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RESEARCH ARTICLE

# Betatrophin: A promising biomarker for metabolic syndrome and diabetes mellitus risk screening in teenagers

Hendra Susanto [1], Aulanni'am Aulanni'am [2,3], Dyah Kinasih Wuragil [3,4], Ahmad Taufiq [5], Sunaryono Sunaryono [5], Jerry Dwi Trijoyo Purnomo [6], Dyah Ika Krisnawati [7], Moch Sholeh [8]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Malang, Indonesia
[2]Department of Chemistry, Brawijaya University of Malang, Malang, Indonesia
[3]Biosains Institute, Brawijaya University, Malang, Indonesia
[4]Laboratory of Veterinary Biochemistry, Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia
[5]Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Malang, Indonesia
[6]Department of Statistics, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia
[7]Department of Nursing, Faculty of Nursing and Midwifery, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia
[8]Department of Biomedical Science, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

*Correspondence:*
Hendra Susanto
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jalan Semarang 05 Malang, East Java Province – 65145, Indonesia

Email: hendrabio@um.ac.id

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## Abstract

Metabolic syndrome (MetS) and diabetes mellitus (DM) have become primary concerns worldwide, especially among the younger population. The Indonesian Boarding School model (IBS/Boarding School) is a large education system with a significant number of pupils (teenagers) and has the potential to become a center for metabolic disease, particularly among teenagers, due to their daily intake. This study aimed to provide a baseline screening for MetS and the risk of DM development in Boarding School teenagers. During this observational study, 90 healthy serological samples were obtained from senior and junior high school students. The circulating level of betatrophin was measured using a human betatrophin ELISA kit. Additionally, metabolic syndrome and DM screening data were analyzed using a rapid reverse-flow immunochromatography kit for 65 kDa glutamic acid decarboxylase (GAD65). Among the 90 healthy students, a high prevalence of GAD65 was observed, indicating a potential risk factor for metabolic diseases. Furthermore, higher serum betatrophin levels were observed in the samples. The circulating level of betatrophin was found to have a significant correlation with age, gender, body mass index (BMI), systolic blood pressure (SBP), fasting blood glucose (FBG), sleeping duration, and duration of stay at Boarding School ($p < 0.05$). Betatrophin emerged as a potential predictor of BMI, SBP, and FBG in students ($p < 0.05$). Both betatrophin and GAD65 have shown promise as future biomarkers, opening up a new avenue for assessing metabolic syndrome and the risk of DM. This underscores the importance of future programs in Boarding Schools focusing on MetS and DM prevention management, making the audience feel the significance of their work in addressing these pressing health issues.

## 1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder caused by long-term hyperglycemia, hypertriglyceridemia, and dynamic alteration in body weight (1). The increasing incidence of DM triggers a significant health problem, not only

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in Western countries but also in developing countries. The prevalence of DM worldwide continues to rise significantly, with a notably high mortality rate among adolescents. As of 2021, 537 million adults (aged 20-79 years) are living with diabetes, which represents approximately 1 in 10 people. This number is predicted to rise to 643 million by 2030 and 783 million by 2045. Over three-quarters of adults with diabetes live in low- and middle-income countries. Additionally, 541 million adults have Impaired Glucose Tolerance (IGT), placing them at high risk of developing type 2 diabetes (1). The International Diabetes Federation (IDF) estimates that approximately 90 million adults aged 20-79 years in the Western Pacific region, which includes many Asian countries, will be living with diabetes in 2021. This number is projected to rise to 150 million by 2045. The prevalence of diabetes among adults in the ASEAN region varies but is generally on the rise. For example, the IDF reported that about 11% of adults in Southeast Asia were living with diabetes as of 2021. In Indonesia, one of the countries with the highest diabetes prevalence rates in Southeast Asia, around 10.7% of adults are estimated to have diabetes, translating to approximately 16 million people living with diabetes in the country (1).

