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The Effect of T cell Activation on the Immune Responses to Hepatitis A Vaccine in Children with Obesity: A Preliminary Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Obesity is a chronic inflammation, effects immune dysregulation. Hepatitis a vaccine (HAV) are recommended in obesity with NAFLD. Previous studies reported that obesity had poor immune response to live vaccine. There is limited data on live attenuated HAV, especially in children with obesity and cellular immunity.
Objectives: To compare antigen specific Interferon gamma+ T cell activation in obesity and healthy children at pre and post live attenuated HAV.
Methods: A retrospective pilot study, obtained blood samples of children and adolescents (212 participants) from previous study. Participants with either immunodeficiency or HAV immunization were excluded. Participants who met criteria; aged 8-12 years and BMI less than 1 SD or more than 2 SD, were included and divided into 2 groups; obese and healthy group. H2 strain, freeze dried, live attenuated HAV (MEVAC-A) were injected 0.5 ml in all participants. Blood for Interferon gamma+ T cell stimulation were collected at pre- and post-vaccination and measured via ELISPOT technique.
Results: 20 participants were included, mean age was 10.2 years. Overall Interferon gamma+ T cell activation demonstrated immunogenicity. However, there were no statistically significant difference of Interferon gamma+ T cell activation in both groups. Interferon gamma+ T cell stimulation GMR (95% CI) at concentration of 2,5,10 mcg/ml of HAV antigen at pre- and post-vaccination for healthy group were 0.66 (0.23-1.92), 1.18 (0.47-2.96), and 1.19 (0.55-2.57), and obese group were 1.06 (0.39-2.85), 1.15 (0.57-2.32), and 1.28 (0.76-2.18), respectively.
Conclusion: Single dose live attenuated HAV is safe and has high immunogenicity in children with obesity.

Keywords: Obesity; live attenuated hepatitis A vaccine; hepatitis A vaccine; T cells; cellular immunity; children.

1. INTRODUCTION

The prevalence of obesity is increasing worldwide and is associated with the comorbidities of metabolic syndrome, insulin resistance and atherosclerosis [1]. Recently, obesity is believed to be a chronic inflammation state which effects immune dysregulation; alteration of proinflammatory leukocyte phenotypes, loss of lymphoid tissue integrity, and alteration of leukocyte populations distribution, leads to impaired immunity [2]. Obesity is a state of immunosuppressive condition which immunity and antiinflammation are dysregulation, later on, effects impaired cytokine function and impaired T cell regulation [3].

In obesity, especially in obesity with underlying of NALFD (Non-Alcoholic Fatty Liver Disease) are recommended to receive hepatitis A vaccine in order to prevent chronic hepatitis [4].

Nowadays, Hepatitis A vaccine is available in two forms, inactivated vaccine and live attenuated vaccine. Live attenuated hepatitis A vaccine is activated by pathogen association pattern, inducing danger signals, changing in receptors, and, activation of B cell and T cell [5]. Live attenuated hepatitis A has been recommended as a part of Chinese Expanded Program of Immunization since 2008 in order to prevent Hepatitis A virus spreading [6]. Additionally, Indian Academy of Pediatrics (IAP) has been recommended H2-strain live attenuated hepatitis A vaccine single dose instead of double dose since 2014 [7].

On the contrary, there are some studies reporting that obesity has poorer immune response in vaccine than normal population. Weber, DJ et al, was the first study of humoral immune response in obesity, they reported that obesity, advanced age and high BMI level were associated with poor immune response in Hepatitis B vaccine [8]. In 2011, Sheridan, PA et al, stated that obesity associated with poor immune response, both humoral and cell mediated immune response, in trivalent influenza vaccine [9]. Many studies demonstrated that, in cellular immune response after vaccination, obesity population has decreased NK cytotoxicity, serum cytokines, and Interferon gamma levels. Furthermore, obesity population showed that both CD8+ and CD4+ T cell activity are decreased function in long term immune response [10,11].

Currently, there is limited data on immunogenicity on live attenuated hepatitis A vaccine in children with obesity. The latest study was in 2020, Dumrisilp, T et al., argued that there

was no significant difference in humoral immune response on live attenuated Hepatitis A vaccine in both obesity and normal weight population [12].

