CONTRIBUTION

All of the authors are equally contributed to the study from the study framework, data gathering, data analysis, until reporting the result of the study.

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