DM is caused by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both (1,2). The etiology of diabetes can be classified into several types, primarily Type 1 and Type 2 diabetes, along with gestational diabetes and other specific types (2). DM is also a challenging disease that contributes to metabolic complications (2). Current data show that metabolic syndrome is associated with the development of DM (3) and is linked to the rising prevalence of diabetes in the younger population (4,5). DM is also associated with alterations in the cardiovascular system (6,7), lower levels of high-density lipoprotein (HDL) (8), and significantly increased mortality in affected patients (9,10). The relationship between obesity and the risk of developing DM is complex and multifaceted (11,12). Lifestyle modifications, such as improved diet and increased physical activity, play a crucial role in reducing the risk of diabetes and improving overall metabolic health (13).

One potential serological biomarker for lipid metabolism and metabolic syndrome development is lipase or betatrophin (ANGPTL-8). Elevated levels of this liver-derived hormone have been confirmed in individuals with hypertriglyceridemia (14,15), metabolic syndrome in young women (16), obesity (17–19), new-onset diabetes (20), nephropathy (21), and pancreatic cancer patients with diabetes (22). In contrast, recent data from our previous non-clinical studies shows that physical activity intervention through a specific exercise model in younger individuals with obesity can decrease circulating levels of betatrophin (23). Therefore, it is crucial to stress the importance of early detection of metabolic syndrome, diabetic symptoms, and physical activity interventions. These could effectively support the future development of metabolic abnormalities prevention programs, particularly for the younger generation.

The initial risk screening for DM in younger Boarding School populations can be assessed by measuring betatrophin levels and an additional biomarker $GAD_{65}$ protein, in circulation. In clinical investigation, elevated serum $GAD_{65}$ levels indicate that asymptomatic individuals may develop DM (24). This enzyme has been considered a potential biomarker of β-cell dysfunction in both types of DM (24,25). Previous studies have suggested that $GAD_{65}$ has excellent sensitivity for detecting the risk of DM (26,27). Additionally, the dynamic physiological perturbations related to lipid metabolism and metabolic syndrome could provide valuable information to prevent future negative impacts on the population.

Although some previous studies have investigated preliminary data on the medical profiles of Boarding School students, limited data is available on metabolic syndrome and DM risk screening in this younger population. This study provides an initial approach to the prevention of metabolic diseases and the risk of diabetes mellitus, particularly for the younger population in Boarding Schools. Moreover, this study could offer a novel, rapid screening method for metabolic syndrome and DM risk symptoms to support future policies for healthy Boarding School programs as part of the extensive education system in Indonesia.

## 2. MATERIALS AND METHODS

### 2.1. Study Population

The study population was part of an ongoing non-clinically observational study. Our study was conducted in collaboration between the Department of Biology, Faculty of Mathematics and Science, Universitas Negeri Malang; the Boarding School Zainul Hasan (PZH), Genggong-Probolinggo; and Biosains Institute of Brawijaya University, Indonesia (23). The enrolled participants were 90 healthy students (without a history of DM related to hyperglycemia and hyperlipidemia) from Junior High School and Senior High School students at PZH. In total, there were 58 male students and 34 female students. This preliminary study was approved by the Research Ethics Committee of Sekolah Tinggi Ilmu Kesehatan (STIKES) Hafshawaty Boarding School Zainul Hasan (PZH) with certificate number KEPK/256/STIKes-HPZH/X/2022. All enrolled participants signed the informed consent form, and samples were collected using a randomized sampling technique at both schools.

### 2.2. Data Collection

All participants in the study underwent overnight fasting prior to data collection. Anthropometric measurements and blood pressure were recorded following standard protocols. Fasting blood glucose levels were measured using a rapid fasting blood glucose test (Easy Touch GCU or Glucose, Cholesterol, Uric Acid Meter by Bioptic Technology Company, USA).