In addition, the effects of obesity on cell mediated immune response to live attenuated hepatitis A vaccine, especially in children, have not been characterized. This is the first study that focus on cell mediated immune response to live attenuated hepatitis A vaccine in children with obesity. The primary objective of our study is to compare antigen specific Interferon gamma+ T cell activation in obesity and healthy children at pre- and post- live attenuated Hepatitis A vaccination. Secondary objective is to determine the association of Interferon gamma + T cell activation in obesity and healthy children at pre- and post-live attenuated Hepatitis A vaccination.

2. MATERIALS AND METHODS

2.1 Study Population and Samples

This study was a retrospective pilot study conducted between April 2021 and December 2021. Our study obtained blood samples from former study in 2020, Impact of Obesity and Being Overweight on Immunogenicity to Live Attenuated Hepatitis A Vaccine in Children and Young Adults by Dumrisilp, T et al. [12]. Total 212 of children and adolescents from primary schools and one university in Bangkok were included.

Participants who had underlying of immunodeficiency, were taking any immunosuppressive drugs, and were previously immunized with hepatitis A vaccine were excluded.

Participants were met criteria; aged between 8-12 years old and has body mass index (BMI) less than 1SD (standard deviation) or BMI more than 2SD, only twenty participants were included.

Twenty participants were divided into 2 groups for study analysis; obesity and normal weight groups according to their body mass index (BMI) from WHO Child Growth Standards criteria [13]. Group 1 consists of children with obesity who has BMI more than 2SD, and Group 2 consists of children with normal weight who has BMI less than 1SD.

2.2 Data and Measurements

Demographic data including age, gender, weight, height, body mass index (BMI), waist circumference, which indicates truncal obesity, and underlying disease were collected in both groups. Blood samples before vaccination and at 8-9 weeks post vaccination in both groups were collected in ACD (Acid Citrate Dextrose) tube and kept for analysis at the Allergy and Immunology Laboratory Unit, Faculty of Medicine, Chulalongkorn University (Fig. 1).

This study was registered in the Thai Clinical Trials Registry (TCTR) (ID number 20210825005) and was funded by Chulalongkorn University under Ratchadapisek Sompoch Endowment Fund.

2.3 Study Vaccine

All participants were vaccinated H2-strain, freeze dried, live attenuated hepatitis A vaccine (MEVAC-A) 0.5 ml subcutaneous over the deltoid muscle of upper arm. MEVAC-A was manufactured by Zhejiang Pukang Biotechnology, China and imported by Biogenetech, Co. Ltd.

2.4 Assessment of Immunogenicity

Blood samples were taken at pre-vaccination and at 8-9 weeks after vaccination and were kept in ACD (Acid Citrate Dextrose) tube at the Allergy and Immunology Laboratory Unit. Blood samples were extracted into PBMCs (Peripheral blood mononuclear cells) via Ficoll-Hypaque Density Gradient Centrifugation and T cell medium, respectively. The PBMCs were analyzed, stimulated with recombinant Hepatitis A antigen VP3 at concentration of 2,5, and 10 mcg/ml and quantified T cell by enzyme-linked immunosorbent assay.

Interferon gamma+T cell stimulation level to Hepatitis A antigen at concentration of 2,5,10 mcg/ml more than negative control were considered positive immunological level.

2.5 Statistical Analysis

Statistical analysis was performed using STATA 15.1(StataCorp, College Station, Tx, USA). Categorical data were expressed as number and percentage. Continuous data were expressed as mean with standard deviation (SD), whereas GMR values were expressed as mean with 95% confidence interval (95% CI).