This device is designed to measure blood sugar, cholesterol, and uric acid levels, with each parameter indicated by the color of the corresponding chip and strip (green for blood sugar, blue for cholesterol, and yellow for uric acid). To use the device, the battery was first installed in the appropriate compartment, and the device was turned on. Upon activation, the date format appeared on the device screen before automatically turning off after a few moments. To measure blood sugar, the green chip was attached to the back of the device, and the green sugar strip was inserted at the top. The chip code display then appeared on the screen, followed by a flashing blood drop icon. A blood drop was applied to the strip, aligning with the arrow at the end of the strip. Results were displayed within a few seconds. The same procedure was followed for measuring cholesterol and uric acid, using the appropriate chips and strips for each parameter. Total cholesterol levels were quantified using the Lipid Pro test Kit (Infopia Co., Ltd, South Korea). Additionally, a 2 mL whole blood sample was drawn from the cubital vein for reverse-flow immunochromatography assay and Enzyme Linked-Immunosorbent Assay (ELISA). The blood sample was allowed to stand at room temperature for 30-60 minutes and before being centrifugated at 3000 rpm for 20 minutes. The resulting serum was stored at -86ºC for subsequent serological detection $GAD_{65}$ protein and betatrophin measurement via ELISA.

## 2.3. The Serological Detection of Betatrophin and Human-Recombinant $GAD_{65}$ KDa

Betatrophin expression at the protein level was quantified using a human ELISA kit for lipases or betatrophin (EIAab Lab, China, with catalog No. E11644h). All reagents were brought to room temperature before use. The standard was first reconstituted by adding 120 µL of the standard stock (12.8 ng/mL) and 120 µL of standard diluent to generate a 6.4 ng/mL standard solution. The standard was allowed to sit for 15 minutes with gentle agitation before making serial dilutions. Duplicate standard points were prepared by serially diluting the standard stock solution (6.4 ng/mL) 1:2 with standard diluent, resulting in a solution of 3.2 ng/mL, 1.6 ng/mL, 0.8 ng/mL, and 0.4 ng/mL. Standard diluent served as the zero standard (0 ng/mL). Furthermore, 20 mL of Wash Buffer Concentrate (25x) was diluted with deionized or distilled water to yield 500 mL of 1x Wash Buffer. If crystals were observed in the concentrate, they were gently mixed until completely dissolved. The assay was performed at room temperature, and the appropriate number of strips was determined. The strips were inserted into the frames for use. Firstly, 50 µL of the standard solution was added to the standard wells (no biotinylated antibody was added to the standard well, as the standard solution already contained biotinylated antibody). Secondly, 40 µL sample was added to the sample wells, followed by 10 µL of anti-BDNF antibody. Then, 50 µL of streptavidin-HRP was added to the sample and standard wells (excluding the blank control well). The wells were mixed well and covered with a sealer, and the plate was incubated for 60 minutes at 37°C. After incubation, the sealer was removed, and the plate was washed 5 times with wash buffer. Each well was soaked with 300 µL wash buffer for 30 seconds to 1 minute during each wash. Each well was aspirated or decanted for automated washing and washed 5 times with wash buffer. The plate was then blotted onto paper towels or other absorbent material. Subsequently, 50 µL Substrate Solution A was added to each well, followed by 50 µL Substrate Solution B. The plate was incubated for 10 minutes at 37°C in the dark, with a new sealer covering the plate. After incubation, 50 µL Stop Solution was added to each well, causing the blue color to change to yellow. Each well's optical density (OD) was determined immediately using a microplate reader (ELISA Reader) set to 450 nm within 10 minutes after adding the stop solution. The results were calculated by constructing a standard curve, plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and drawing a best-fit curve through the points on the graph. This calculation was performed using computer-based curve-fitting software, and the best-fit line was determined through regression analysis, ensuring the thoroughness and reliability of the results.

For GAD65 KDa detection, approximately 5 µL of serum sample was dropped onto a Biosains Rapid Test GAD65 (Biosains Lab, Universitas Brawijaya-Indonesia). The kit, an early diagnostic test for diabetes, is ready for mass production and marketing collaboration with Biofarma Company (Indonesia). The diagnostic kit, with its excellent sensitivity (100%) and a specificity of 91.67%, has successfully passed laboratory and field patient tests, demonstrating its reliability. The Biosains Rapid Test GAD65 is designed to detect Diabetes Mellitus (DM) types 1, 1.5 (Also known as Latent Autoimmune Diabetes in Adults or LADA), and 2 by identifying the presence of GAD65 autoantibodies, early markers of pancreatic beta-cell damage. This reliable test can detect the onset of autoimmune diabetes, making it suitable for use in infants and children with a family history of diabetes, and as an early screening tool for DM, it can significantly improve the prevention and management of the disease, particularly for families with a history of DM.