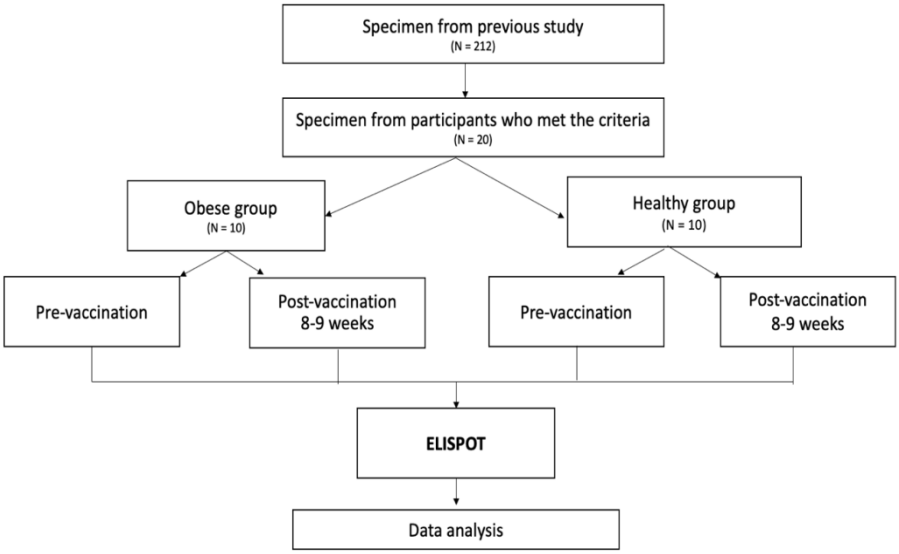

Fig. 1. Assembly chart for blood specimen collection of subjects in 2 groups

Comparison between two groups were analyzed using Two-sample independent t-test for mean, Wilcoxon rank sum test for median and Chi-square or fisher exact test for proportion. Association between factors were analyzed by simple linear regression. P values less than 0.05 were considered to be statistically significant.

3. RESULTS

3.1 Participants

From the twenty participants, mean with SD were 10.2 (0.8). Ten participants were allocated into obese group and the remaining ten participants were allocated into healthy group. For obese group, mean with SD of weight, BMI, and waist circumference were 68.2 (13), 30.7 (4.9), and 92.5 (10.2), respectively. For healthy group mean with SD of weight, BMI, and waist circumference were 30.3 (5.9), 16 (1.4), and 57.8 (4.3), respectively.

There was no statistically significant difference in age between both groups. However, in obese group, there were participants with acanthosis, truncal obesity, and underlying of nonalcoholic fatty disease (NFALD) 90%, 100%, and 40%, respectively (Table 1).

3.2 Pre- and Post-vaccination Immunogenicity

Overall Interferon gamma+ T cell stimulation to Hepatitis A antigen at concentration of 2,5,10 mcg/ml had positive immune level in both groups.

Table 1. Baseline characteristics

	Total (N=20)	Normal (N=10)	Obese (N=10)	P-value
Age in years, mean (SD)	10.2 (0.8)	10.2 (0.4)	10.2 (1)	0.99
Female, n(%)	10 (50)	5 (50)	5 (50)	0.99
Weight, mean (SD)	49.3 (21.8)	30.3 (5.9)	68.2 (13)	<0.001
BMI, mean (SD)	23.3 (8.3)	16 (1.4)	30.7 (4.9)	<0.001
BMI Z score				
• **Mean (SD)**	1.6 (2.4)	-0.5 (0.7)	3.7 (1.2)	<0.001
• **Median (IQR)**	1.3 (-0.5 to 3.7)	-0.5 (-1.2 to 0.04)	3.7 (2.7 to 4.2)	<0.001
Waist circumference, mean (SD)	75.2 (19.4)	57.8 (4.3)	92.5 (10.2)	<0.001
Acanthosis nigricans, n(%)	9 (45)	0	9 (90)	<0.001
Truncal obesity, n(%)	10 (50)	0	10 (100)	<0.001
NFALD, n(%)	4 (20)	0	4 (40)	0.08

In obese group, Interferon gamma+ T cell stimulation GMR (95% CI) at concentration of 2,5,10 mcg/ml of Hepatitis A antigen at pre-and postvaccination were 0.66 (0.23-1.92), 1.18 (0.47-2.96), and 1.19 (0.55-2.57), respectively. In healthy group, Interferon gamma+ T cell stimulation GMR (95% CI) at concentration of 2,5,10 mcg/ml of Hepatitis A antigen at pre-and postvaccination were 1.06 (0.39-2.85), 1.15 (0.57-2.32), and 1.28 (0.76-2.18), respectively (Table 2, Table 3).

Comparison of Interferon gamma+ T cell stimulation at concentration of 5,10 mcg/ml of Hepatitis A antigen between pre-and post-vaccination, post-vaccination expressed higher level of T cell stimulation than pre-vaccination in both obese and healthy groups. Otherwise, there were no statistically significant difference between pre-and post-vaccination (Fig. 2.2, Fig. 2.3).

For healthy group, Interferon gamma+ T stimulation at concentration 2 mcg/ml of Hepatitis A antigen at pre-vaccination had positive correlation to Interferon gamma+ T stimulation at post-vaccination (Pearson correlation 0.76, p value 0.01) (Fig. 3.1).

However, comparison of Interferon gamma+ T cell stimulation GMR (95% CI) at concentration of 2,5,10 mcg/ml of Hepatitis A antigen at pre-and postvaccination between obese and healthy group were no statistically significant difference (Table 4).

Moreover, simple linear regression demonstrated that influencing factors (gender, BMI, waist circumference, obesity complications) were not associated with Interferon gamma+ T cell stimulation at any concentration of Hepatitis A antigen.

4. DISCUSSION

In 2006, Eliakim, A. et al. reported that children with obesity were linked to poor humoral immune response to tetanus vaccine [14]. As well as, Painter, SD et al, demonstrated that obesity and high BMI level were predictive factors of poor humoral immune response to hepatitis B vaccine in adults [10].

However, the result of our study was unexpected, Interferon gamma + T cell activation demonstrated positive immune level in obese and healthy group. Post-vaccination in both obese and healthy groups expressed higher level of T cell stimulation to Hepatitis A antigen at concentration of 5,10 mcg/ml than pre-vaccination. Unfortunately, there were no statistically of Interferon gamma+ T cell stimulation GMR (95% CI) at concentration of 2,5,10 mcg/ml of Hepatitis A antigen at pre-and post-vaccination between both groups.

Recently, Gasmi, A. et al. stated that obesity was associated with increased in macrophage phagocytic activity and the proinflammatory cytokines synthesis including interferon-gamma [15]. Therefore, this may support our study that children with obesity are likely to have potentially expressed interferon gamma+ T cell stimulation as well as heathy group.

According to former study of Dumrislip, T. et al. demonstrated that after single dose of live attenuated HAV were injected, anti HAV titer in both obese and normal weight groups were determined as seroprotective level. Also, there no statistically significance of anti HAV titer between obese and normal weight group [12]. In addition, Lim, et al. reported that obesity was not associated with HAV immunogenicity after a single dose of HAV vaccine in Korean adolescent subjects [16]. This may suggest that obesity is not effect on humoral immune response and cell mediated immune response to live attenuated HAV in children and adolescents.

On the other hand, there are other influencing factors that effects hepatitis A vaccine immunogenicity apart from humoral and cell mediated immune response including age, vaccine itself, and HAV receptor on T cell. In Austria, 2008, Garner-Spitzer, E. et al, reported that the majority of population were hepatitis A vaccine responder, whereas, only two percents of population expressed antibody titer below protective level, were considered hepatitis A vaccine non/low responder. In hepatitis A vaccine non/low responder had low antibody titer, low T cell function, and low Hepatitis A cellular receptor 1 (HAVcr-1) expression on CD4+T cell surface. Thus, Hepatitis A cellular receptor may play a prediction marker role of immune responsiveness to hepatitis A. Future studies may focus on Hepatitis A cellular receptor in obese population [17].

Live attenuated HAV itself has high immunogenicity especially in children. Faridi, MM et al., demonstrated that the majority of subjects achieved seroprotective level at 6 months

following single dose of the HAV vaccine and had highest immunogenicity in early age group (age 18-24 months) [18]. Similarly, Zhang, X et al. reported that after a single dose of live attenuated HAV vaccine, school aged children had anti HAV titer persisted as seroprotective level at least 24 months [19]. Previous studies stated that a single dose of live attenuated HAV vaccine could induce both humoral and cell-mediated immune responses [7,20,21].

In conclusion, the immunogenicity of live attenuated HAV does not depends on humoral and cell mediated immune response alone. There are some factors that may affected live HAV immunogenicity such as age, cytokine function, and HAV receptor on T cell that should be investigated in further study. However, after a single dose of live attenuated HAV, children with obesity had seroprotective level in both humoral and cell mediated immune response [6,22,23].

Furthermore, this is the first time reported that T cell stimulation to live attenuated hepatitis A vaccine in children with obesity.

Limitation of this study is the small amount number of participants. Additional studies may focus on the cytokine and other factors that effects T cell function, especially Granzyme B level or HAV receptors on T cells, to other live attenuated vaccines.

5. CONCLUSION

Single dose live attenuated hepatitis A vaccine is safe and has high immunogenicity in children with obesity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

SUPPLEMENTARY MATERIAL

Supplementary Material is available in this link: https://www.journalaji.com/index.php/AJI/libraryFiles/downloadPublic/4

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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