In order to perform the GAD65 Rapid Test, the kit was kept at room temperature and gently mixed with the buffer for 20 to 30 minutes before use. About 20 µL of blood serum was dropped onto the absorbent paper (sample pad) of the kit, and the serum was allowed to travel to the observation section, which contains a nitrocellulose membrane. One drop of Buffer A was applied to the sample pad, one drop of buffer B to the signal reagent area (a polyester membrane), and one additional drop of buffer A to the signal reagent area. The kit was attached using a clip, and the results could be developed for 20-30 minutes. Two red lines in the control and test line areas indicated positive results, while a single red line in the control area was considered harmful. The results obtained were analyzed using descriptive statistical methods based on the outcomes of the GAD65 Rapid Test.

### 2.4. Data Analysis

Data distribution was assessed using the Kolmogorov-Smirnov normality test. Continuous variables were presented as mean ± standard deviation (SD). The data were compared between groups using a parametric unpaired t-test. Pearson's product-moment correlation and univariate linear regression analysis were used to examine associations between parameters, utilizing IBM SPSS v25. Data visualizations were generated using the GraphPad Prism V5 application. A significance level of 5% was considered for all statistical analyses.

## 3. RESULTS AND DISCUSSION

### 3.1. Baseline characteristics of the study participants

The baseline characteristics of the students in senior and junior high school are shown in Table 1. Most students were between 14 and 18 years old, and the majority had blood type O. Interestingly, the proportion of underweight students was similar in both groups. More than half of senior high school students were categorized as overweight or obese, while the younger group predominantly had a normal BMI. The increasing prevalence of overweight and obesity among teenagers is influenced by various factors, including dietary habits, socioeconomic conditions, and environmental changes (28). It's crucial to address this issue with comprehensive public health strategies that focus on nutrition education and promoting active lifestyles among adolescents (29,30). Continued research is essential to monitor trends and develop effective interventions tailored to specific populations, but the first step is to implement these strategies to combat the rising rates of adolescent obesity.

Similar patterns of systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBG), and total cholesterol (TC) were observed in both junior and senior high school groups. In addition, senior students reported a more extended stay at the Boarding School than junior students. Higher body mass index (BMI) in adolescents is associated with elevated SBP and DBP. A study involving 3,862 adolescents found that increased arterial stiffness (measured by carotid-femoral pulse wave velocity) was linked to a higher risk of elevated SBP and DBP, particularly in those with overweight or obesity (31). Another study indicated that adolescents who were overweight or obese had a significantly higher prevalence of hypertension (32). Engaging in structured physical activities has been shown to reduce blood pressure positively among overweight adolescents (33,34). Overweight teenagers are also at increased risk for impaired glucose metabolism. While specific studies directly linking FBG levels to overweight status in teenagers were not identified in the search results, obesity is a known risk factor for developing insulin resistance and type 2 diabetes, both of which are often associated with elevated FBG levels (35-37). Dyslipidemia is prevalent among overweight adolescents. A study showed that 64% of obese teenagers had elevated total cholesterol levels, with significant reductions observed after dietary and physical activity interventions. This suggests that obesity management can improve lipid profiles (38-40).

Interestingly, although senior students had longer sleep durations (4–5 hours/day), the prevalence of overweight and obesity was still higher than in the younger group. Additionally, despite the younger students having similar resting time of 4–5 hours/day, this group was predominantly characterized by normal BMI and blood pressure. Insufficient sleep has been linked to various health risks, particularly in adolescents, where it may contribute to the development of overweight and obesity. Research indicates that promoting regular and sufficient sleep can serve as a cost-effective strategy for preventing these issues (41,42). By encouraging good sleep hygiene and establishing consistent sleep patterns, health interventions can potentially mitigate the risk of weight gain during this critical developmental stage (43,44). This approach not only fosters long-term well-being in adolescents, but also stresses the importance of addressing immediate health concerns, urging the audience to take action.

When it comes to smoking habits, it's important to note that younger students at the Boarding School are more likely to smoke than their senior counterparts. This trend is not unique to our school, as other studies have also found a significant relationship between overweight status and smoking habits in teenagers. These habits are not just a matter of personal choice, but are heavily influenced by psychosocial factors, peer dynamics, and misconceptions about weight control (45-47). Addressing these complex issues through education, support, and policy changes is crucial for promoting healthier lifestyles among adolescents.

### 3.2. Betatrophin expression in younger population of boarding school

The serological test of betatrophin/lipasin/ANGPTL-8 protein expression in the Boarding School teenagers' samples has unveiled findings with promising future applications. It was found that circulating betatrophin levels were higher in the older group compared to the younger group (Figure 1). The serum level of betatrophin was significantly higher in senior high school students than in junior high school students ($p < 0.05$), especially in adolescents classified as overweight or obese (Table 1). In our previous study, we observed that betatrophin levels in plasma increased in young women who were obese and decreased following physical exercise interventions (23). Other research also suggests that betatrophin levels can be altered in obese individuals. Studies have shown that circulating betatrophin levels are often elevated in obese teenagers compared to their lean counterparts. This increase may serve as a compensatory mechanism in response to

insulin resistance and metabolic dysregulation commonly seen in obesity (48). Betatrophin levels in plasma have also been found to significantly correlate with BMI and endurance-related oxygen consumption (23).

**Table 1.** Participants baseline characteristics

| Parameter | Junior High School (n = 44) | Senior High School (n = 46) |
|---|---|---|
| Age (years) | | |
| 13 | 2 | 0 |
| 14 | 15 | 0 |
| 15 | 24 | 0 |
| 16 | 3 | 4 |
| 17 | 0 | 13 |
| 18 | 0 | 29 |
| Blood type | | |
| A | 5 | 4 |
| B | 6 | 9 |
| AB | 2 | 4 |
| O | 31 | 29 |
| Height (m)* | 1.57 ± 0.07 | 1.61 ± 0.07 |
| Weight (kg)* | 51.60 ± 2.84 | 69.30 ± 2.24 |
| BMI categories (kg/m$^2$) | | |
| Underweight | 19 | 13 |
| Normal | 17 | 6 |
| Overweight | 6 | 7 |
| Obese | 2 | 20 |
| SBP (mmHg) | | |
| > 130 | 13 | 4 |
| < 130 | 31 | 42 |
| DBP (mmHg) | | |
| > 85 | 5 | 5 |
| < 85 | 39 | 41 |
| FBG (mg/dL) | | |
| > 100 | 16 | 13 |
| < 100 | 38 | 33 |
| TC (mg/dL) | | |
| > 170 | 7 | 5 |
| < 170 | 37 | 41 |
| Duration of stay at Boarding School (years) | | |
| 6–7 | 0 | 27 |
| 4–5 | 0 | 8 |
| 2–3 | 43 | 11 |
| < 2 | 1 | 0 |
| Sleeping duration (hours/day) | | |
| > 7 | 0 | 0 |
| 6–7 | 12 | 39 |
| 4–5 | 36 | 7 |
| < 4 | 6 | 0 |
| Smoking | | |
| Yes | 27 | 10 |
| No | 17 | 36 |

Abbreviations: *) Data are presented as mean SD. Systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBG), and total cholesterol (TC).

Furthermore, betatrophin was significantly elevated in a case study involving 109 patients with type 2 diabetes mellitus (T2DM) at various stages of albuminuria. Betatrophin may serve as a novel endocrine regulator involved in developing diabetic nephropathy (DN) (21). Betatrophin is believed to influence the development of insulin-producing beta cells in the pancreas (49, 50). Its role in lipid metabolism suggests it may also affect triglyceride levels and overall fat storage (51). The expression of ANGPTL8 is closely linked to the regulation of lipoprotein lipase, an enzyme crucial for breaking down fats in the bloodstream (52-54). In general, betatrophin plays a crucial role in the metabolic health of obese teenagers by influencing insulin secretion and lipid metabolism. Interventions such as regular exercise can enhance its function, leading to improved metabolic outcomes and reduced risks associated with obesity. This potential for improved metabolic outcomes offers hope and optimism for the future of obesity management. Regular monitoring and lifestyle changes are essential for managing obesity-related complications in this demographic (55,56).

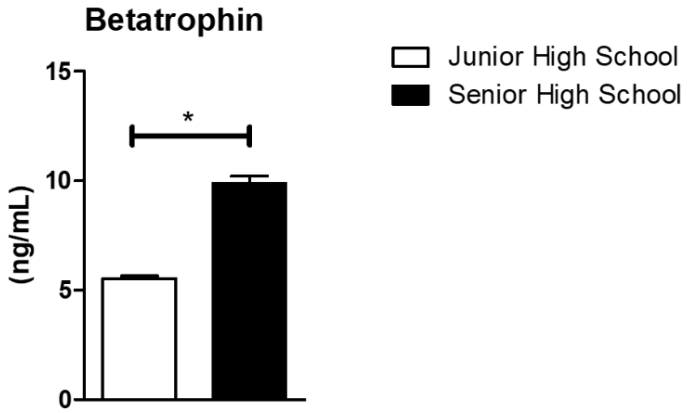


**Figure 1.** The Protein Level of Betatrophin in Junior and Senior High School Islamic Boarding School Students. * Significant vs placebo ($p \leq 0.05$).

In this observational study, we explored the potential correlations between betatrophin and various health parameters from our baseline data. Our findings revealed significant associations between the serum level of betatrophin and age (r = 0.592, p = 0.000), gender (r = 0.655, p = 0.000), BMI (r = 0.278, p = 0.012), SBP (r = –0.311, p = 0.0005), FBG (r = –0.233, p = 0.047), sleeping time (r = 0.340, p = 0.002), and duration of stay (r = 0.567, p = 0.000) (Table 2). However, we did not find any significant correlation between betatrophin and other parameters, including blood type, DBP, and total cholesterol, providing reassurance about the specificity of our findings.

**Table 2.** Univariate correlation with the betatrophin in subjects

| | Univariate Correlation | |
|---|---|---|
| | ***r*** | ***P-value*** |
| Age | 0.592 | 0.000* |
| Gender | 0.655 | 0.000* |
| Blood Type | -0.152 | 0.178 |
| BMI | 0.278 | 0.012* |
| SBP | -0.311 | 0.005* |
| DBP | -0.018 | 0.877 |
| Total Cholesterol | -0.022 | 0.845 |
| FBG | -0,233 | 0.047* |
| Sleeping Duration | 0.340 | 0.002* |
| Duration of Stay | 0.567 | 0.000* |

*Significance at level 0.05

Betatrophin levels may vary with age, potentially reflecting changes in metabolic processes and insulin sensitivity over time. Older adults might exhibit different betatrophin responses than younger individuals due to age-related metabolic changes (57). Studies have indicated that gender can impact betatrophin levels, with some research suggesting that females may have higher levels than males, possibly due to hormonal differences (58). Higher BMI is often associated with increased levels of betatrophin. Obesity can alter metabolic pathways, potentially elevating betatrophin as a compensatory response to insulin resistance (59). Elevated SBP may also correlate with changes in betatrophin levels, as hypertension is commonly associated with metabolic syndrome, which could influence the secretion of this protein (60).

FBG levels, as critical indicators of metabolic health, are significantly influenced by higher fasting glucose levels, particularly in individuals with insulin resistance or diabetes (57). The role of educational background in reflecting socioeconomic status and lifestyle choices that affect overall health is also important. While a direct correlation between the duration of education and betatrophin levels has not been established, it is worth noting that better education often leads to healthier lifestyle choices. This potential for positive change underscores the importance of education in indirectly impacting metabolic markers.

Moreover, our study utilized linear regression analysis to extend and clarify the independent association between betatrophin and metabolic syndrome parameters. Notably, our data revealed a strong association between betatrophin predictors and BMI (Beta = 0.636, p = 0.012), SBP (Be-ta = –1.515, p = 0.005), and FBG (Beta = –1.331, p = 0.047) (Table 3). As previously discussed, betatrophin is a significant predictor of BMI, SBP, and FBG, highlighting its potential in assessing metabolic health and the risk of developing related diseases. This promising potential should inspire hope for the future implications of our research among our colleagues in the field.

**Table 3.** Univariate linear regression analysis

| Response | Predictor | Estimate (B) | p-value | 95% CI |
|---|---|---|---|---|
| BMI | Betatrophin | 0.636 | 0.012* | [0.141–1.130] |
| SBP | Betatrophin | −1.515 | 0.005* | [(−2.560) – (−0.471)] |
| FBG | Betatrophin | −1.331 | 0.047* | [(−2.644) – (−0.019)] |

* Significant with $p \leq 0.05$

Studies have shown that elevated betatrophin levels may be associated with insulin resistance, a key component of metabolic syndrome. Insulin resistance leads to increased blood sugar levels and can contribute to the development of T2DM (61). Betatrophin levels are often higher in obese individuals, which may reflect the body's attempt to compensate for insulin resistance and impaired glucose metabolism associated with excess adiposity (14,17,19,21,22,48). Betatrophin is also linked to lipid metabolism, which is often disrupted in metabolic syndrome. Higher betatrophin levels might correlate with dyslipidemia (abnormal lipid levels), further complicating metabolic health (56,58). Betatrophin levels can be measured in clinical settings using techniques such as enzyme-linked immunosorbent assay (ELISA) or mass spectrometry, providing a practical way to assess its role in metabolic disorders.

Other research indicates that individuals with T2DM may have altered betatrophin levels compared to healthy individuals. Some studies suggest that lower betatrophin levels could indicate beta-cell dysfunction, a hallmark of diabetes (19,21,25). There is evidence that betatrophin may play a role in glycemic control, potentially influencing the progression of diabetes. Elevated betatrophin levels have been observed in response to glucose challenges, suggesting a compensatory mechanism in the presence of hyperglycemia (37). Due to its association with insulin sensitivity and beta-cell function, betatrophin is being explored with great promise as a potential biomarker for assessing the risk of developing T2DM and monitoring metabolic health (35,52).

## 3.3. The screening of diabetes mellitus risk by GAD65 rapid test kit

Our research on diabetes risk screening in students involved performing GAD65 tests in both junior and senior high school students. The significant negative and positive results for the GAD65 reverse flow immunochromatography rapid test, as shown in Figure 2, have practical implications for diabetes detection in students. The percentage of individuals with positive test results for GAD65 was notably higher in both groups of the younger population, as depicted in Figure 3.

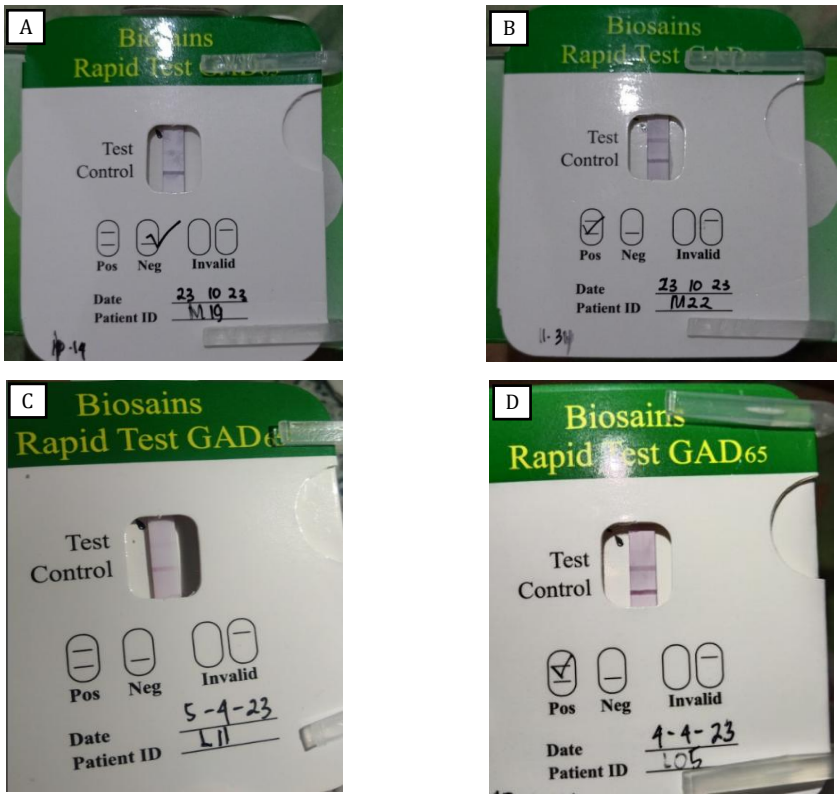


**Figure 2.** The representative results of anti-GAD65 autoantibodies detection rapid test kit. (A) Negative sample of Senior Junior School Students. (B) Positive sample of Junior High School Students. (C) Negative sample of Senior High School Students. (D) Positive sample of Senior High School Students.

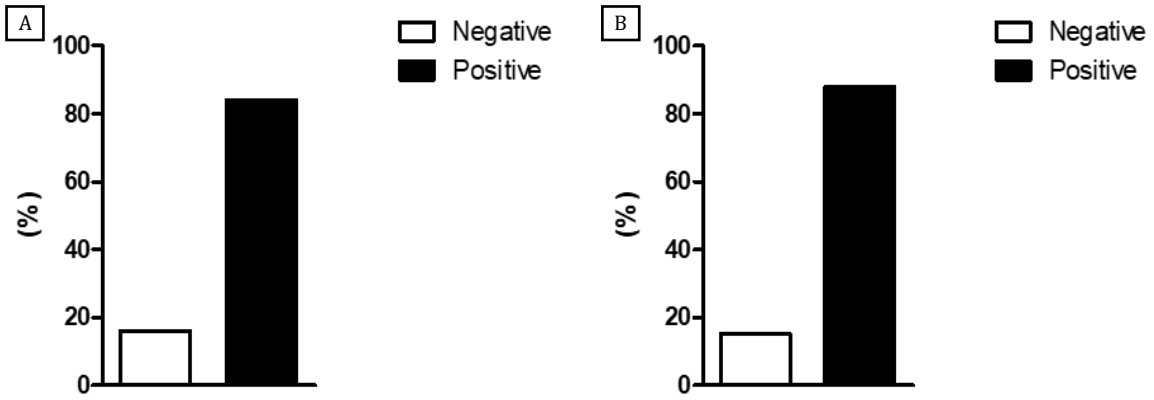


**Figure 3.** Percentage of Negative and Positive Samples for $GAD_{65}$-based test. (A) Junior School Students, (B) Senior High School Students.

GAD65's role in the interplay between obesity and metabolic syndrome, its involvement in insulin secretion, potential autoimmune responses, and inflammatory processes, all highlight its importance in understanding these complex conditions (62-64). Our previous study on 276 healthy subjects and 51 type 1 diabetes showed precise results that our GAD65 rapid test can detect DM (65). In the clinical implications, the potential of GAD65 antibodies as a biomarker for distinguishing between Type 1 and Type 2 diabetes, particularly in atypical cases, is a significant clinical implication. Understanding the role of GAD65 in metabolic syndrome and diabetes could lead to innovative therapeutic strategies to enhance insulin sensitivity and preserve beta-cell function (25,26,66).

## 4. CONCLUSIONS

In summary, we have identified that betatrophin is related to metabolic syndrome and risk of diabetes in students of Boarding School. Thus, we recommend betatrophin biomarkers for sustainable screening of individuals and as valuable predictors of newly onset metabolic syndrome and diabetic development in future clinical settings.

**Author contributions:** HS, AA: Conceived the research and contributed to the research project design, the data interpretation, and writing of the manuscript. DKW, DIK, MS: Contributed to the participant recruiting and drafting of the paper. JDTP, AT, SS: Contributed to the analysis of the clinical data. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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**Ethics statement:** The implementation of this research has been approved by the health research ethics committee of the Sekolah Tinggi Ilmu Kesehatan Hafshawaty Boarding School Zainul Hasan (School for Health Sciences) with number KEPK/256/STIKes-HPZH/X/2022. All respondents have agreed to contribute to this research and obtained permission from the leadership of the Zainul Hasan Geng-gong Boarding School, Probolingo, East Java Province, Indonesia.

**Conflict of interest:** We declare no potential conflicts of interest relevant to this article to report.

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